Non-Steroidal Anti-Inflammatory Drug Effects On Core Temperature, Hydration, Gastrointestinal Distress, And Performance In Exercising Humans

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NON-Steroidal ANti-INFLAMMATory DRUG EFFECTS ON CORE TEMPERATURE, HYDRATION, GASTROINTESTINAL DISTRESS, AND PERFORMANCE IN EXERCISING HUMANS

by

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DEDICATION

To the Lord, Our God.

I would have nothing without Him. All joy, love, patience, and success in my life and surrounding this project is because of Him who blessed me. He is by my side in my weakness, turmoil, frustration, doubt, and anger. He does not give up, but challenges, encourages, and guides. I offer this and everything I do to Him.

Credo in unum Deum, Patrem omnipotentem, factorem caeli et terrae, visibilibum omnium et invisibilium, Et in unum Dominum Iesum Christum, Filium Dei unigenitum, et ex Patre natum, ante omnia saecula, Deum de Deo, lumen de Lumine, Deum verum de Deo vero, genitum, non factum, consubstantialem Patri: per quem omnia facta sunt.

Qui propter nos homines et propter nostram salutem descendit de caelis. Et incarnatus est de Spiritu Sancto ex Maria Virgine, et homo factus est. Crucifixus etiam pro nobis sub Pontio Pilato; passus et sepultus est, et resurrexit tertia die, secundem Scripturas, et ascendit in caelum, sedet ad dexteram Patris. Et iterum venturus est cum gloria, iudicare vivos et mortuos, cuius regni non erit finis.


Amen.
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ABSTRACT

Naproxen is a commonly used non-steroidal anti-inflammatory drug designed to relieve pain and inflammation. Due to potentially adverse effects on the gastrointestinal (GI) tract, cardiovascular and immune system, and renal function, naproxen may negatively affect thermoregulation, fluid and electrolyte balance, and performance during exercise, particularly in the heat. Therefore, the purpose of this study was to determine the effects of naproxen on core temperature (Tc), inflammation, hydration, GI distress, and performance during cycling in a hot environment. We utilized a double-blind, randomized and counterbalanced, cross-over design to determine the effects of naproxen (dose = 3220 mg naproxen sodium pills) or placebo (3 cellulose pills) on the dependent variables: Tc, interleukin-6 (IL-6), GI distress symptoms, fecal occult blood, plasma sodium and potassium concentration, plasma osmolality, urine osmolality, urine specific gravity, percent change in body mass, urine volume, fluid volume, heart rate, blood pressure, rate of perceived exertion, and distance during a 10 min time trial. Participants (n = 11, age = 27.8 ± 5.7 yrs, weight = 79.1 ± 17.9 kg, and \( \dot{V}O_{2\text{max}} = 41.4 ± 5.7 \text{ mL/kg} \)) completed 4 conditions: 1) placebo and ambient (Control); 2) naproxen and ambient (Npx); 3) placebo and heat (Heat); and 4) naproxen and heat (NpxHeat) separated by a minimum of 7 days. Dependent measures were taken pre-, during, post-, and 3 hrs post- a 90 min cycling protocol. We found no statistically significant differences between experimental conditions for any dependent variable. Exercise induced significant overall increases pre- to post- for Tc, IL-6, plasma potassium, heart rate, blood pressure, perceived exertion, and GI distress.
Compared to placebo, naproxen generally led to higher fluid intake and lower urine volume. Mean distance traveled was highest during Npx (3.3 ± 0.8 miles) and lowest in NpxHeat (2.8 ± 0.8 miles). Participants experienced greater GI distress during Heat, especially day post-exercise. During exercise, GI symptom occurred in 64% of trials. Interestingly, dizziness and headache were only reported during placebo conditions, with generally less systemic symptoms during exercise with naproxen use. In conclusion, our results do not support our hypotheses that naproxen would increase Tc, IL-6, and GI distress, alter fluid-electrolyte balance, and decrease performance compared to placebos.
TABLE OF CONTENTS

DEDICATION ....................................................................................................................... iii

ACKNOWLEDGEMENTS ........................................................................................................ iv

ABSTRACT ............................................................................................................................v

LIST OF TABLES ....................................................................................................................x

LIST OF FIGURES ................................................................................................................. xi

LIST OF SYMBOLS .............................................................................................................. xii

LIST OF ABBREVIATIONS ................................................................................................... xiii

CHAPTER 1 OVERALL INTRODUCTION ...................................................................................1

CHAPTER 2 NAPROXEN EFFECTS ON CORE TEMPERATURE AND INFLAMMATION DURING CYCLING IN THE HEAT ..........................................................8

ABSTRACT ...................................................................................................................9

INTRODUCTION ..........................................................................................................10

METHODS ..................................................................................................................11

RESULTS ....................................................................................................................17

DISCUSSION ...............................................................................................................19

REFERENCES .............................................................................................................25

CHAPTER 3 NAPROXEN EFFECTS ON HYDRATION AND ELECTROLYTE MEASURES DURING CYCLING IN THE HEAT ...............................................................36
APPENDIX C: TAKE HOME INSTRUCTIONS ................................................................. 223

C.1. FIRST TRIAL E-MAILS 4 DAYS PRE-DATA COLLECTION ............................ 223
C.2. REMINDER EMAIL DIET AND ACTIVITY LOG .............................................. 224
C.3. SUBSEQUENT TRIALS E-MAILS 4 DAYS PRE-DATA COLLECTION ............... 224
C.4. PRE-DATA COLLECTION TAKE HOME INSTRUCTIONS .......................... 225
C.5. POST-DATA COLLECTION TAKE HOME INSTRUCTIONS ......................... 226

APPENDIX D: GASTROINTESTINAL SYMPTOMS INDEXES ................................. 228

D.1. GI SYMPTOM INDEX DURING EXERCISE .................................................. 228
D.2. GI SYMPTOM INDEX PRE- AND POST- EXERCISE .................................... 229

APPENDIX E: DATA COLLECTION PROCEDURE CHECKLISTS .......................... 230

E.1. INFORMATION SESSION .............................................................................. 230
E.2. V̇O₂MAX TESTING ...................................................................................... 230
E.3. PRE-TRIAL APPOINTMENT .......................................................................... 232
E.4. POST-TRIAL APPOINTMENT ...................................................................... 233
E.5. DATA COLLECTION SESSION ................................................................... 233

APPENDIX F: ADDITIONAL TABLES AND FIGURES .............................................. 240
LIST OF TABLES

Table 2.1 Participant Demographics .................................................................29

Table 3.1 Hydration Measures, Fluid Volume, and Urine Volume for Experimental Conditions .................................................................59

Table 3.2 Plasma Electrolytes for Experimental Conditions ......................60

Table 4.1 Participant Demographics .................................................................86

Table 4.2 Performance Measures during Cycling for Experimental Conditions ....87

Table 4.3 Mean and Maximum Gastrointestinal Symptom Scores for Experimental Conditions .................................................................88

Table 4.4 Mean and Maximum Gastrointestinal Symptom Scores for Experimental Conditions during Exercise .................................................................89

Table F.1. Fluid Volume and Urine Volume for Gender by Experimental Conditions ........................................................................................................245

Table F.2. Diet and Exercise Overall and for Gender ......................................247

Table F.3. Rate of Perceived Exertion during Cycling for Gender by Experimental Conditions .................................................................248

Table F.4. Distance and Max Heart Rate during Cycling for Gender by Experimental Conditions .................................................................249

Table F.5. Mean, Maximum, and Serious (> 4) Gastrointestinal Symptom Scores for Experimental Conditions .................................................................250

Table F.6. Mean and Serious (> 4) Gastrointestinal Symptom Scores for Experimental Conditions during Exercise .................................................................251
LIST OF FIGURES

Figure 2.1 Mean Core Temperature during Cycling and Rest for Experimental Conditions .................................................................30

Figure 2.2a-d Regression and Participant Core Temperature during Cycling ..................31

Figure 2.3 Mean IL-6 Pre-, Post-, and 3 Hrs Post-Cycling for Experimental Conditions .................................................................35

Figure 3.1 Experimental Procedures Schematic .................................................................61

Figure 5.1 Working Model for Overall Study .................................................................186

Figure 5.2 Experimental Procedures Schematic ..............................................................187

Figure F.1 Male Mean Core Temperature during Cycling and Rest for Experimental Conditions .................................................................240

Figure F.2 Female Mean Core Temperature during Cycling and Rest for Experimental Conditions .................................................................241

Figure F.3 Mean Core Temperature during Cycling and Rest for Gender .................242

Figure F.4 Mean IL-6 Pre-, Post-, and 3 Hrs Post-Cycling for Males .............................243

Figure F.5 Mean IL-6 Pre-, Post-, and 3 Hrs Post-Cycling for Females .........................244

Figure F.6 Mean Systolic BP Pre-, Post-, and 3 Hrs Post-Exercise for Experimental Conditions by Gender .................................................................246
LIST OF SYMBOLS

α  alpha
β  beta
χ²  chi-squared
°C  degree Celsius
=  equal
λ  gamma
<  less than
%  percent
±  plus and minus
LIST OF ABBREVIATIONS

APC ................................................................................................... antigen presenting cell
ADH .................................................................................................... anti-diuretic hormone
ATP ................................................................................................... adenosine triphosphate
BP ................................................................................................................... blood pressure
BM ........................................................................................................................body mass
bpm ............................................................................................................. beats per minute
cAMP ............................................................................................cyclic adenosine monophosphate
CI............................................................................................................. confidence interval
CNS ................................................................................................... central nervous system
Control ............................................................................ placebo and ambient environment
COX ............................................................................................................. cyclooxygenase
Cr-EDTA.................................................. chromium-labelled ethylenediaminetetra-acetate
Da......................................................................................................................Dalton
DNA ................................................................................................... deoxyribonucleic acid
eg............................................................................................................... for example
EHI ...................................................................................................... exertional heat illness
EHS ..................................................................................................... exertional heat stroke
EP........................................................................................................ E prostanoid receptor
FIT............................................................................................................fecal immunological test
ng...........................................................nanogram
NKC ............................................................natural killer cell
Npx............................................................naproxen and ambient environment
NpxHeat .....................................................naproxen and hot environment
NSAID .......................................................non-steroidal anti-inflammatory drug
PEG ..........................................................polyethylene glycol
PG ...............................................................prostaglandin
pH .............................................................acid base balance
PK ..............................................................plasma potassium concentration
PNa .............................................................plasma sodium concentration
PK .............................................................prostaglandin
Posm ............................................................plasma osmolality
pg ...............................................................picogram
RH .............................................................relative humidity
RNA ...........................................................ribonucleic acid
ROS ...........................................................reactive oxygen species
RPE ...........................................................rate of perceived exertion
RR ...............................................................relative risk
SIR ............................................................systemic inflammatory response
T4 ..............................................................helper T lymphocyte
T8 ..............................................................cytotoxic T lymphocyte
Tc ...............................................................core temperature
TNF ............................................................tumor necrosis factor
Treg .............................................................. regulatory T cells
TX .............................................................. thromboxane
Uosm ........................................................... urine osmolality
Usg .............................................................. urine specific gravity
Uvol ............................................................ urine volume
\( \dot{V}O_{2\text{max}} \) ........................................ maximum oxygen consumption
\( \dot{V}O_{2\text{peak}} \) ......................................... peak oxygen consumption
yr ............................................................. year
CHAPTER 1

OVERALL INTRODUCTION

Intense exercise places strain on multiple physiological systems that work to maintain core temperature (Tc), cardiovascular function, and fluid and electrolyte balance. When these systems become less efficient, individuals present with signs and symptoms associated with health and performance decrements. For example, when the body fails to thermoregulate, Tc increases. If this increase occurs beyond a certain point, typically above 40°C, cells begin to die and a systemic inflammatory response (SIR) can ensue, leading to organ failure and death.\textsuperscript{1,2} Therefore, when thermal stress is added to intense exercise the demand on these systems increases.

Importantly, the thermoregulatory, cardiovascular, and fluid-electrolyte systems work concurrently to maintain homeostasis. Changes in these three systems often depend on exercise intensity. In general, the cardiovascular system responds by shunting blood, approximately 80\%, away from the gastrointestinal (GI) organs to working muscles.\textsuperscript{3} As exercise continues, the cardiovascular system will maintain blood pressure (BP) and cardiac output by increasing heart rate (HR). At the same time, working muscles are producing a large amount of metabolic heat, which increases Tc. To mitigate this, blood is shunted to peripheral vessels to dissipate heat through the skin.\textsuperscript{3} If an individual maintains hydration the cardiovascular system is more capable of keeping up with the exercise and/or thermal demands. However, when dehydration occurs plasma volume decreases, which
stimulates the renin-angiotensin-aldosterone system to release hormones (ie, aldosterone and vasopressin) that act on the kidneys and blood vessels. In the kidneys, aldosterone causes sodium reabsorption. As a powerful cation, sodium causes water to be reabsorbed, subsequently increasing plasma volume and BP. In a similar way, vasopressin, also known as anti-diuretic hormone, causes kidneys to reabsorb water. Finally, blood continues to be shunted from non-working muscles and the GI through vasoconstriction, which assists in increasing BP. As long as the body’s systems can maintain function, an individual can continue exercise until he/she voluntarily stops. When one or more of these systems begin to fail the person either continues exercise while experiencing signs and symptoms (eg, headache, tachycardia, fatigue, abdominal cramping) or the person involuntarily stops exercise (eg, loss of consciousness, cardiac failure).

Indications for thermoregulatory failure may present as an exertional heat illness (EHI), such as heat syncope, heat exhaustion, or, in extreme cases, heat stroke (EHS). Milder symptoms, typically seen with syncope or exhaustion, include lightheadedness, dizziness, fatigue, nausea, and headache. Cardiovascular failure often accompanies EHS. Individuals present with more serious symptoms, such as central nervous system dysfunction (ie, loss of consciousness, irritability, or irrational behavior), multi-organ failure, or death.

Fluid-electrolyte imbalances during intense exercise and thermal strain include hypohydration, hyperkalemia (plasma potassium > 5 mmol/L), and hyponatremia (plasma sodium concentration < 135 mmol/L). One big concern for hypohydration is how it affects the other systems. Symptoms for hypohydration vary on severity (mild to extreme hypohydration), and are similar to those of EHIs (ie, dizziness, headache, fatigue).
Hypohydration, as little as 2% body mass loss, can perpetuate Tc increase because of increased cardiovascular strain.\textsuperscript{8,9} Elevated plasma potassium during exercise primarily comes from working skeletal muscle releasing potassium.\textsuperscript{6} When systems are functioning appropriately, particularly the kidneys, potassium can be cleared from the blood before detrimental effects occur. This additionally supports the need to maintain hydration in order to maintain renal function, since hyperkalemia can lead to extreme cardiac failure (ie, stroke and heart attack).\textsuperscript{6} Sodium is essential for many physiological functions. When plasma sodium levels are depleted an individual may experience many of the same symptoms of hypohydration and EHIs (ie, headache, dizziness, weakness, and nausea). During severe hyponatremia, the excessive water intake and dramatic drop in sodium can lead to cerebral and pulmonary edema, central nervous system dysfunction, seizure, and death.\textsuperscript{7} The primary etiology for hyponatremia is consuming hypotonic fluids in excess of fluid lost through sweat and respiration. Hyponatremia can also be induced from inappropriate release of vasopressin, causing water retention and diluting sodium, or excessive sodium loss through sweat.\textsuperscript{7}

Together, the cardiovascular, thermoregulatory, and fluid-electrolyte systems are critical in maintaining health and performance during activity and can be influenced by two other important systems – the GI and immune. Major effects on the GI during intense exercise include hypoxia from shunted blood, disruption in epithelial barrier integrity,\textsuperscript{10} and direct damage from mechanical vibrations and increased abdominal pressure.\textsuperscript{11} These can upregulate immune responses and lead to SIR. As previously mentioned, SIR is seen during EHS. The similarities between the two has led to a closer examination of EHS etiology, going beyond the early theory of cells simply becoming “too hot”, to what now
some consider a dual model for developing EHS. The first model suggests hyperthermia is caused by exercise in the heat and exacerbated by GI ischemia, inflammatory cytokine release, and endotoxin release. Exercise continues until the body is overloaded by endotoxin and pyrogenic cytokines, which cause cell necrosis, decreased plasma volume, fever, and eventually SIR and EHS. The second model suggests that regardless of environment, prolong intense exercise suppresses the immune system. The individual, exercising with a blunted immune response and/or subclinical infection, exercises but can no longer handle the demands to maintain cardiac function and Tc. Inflammatory responses drive endotoxin release from the GI and, like the first model, SIR and EHS can occur. This latter model helps explain why some individuals can experience EHS in cooler environmental temperatures or with Tc below 40.5°C.

Presumably, any factor that can perpetuate cardiovascular failure, thermoregulatory failure, fluid-electrolyte imbalance, or increase immune and GI responses can predispose an individual to developing EHI. There are a number of known intrinsic and extrinsic risk factors for EHS. Extrinsic variables (ie, equipment, clothing, rest breaks, nutrition, water access) are more easily controlled. On the other hand, intrinsic variables are more difficult to understand and may be unknown to the individual or healthcare professionals working with physically active individuals. For instance, previous or current illness can elicit an immune response causing subclinical fever and inflammation, which would decrease thermal tolerance.

Some medications stimulate metabolic heat production or alter cardiovascular function and subsequently fluid-electrolyte balance. Non-steroidal anti-inflammatory
drugs (NSAIDs) are not currently considered a risk factor for EHS. This is primarily attributed to their association with being anti-pyretic and anti-inflammatory. Another reason is that existing literature results are conflicting. It is difficult to make conclusions because of the wide variety of NSAIDs available and the doses used. In theory and despite their anti-pyretic and anti-inflammatory affects, NSAIDs may be a risk factor due to the adverse effects they have on the GI epithelial lining, cardiovascular system, and renal function. Regardless of the type of NSAID, research has established they directly damage the GI, causing ulcers, hemorrhaging, and increased barrier permeability.\textsuperscript{13,14} Gastrointestinal damage causes an inflammatory response, upregulating cytokine release.\textsuperscript{15} NSAIDs promote vasoconstriction in both the kidneys and peripheral blood vessels. Renal vasoconstriction decreases urine volume by retaining fluid, which dilutes plasma sodium. Peripheral vasoconstriction decreases the effectiveness to eliminate metabolic heat production, increasing Tc. Finally, both vasoconstriction and fluid retention increase BP.\textsuperscript{7,16} It can be presumed, through these adverse effects, NSAIDs have several pathways to promote either model of EHS.

The little research available and conflicting results makes it difficult to determine whether NSAIDs are risk factors for EHS. Some studies have found NSAIDs have no effect on Tc during exercise,\textsuperscript{17,18} while others have found they increase Tc.\textsuperscript{19} No research has examined naproxen’s effects on Tc. Presently very little research on NSAIDs using both males and females and comparing ambient and hot conditions exists. The purpose of this study was to contribute to the body of literature in this area by examining naproxen on thermoregulation, cardiovascular function, and fluid-electrolyte balance during exercise. Identifying and understanding potential risk factors for EHS may increase awareness and
improve prevention techniques for physically active persons and those working with these individuals

REFERENCES


CHAPTER 2

NAPROXEN EFFECTS ON CORE TEMPERATURE AND INFLAMMATION DURING EXERCISE IN THE HEAT

1

ABSTRACT

**Purpose:** Existing literature regarding non-steroidal anti-inflammatory drugs (NSAIDs) on core temperature (Tc) during exercise is limited and conflicting. Our aim was to determine the effects of naproxen, a common NSAID, on Tc and interleukin-6 (IL-6) in hydrated, exercising humans. **Methods:** We utilized a double-blind, randomized and counterbalanced, cross-over design to determine effects of a 24 hr naproxen dose (220 mg naproxen/dose) and placebo (0 mg naproxen/dose) on Tc and IL-6 during cycling in a hot or ambient environment. Participants (n = 11; 6 male, 5 female) completed 4 conditions: 1) placebo and ambient (Control); 2) placebo and heat (Heat); 3) naproxen and ambient (Npx); and 4) naproxen and heat (NpxHeat). Participants cycled for 90 min then rested 3 hrs in an ambient environment. Throughout exercise, participants drank water (3.5 ml/kg) every 15 min to maintain euhydration. Dependent measures were taken pre-, during, post- and 3 hrs post-cycling. **Results:** Overall Tc significantly increased pre- to post-cycling (37.1 ± 0.4°C to 38.2 ± 0.3°C, P < 0.001) and decreased during rest (P < 0.001), but neither rate of change or maximum Tc were significantly different between conditions. IL-6 increased pre- to post-exercise (0.54 ± 0.06 pg/ml to 2.46 ± 0.28 pg/ml, P < 0.001), and remained significantly higher than pre- at 3 hrs post- (1.17 ± 0.14 pg/ml, P = 0.001). No significant IL-6 differences were found between conditions. **Conclusion:** A 24 hr dose of over the counter strength naproxen did not significantly affect Tc or IL-6 among hydrated males and females cycling in hot and ambient conditions. **Key Words:** cytokine, exertional heat illness, NSAIDs, thermoregulation
INTRODUCTION

Intense physical activity and/or thermal stress is known to induce gastrointestinal (GI) damage and a number of inflammatory responses. Damage to the GI epithelial barrier lining increases GI permeability and causes inflammatory cytokine release. Maintaining GI barrier integrity mitigates potentially toxic products, such as bacterial lipopolysaccharide (ie, endotoxin), from moving from the gut into blood circulation. When integrity is compromised these products can enter the blood and cause severe conditions, such as endotoxemia and systemic inflammatory response (SIR).

Symptoms associated with SIR are similar to those seen with exertional heat illness (EHI), particularly exertional heat stroke (EHS). For example, both EHS and SIR can present with vomiting, dehydration, cell necrosis, cardiovascular compromise, and multi-organ failure. Additionally, both conditions can be driven by GI distress or compromised immune function. An individual exercising with an underlying inflammatory response is at greater risk for EHI due to thermal intolerance.

Exertional heat stroke is identified by two key characteristics: 1) a Tc ≥ 40.5°C and 2) altered central nervous system function. It is important to note that 40.5°C is not a "golden number". It remains elusive why some individuals can participate in intense activity with Tc above 40.5°C without developing EHS, and other individuals experience EHS below this temperature. Extensive scientific literature has examined risk factors, ways to prevent, and ways to treat EHS. However, preventable EHS deaths still occur. Individual variability among EHS cases suggests there are a number of less
understood intrinsic factors. Additional extrinsic variables, such as non-steroidal anti-inflammatory drugs (NSAIDs), may also play a role.

NSAIDs reduce fever, inflammation, and pain by inhibiting cyclooxygenase (COX) derived prostaglandins (PGs) that induce these responses. However, NSAIDs are also associated with a number of adverse effects on the GI and immune systems. These adverse events are closely associated with developing SIR and EHI. Like intense exercise and thermal stress, NSAIDs increase GI permeability,(12,13,25) cause GI distress(6,14) and induce inflammatory cytokines,(17,28,32) Despite these similarities, limited research has evaluated NSAID effects on Tc during exercise, particularly during thermal stress. Older studies found sodium salicylate(11) and aspirin(7) had no effect on Tc compared to placebos. However, a more recent investigation using aspirin showed significant Tc increases due to blunted skin blood flow.(4) Our study sought to determine acute dose effects of a commonly used over the counter NSAID, naproxen, on Tc and the inflammatory cytokine interleukin-6 (IL-6). We hypothesized naproxen would increase Tc and plasma IL-6 levels significantly during exercise in the heat compared to placebo controls.

METHODS

Participants

Eleven moderately trained, healthy, non-heat acclimatized participants (6 male, 5 female) were recruited from the university and surrounding community to complete this study. Participant demographical information is presented in Table 2.1. Prior to participation, participants read and signed an Institutional Review Board approved informed consent form (Appendix A). Inclusion criteria was determined by a health and
injury history questionnaire (Appendix B) and \( \dot{\text{VO}}_2\text{max} \) test (Appendix E.2). Participants were free from cardiovascular, respiratory, and metabolic disorders; musculoskeletal disorders preventing cycling exercise; fluid and electrolyte balance disorders; and GI and swallowing disorders. Participants were also asked about current over the counter and prescription medication use to ensure no potentially harmful interactions. Any participant currently taking analgesic or anti-inflammatory medication was asked to cease the medication for the study duration. Participants were asked about previous EHI history, but this was not an exclusion criteria. Lastly, females completed questions regarding menstrual cycle. Participants completed a graded cycling \( \dot{\text{VO}}_2\text{max} \) test to ensure they were moderately trained (male \( \dot{\text{VO}}_2\text{max} \) between 35-40 mL/kg and female \( \dot{\text{VO}}_2\text{max} \) between 32-40 mL/kg).(21)

**Experimental Conditions**

Four experimental conditions were completed in a randomized, counter-balanced order. A minimum of 7 days separated each trial. Prior to beginning exercise, participants took a 24 hr dose (3 capsules) of placebo or naproxen. Participants consumed 1 capsule at 16 hrs, 8 hrs, and 0 hrs before data collection. All participants were given specific take home instructions to follow 24 hrs prior to data collection, including directions for taking each capsule with 1 8oz glass of water and not with food. All trials took place in an environmental chamber (hot = 35.7 ± 1.3°C, 53.2 ± 3.2% relative humidity [RH]) or laboratory (ambient = 22.7 ± 1.8°C, 52.4 ± 5.5% RH).

**Control.** Participants in the Control condition were given 3 placebo capsules (cellulose) and exercised in an ambient environment.
Naproxen (Npx). Participants in the Npx condition were given 3 naproxen capsules (220 mg naproxen sodium/dose) and exercised in a hot environment.

Placebo and Heat (Heat). Participants in the Heat condition were given 3 placebo capsules (cellulose) and exercised in a hot environment.

Naproxen and Heat (NpxHeat). Participants in the NpxHeat condition were given 3 naproxen capsules (220 mg naproxen sodium/dose) and exercised in a hot environment.

Instruments and Protocols

Anthropometric Measures. Weight and body fat percentage was measured using a bioelectrical impedance analysis scale (Tanita SC-331S Body Composition Monitor, Tanita Co., Tokyo, Japan). Height was self-reported.

Health and Injury History Questionnaire (Appendix B). Details in Chapter 3.

Cycle Exercise Protocol. Participants completed a 90 min cycle protocol on a stationary bike (Monark Ergomedic 828E, Monark Exercise AB, Vansbro, Sweden). Before starting a 3 min warm-up, participants were provided their target heart rate (HR) corresponding to 70% $\dot{V}O_2_{\text{max}}$ to maintain during 80 min of cycling. Heart rate was monitored using Polar HR monitors (Polar Electro Inc., Lake Success, NY). Monitors were within the participant’s visual and researchers provided verbal cues to assist participants in maintaining HR throughout. Following 80 min, participants cycled 10 min at maximum effort. Research assistants gave each participant verbal encouragement. The exercise protocol ended with a 5 min cool down.

Inflammatory Cytokines. We collected blood from a cubital vein pre-, post-, and 3 hrs post-exercise. Two 6 ml vials were collected into a vacutainer tube and inverted several times to mix. We assessed IL-6 using enzyme linked immunosorbent assay high
sensitivity kits (R&D Systems human IL-6 Quantikine ELISA kit, R&D Systems, Inc., Minneapolis, MN).

**Core Temperature.** Core temperature was measured using rectal thermometry (Doric 450 Series digital thermometer, VAS Engineering, Inc., San Diego, CA). Participants were instructed to insert the probe 10 cm past the anal sphincter. During exercise, Tc was continuously monitored and recorded every 5 min. Participants were not allowed to exceed a Tc > 40°C. During the 3 hr rest period, Tc was recorded at 5 and 10 min post-exercise, then every 15 min until the participant reached baseline (pre-exercise Tc) or the rest period concluded.

**Hydration.** Participants were required to be euhydrated prior to beginning exercise. To maintain hydration during exercise, participants were provided 3.5 ml/kg of water to consume every 15 minutes. Hydration status was measured 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.(26) A clean catch method was used in which the participant was instructed to urinate a small amount before placing the specimen cup in midstream to collect a minimum of 1 ounce of urine. Hydration measures were taken pre-, post-, and 3 hrs post-exercise.

**Diet and Activity Logs.** To control for pre-data collection dietary and physical activity influences, participants were asked to track 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 similar diet and physical activity habits during the 24 hrs prior to each data collection session.
Experimental Procedures

**Information Session.** After all inclusion criteria were met, participants were randomly assigned to experimental conditions by a research assistant. Participants, primary investigators, and primary research assistants were blinded to which condition (naproxen or placebo) participants were completing. Participants were familiarized with the online diet and activity log. The $\dot{V}O_{2\text{max}}$ test was used to familiarize participants with the stationary cycle. Seat and handle bar position were noted to maintain consistency throughout trials.

**Pre-Data Collection.** Seventy-two hrs prior to data collection, participants were sent an email with instructions for completing the diet and physical activity log. Logs included the 72 hrs before and 24 hrs after data collection. Participants were also instructed to refrain from intense, vigorous exercise for 48 hrs pre-trial.(22) Twenty-four hrs prior, participants received written take-home instructions and information was reviewed verbally by a research assistant. Instructions included when to take 2 capsules (placebo or naproxen), refrain from consuming alcohol and exercising, and to maintain normal sleep behaviors. Participants were also instructed to consume fluids, such as water, to ensure euhydration upon arriving for data collection. Lastly, participants were instructed to consume a small meal at least 2 hrs before arriving to ensure no food was in the stomach.

**Data Collection Session.** Once at the laboratory, participants were verbally asked if they took the 2 pills before taking the 3rd pill. Baseline HR, Tc, and Usg were measured prior to blood collection. During the 90 min cycling, participants consumed water every 15 min, and researchers continuously monitored Tc and HR. At the conclusion of the 10 min maximum effort, participants cooled down on the bike for 5 min. Participants then rested
for 5 min where post-blood was collected. Participants provided a urine sample for hydration assessment. During the 3 hr rest period, participants sat in a semi-reclined/seated position in an ambient environment (23°C and 56% RH). Each participant received a standardized non-sucrose snack based on individual weight and were allowed to consume water ad libitum. Throughout the rest, Tc was recorded every 15 min. Blood and urine measures were collected at the rest conclusion. Before leaving, take home instructions were reviewed with the participant regarding the 1 day post diet and activity log.

Statistical Analysis

IBM SPSS Statistics (version XXII; IBM Corporation, Armonk, NY) was used for all analyses. Descriptive statistics (mean and standard deviations) for all dependent variables were calculated. A one-way ANOVA assessed differences in demographics between conditions and gender (eg, age, height, weight, body fat %). Changes in Tc were assessed using regression models and two repeated measures ANOVAs: 1) 4 (condition) x 20 (cycling) and 2) 4 (condition) x 9 (rest). A 4 (condition) x 3 (time) repeated measures ANOVA was used to determine differences in pre-, post-, and 3 hr post-cycling IL-6 and Usg. The same repeated measures ANOVAs were conducted to determine differences in Tc and IL-6 between conditions within genders. Differences for overall Tc between genders was assessed using 2 (gender) x 20 (cycling) and 9 (rest) repeated measures ANOVAs. A 2 (gender) x 3 (time) repeated measures ANOVA determined differences in IL-6. Assumptions of sphericity were verified to determine whether variance in the differences within experimental conditions were equal or significantly different. Greenhouse-Geisser corrections were used for Tc and IL-6 when sphericity was violated. Post hoc analysis was conducted on significant main effects with Bonferroni corrections.
Power calculations were conducted a priori using standard deviations for Tc and IL-6. A sample size of 8 was necessary to achieve a statistical power of 0.8. Our participant number (n =11) is consistent with previously published research. Significance level was set at $P < 0.05$ for all analyses.

**RESULTS**

Sixteen participants began the study. Two participants dropped due to the intensity of the study and 3 dropped due to the time commitment, yielding 11 participants. We identified significant gender differences for height and body fat % (Table 2.1), but no other demographical measures were significantly different. Five of 6 female participants used oral contraceptives. Only one participant reported a previous history of EHI. All participants began experimental trials euhydrated ($Usg = 1.012 \pm 0.005$) and remained euhydrated to post- ($Usg = 1.011 \pm 0.008$) and 3 hr post-exercise ($Usg = 1.007 \pm 0.006$). No significant Usg differences existed between experimental conditions at any time point. Dietary analysis revealed no significant differences in calorie, carbohydrate, protein, sodium, or fat intake between conditions. Physical activity level 24 hrs before remained low to sedentary and was not significantly different between conditions.

**Core Temperature**

Figure 2.1 shows Tc changes throughout exercise and rest for each experimental condition. Overall, Tc significantly increased from pre- ($37.1 \pm 0.4^\circ C$) to post-exercise ($38.2 \pm 0.3^\circ C$, $P < 0.001$). During rest, Tc significantly decreased over time ($P < 0.001$) and reached pre-exercise by 75 min post-exercise. Starting at 60 min cycling, mean Tc was higher in heat trials compared to ambient (Figure 2.1) and remained higher throughout rest.
However, there were no statistically significant Tc differences between experimental conditions at any time point.

Figures 2.2.a-d illustrate individual participants’ Tc, overall mean, and regression statistics for each condition during exercise. During Control and Npx, Tc increased curvilinear during cycling, with a cubic regression model providing the best fit. Both Heat and NpxHeat followed a linear model with no difference in slope between conditions. Similar results were found for Tc during rest, with a curvilinear, cubic model providing the best fit for all conditions and no difference in slope between conditions.

Regarding gender, a significant main effect was found for Tc change over time within males ($P < 0.003$) and females ($P < 0.001$). For males we found no significant Tc differences between conditions (Appendix F, Figure F.1); however, the main effect for Tc and condition during exercise approached significance ($P = 0.075$). Within females there was no significant differences between conditions at any time point (Appendix F, Figure F.2). When combined (Appendix F, Figure F.3.), pre-exercise mean female Tc was significantly higher ($37.3 \pm 0.3^\circ C$) than males ($37.0 \pm 0.4^\circ C$, $P = 0.02$). During cycling, females stayed significantly higher until 15 min. During rest, male Tc was significantly higher at 10 min post-cycling ($38.3 \pm 0.5^\circ C$ versus $38.0 \pm 0.4^\circ C$, $P < 0.02$). Females were significantly higher again at 75 post-cycling ($37.3 \pm 0.3^\circ C$ versus $37.1 \pm 0.4^\circ C$, $P = 0.006$) and remained higher until the end of rest ($37.3 \pm 0.2^\circ C$ versus $36.8 \pm 0.3^\circ C$, $P < 0.001$).

**Cytokines**

Differences in IL-6 between pre-, post-, and 3 hrs post-exercise are presented in Figure 2.3. No significant differences were found between conditions at any time point. A significant change in mean IL-6 occurred over time ($P < 0.001$), with post-exercise IL-6
significantly higher than pre- and 3hrs post- for all conditions. At 3 hrs post-exercise, both Npx (1.06 ± 0.73 pg/ml) and Heat (0.97 ± 0.49 pg/ml) were significantly higher compared to pre- (Npx and Heat mean = 0.44 ± 0.29 pg/ml, P < 0.04).

A significant main effect for IL-6 was found across time for gender (P = 0.001). Additional analysis found no significant difference between genders at any time point between conditions. Within males (Appendix F, Figure F.4) there was a significant main effect over time within each condition (P < 0.04). Post hoc analysis showed only Heat and NpxHeat were significantly greater post-exercise compared to pre-. No other significant differences were found for males. Significant main effects were found within Control and Npx for females (Appendix F, Figure F.5). Post-exercise IL-6 was significantly greater than pre- in the Npx condition. Though not statistically significant, mean post-exercise IL-6 during NpxHeat was higher than all other conditions and remained elevated 3 hrs post-exercise.

DISCUSSION

Due to anti-pyretic effects, NSAIDs have been theorized to decrease or blunt Tc rise during exercise. However, existing literature has been unable to find NSAIDs elicit this effect, instead finding they cause no change(7,11) or may increase Tc.(4) Our study sought to determine whether a 24 hr over the counter dose of naproxen would negatively affect thermoregulation and inflammation during moderate-intense cycling. Compared to a placebo, our results indicated naproxen did not significantly affect Tc or IL-6 in either an ambient or hot environment. These results are consistent with males walking in a hot environment using 7.8 g sodium salicylate(11) and in ambient conditions using aspirin acutely (1½ hrs pre-exercise) or chronically (daily doses for 2 and 7 days).(7) Sodium
salicylate is generally considered less effective than aspirin at inhibiting PGs and is not known for its antipyretic effects.(2) Therefore, it is not surprising taking sodium salicylate would not elicit significant Tc changes.

Lack of significance in max Tc and rate of Tc increase between conditions may be attributed to our dosage. Using low dose aspirin (81 mg daily for 7 days) in older males and females who rested and then cycled 2 hrs at 60% VO$_{2\text{max}}$ significantly increased Tc after resting 30 min in a hot environment and remained significantly higher throughout exercise.(4) The mechanism for Tc increase is attributed to peripheral vasoconstriction,(4) which prevents temperature dissipation. This mechanism is understandable considering aspirin’s efficacy on the cardiovascular system, effectively reducing cardiac events (ie, stroke and heart attack).(18) Compared to aspirin, naproxen has greater selectivity toward COX 2, making naproxen highly effective at reducing pain and inflammation.(3) Unlike aspirin, naproxen reversibly inhibits platelet aggregation,(1) making naproxen less effective at reducing cardiac events. Because we did not measure skin blood flow we are unable to make a definitive statement whether naproxen elicited any effect through vasoconstriction, but based on our lack of Tc differences, we find it unlikely our naproxen dosage caused changes in skin blood flow. With higher dosages (ie, prescription strength) or longer use (ie, 7 days) the possibility exists that we would see similar responses as Bruning et al.(4)

The significant increase in IL-6 pre- to post-exercise in our study was expected and similar to other moderate-intense cycling.(31) Like Tc, we found naproxen did not significantly affect IL-6. These results are consistent with other studies showing neither a 1000 mg aspirin (20) or 400 mg ibuprofen(27) dose significantly increased plasma
inflammatory cytokine concentrations. Aspirin is more selective toward COX 1 than 2, meaning aspirin is less effective at relieving inflammation. (18) Considered equipotent against COX 1 and 2, results on ibuprofen’s effects on exercise driven inflammation is contradictory; though, dosage plays an important role. While 400 mg 2 hrs prior to ultra-distance running made no difference in IL-6 (27), 600 mg the afternoon prior and 1200 mg on the day of a ultra-distance race significantly increased post-race IL-6 compared to controls. (17) The 3 220 mg naproxen doses over 24 hrs prior to exercise in our study did not significantly increase or decrease IL-6 compared to placebos. If dosing was extended across multiple days there may be significant effects.

Controlled laboratory exercise studies often do not measure NSAIDs, Tc, and inflammation together, and even fewer field studies are available. Among a group of ultra-marathon runners (n = 19), 6 participants reported using NSAIDs or other anti-inflammatory supplements (ie, fish oil). (10) Researchers found no correlation with NSAID use and IL-6. The researchers determined there was no effect on Tc; however, temperature was assessed aurally. (10) Aural temperature is known to be an inaccurate Tc assessment in exercising individuals. (5) The reported post-race temperatures (37.0 ± 0.3°C) (10) may not have accurately reflected individuals’ Tc when one considers a person’s normal baseline Tc (37°C ± 1°C) (16) and the environmental race conditions (30-40°C and 31-40% RH). (10)

**Individual Variability**

Knowing a number of factors play a role in thermoregulation during exercise, we attempted to control nutrition, hydration, medication, physical activity, clothing, and illness. Furthermore, our study participants were non-heat acclimatized and moderately
endurance trained. Despite our attempts, a number of factors cannot be controlled and explain some individual variability in existing EHS literature and in our results, particularly when examining genders.

Though not statistically significant, the IL-6 increase in our male participants was greater than females. Also, these concentrations tended to stay elevated at 3 hrs post-exercise. Lack of statistical significance likely comes from the large standard deviations post-exercise among males in all conditions. Timmons et al. (31) compared immune responses in males to females during different menstrual phases using and not using oral contraception. Result indicated no significant differences in IL-6 levels from pre- to post-cycling. (31) Skeletal muscle contraction during exercise produces a high amount of IL-6, which increases plasma IL-6. (30) Our male participants did not weigh significantly more than females, but males had greater muscle mass, as indicated by their significantly lower body fat %. The higher mean IL-6 and large variations in male participants could be caused by muscle induced IL-6. Within the males, IL-6 means were lower post-exercise with naproxen, suggesting there may have been some effects, but this did not continue at 3 hrs.

We identified some overall Tc differences. Both male and female Tc averaged 38.2°C immediately post-cycling, but males continued to increase during the 5 min cool down, reaching 38.4°C and remaining significantly higher 10 min post-cycling. This elevation may be explained by a higher metabolic heat production, (8) less body fat and slightly higher $\dot{V}O_{2max}$. Female pre- and 3 hr post-exercise Tc was significantly higher than males. This is likely due to the naturally higher Tc during the luteal phase (~ 0.3°C higher). (23) We did not control for menstrual cycle, but we did not find significant differences in resting Tc based on the individual’s phase (follicular or luteal). Oral
contraceptives increase $T_c$ during moderate-intense exercise in the heat (15) similar to that seen in non-oral contraceptive users. In our one participant who did not use birth control, we found during the luteal phase, which happened to be when she completed NpxHeat, she experienced her highest post-exercise $T_c$ and IL-6. This was not found during the luteal phase in any other female participant. Further, this individual was the one participant who reported a previous EHI and had the highest body fat % and lowest $\dot{V}O_{2\text{max}}$ among females.

Another interesting consideration for individual confounding risk factors is in our failed trials. A female participant attempting to complete the NpxHeat trial began experiencing hypotension and reported severe nausea. Researchers ceased her trial for safety reasons. Though her pre-trial measures were within normal limits, upon inquiry, the participant reported previous GI illness within a few days of the trial and feeling tired. One male participant was unable to begin a data collection trial due to extreme GI symptoms and illness after initiating the naproxen. The individual withdrew after a later trial due to the intensity of the study. These two examples support the need to use a multifaceted approach to recognize and monitor individuals at-risk for adverse events. Additionally, it is important to inquire about NSAID use in persons who may present with signs and symptoms before or during exercise.

Limitations and Future Research

The primary limitation to this study was using a constant HR during 80 min of cycling rather than using the work rate corresponding to 70\% $\dot{V}O_{2\text{max}}$. The purpose of a constant HR was to allow us to better determine the direct effects of naproxen on dependent measures. If work rate had been used we would expect an increased cardiovascular strain, particularly in the heat, which could increase $T_c$. However, by using a constant HR, the
cycling intensity during heat trials was inherently less than ambient due to cardiovascular alterations (increased HR and BP) in response to thermal stress. With the available data, we are unable to identify specific work rate differences between heat and ambient trials during the 80 min. We acknowledge using constant HR likely limited us from finding significant differences between experimental conditions. Additionally, we could have measured oxygen consumption to better control for work rate during each trial. Many exercise bouts are completed at a set intensity rather than focusing on a target HR; however, our results are useful for individuals who may use a set HR to pace themselves during endurance exercise and then “go all-out” at the finish. Our second limitation is only measuring IL-6, which is a prominent pro-inflammatory cytokine that increases early during immune responses. Another prominent pro-inflammatory cytokine, tumor necrosis factor-alpha increases several hours after exercise.(19) Examining this cytokine at 3 hrs post-cycling and day-post would provide a better idea of naproxen’s effects. Third, diet and physical activity logs indicated participants followed pre-data collection guidelines for physical activity and nutrition. We assume participants were honest with following guidelines and completing the logs.

Future research should examine naproxen and other non-aspirin NSAIDs in higher doses, particularly looking at prescription strength and/or long-term use (ie, 7 days, 14 days, etc.). Research is warranted in hot environments using greater cycling intensity and other exercise modes. Running is known to add strain on the GI tract,(24) which may exacerbate responses. Field studies should be conducted in both long distance cycling and running, as well as field sports where there is alternating high intensity activity and rest periods. Considering GI barrier integrity and inflammation driven SIR closely resembles
models for EHS, research should examine plasma endotoxin concentration and GI permeability related to NSAID use and exercise. We also suggest additional research on potential gender differences. Examining females using and not using oral contraception is warranted as well as during different menstrual phases. Studies in individuals with musculoskeletal injuries or conditions potentially compromising the immune system are pertinent. Finally, future research is needed regarding confounding risk factors, such as sleep deprivation, nutrition, and especially hypohydration. Hypohydration places additional strain on the cardiovascular and thermoregulatory systems, which, if combined with NSAID effects, could promote Tc increase.

**Conclusion**

Our results suggest hydrated males and females completing moderate-intense exercise in either ambient or hot environments taking a 24 hr naproxen dose do not experience increased Tc or IL-6. These results are important for both physically active individuals and those working with persons who may be taking naproxen. While we did not find negative impacts on thermoregulation or inflammation, we encourage individuals to be aware of the potential adverse effects that NSAID may have on the GI tract that could perpetuate thermal intolerance during exercise. This understanding is especially vital in individuals with other confounding risk factors (eg, previous illness, sleep deprivation, repeated intense exercise bouts, hypohydration, etc.).

**REFERENCES**


### Table 2.1. Participant Demographics (M ± SD)

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<tr>
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<th>Aggregate (N = 11)</th>
<th>Male (N = 6)</th>
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<tr>
<td>Age (yrs)</td>
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<td>Weight (kg)</td>
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<td>41.4 ± 5.7</td>
<td>43.6 ± 5.3</td>
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</tbody>
</table>

*aSignificantly different between genders (P < 0.04)*
Figure 2.1. Mean Core Temperature during Cycling and Rest for Experimental Conditions

Significant change in Tc during exercise ($F_{1,767.3} = 150.5, P < 0.001$) and rest ($F_{2.018.5} = 201.6, P < 0.001$)
Figure 2.2.a. Regression and Participant Core Temperature during Cycling.
Bold line represents mean Tc for condition. Shaded area indicates 10 min cycling at maximum effort. Final 5 min is an active cool down. Cubic regression equation: $\hat{y} = 0.03x + 37.3$; $r^2 = 0.59$; 95% CI = 37.1, 37.5; $P = 0.001$. 
Figure 2.2.b. Regression and Participant Core Temperature during Cycling.
Bold line represents mean Tc for condition. Shaded area indicates 10 min cycling at maximum effort. Final 5 min is an active cool down. Cubic regression equation: \( \hat{y} = 0.02x + 37.4; r^2 = 0.58; 95\% \text{ CI} = 37.2, 37.6; P = 0.002. \)
Figure 2.2.c. Regression and Participant Core Temperature during Cycling.
Bold line represents mean Tc for condition. Shaded area indicates 10 min cycling at maximum effort. Final 5 min is an active cool down. Linear regression equation: $\hat{y} = 0.01x + 37.4; r^2 = 0.54; 95\% \text{ CI } = 37.2, 37.6; P < 0.001$. 
Figure 2.2.d. Regression and Participant Core Temperature during Cycling.
Bold line represents mean Tc for condition. Shaded area indicates 10 min cycling at maximum effort. Final 5 min is an active cool down. Linear regression equation: \( \hat{y} = 0.01x + 37.4; \) \( r^2 = 0.56; \) 95% CI=37.2, 37.6; \( P < 0.001. \)
Figure 2.3. Mean IL-6 Pre-, Post-, and 3 Hrs Post-Cycling for Experimental Conditions
N = 7. Significant change over time ($F_{2,48} = 41.8$, $P < 0.001$). \(^a\)Significantly higher than pre- within condition ($P < 0.04$). \(^b\)Significantly lower than post- within condition ($P < 0.04$).
CHAPTER 3

NAPROXEN EFFECTS ON HYDRATION AND ELECTROLYTE MEASURES DURING CYCLING IN THE HEAT

ABSTRACT

Context: Non-steroidal anti-inflammatory drugs (NSAIDs) can cause a number of adverse effects, including impaired fluid-electrolyte balance. There is a lack of controlled laboratory studies examining NSAID effects on fluid and electrolyte regulation during exercise.

Objective: To examine the effects of naproxen, a commonly used NSAID, on hydration and electrolyte measures in hydrated humans cycling in a hot environment.

Design: Double-blind, randomized and counterbalanced, cross-over design.

Setting: Laboratory.

Patients or Other Participants: A total of 11 moderately endurance trained volunteers (6 male and 5 female; age = 27.8 ± 6.5 yrs, weight = 79.1 ± 17.9 kg, height = 177 ± 9.5 cm, $\dot{V}O_{2\text{max}} = 41.4 ± 5.7$ mL/kg) completed this study.

Intervention(s): Participants were randomly assigned to complete 4 trials: 1) placebo and ambient (Control); 2) placebo and heat (Heat); 3) naproxen and ambient (Npx); and 4) naproxen and heat (NpxHeat).

Main Outcome Measure(s): Hydration (plasma osmolality, urine osmolality, urine specific gravity, and body mass change), electrolyte (plasma sodium and plasma potassium concentration), and cardiovascular (heart rate and blood pressures) measures were taken pre-, post- and 3 hrs post-90 min cycling. We also measured fluid volume (Fvol) and urine volume (Uvol).

Results: We found no statistically significant differences between experimental conditions for hydration, electrolyte, or cardiovascular measures. All participants began hydrated and became more hydrated throughout trials. Mean aggregate plasma sodium was < 135
mmol/L at pre-, post-, and 3 hrs post-exercise, but did not significantly decrease over time. Overall plasma potassium significantly increased pre- to post-exercise ($P = 0.02$). Though not statistically significant, mean Fvol was greater and Uvol lower during naproxen trials compared to placebos.

**Conclusion:** A 24 hr naproxen dose did not significantly alter hydration or electrolyte measures during 90 min cycling in either ambient or hot conditions. The trend for naproxen to increase Fvol and decrease Uvol suggests the start of fluid retention, which should be a concern for individuals at risk for hyponatremia or with pre-existing cardiovascular conditions (ie, hypertension).

**Key Words:** NSAID, urine specific gravity, plasma osmolality, plasma sodium, urine osmolality

**INTRODUCTION**

Non-steroidal anti-inflammatory drugs (NSAIDs) include a variety of prescription and non-prescription medications including aspirin, ibuprofen, indomethacin, and naproxen. An individual drug’s effectiveness and side effects are dependent on its chemical makeup and selectivity toward inhibiting cyclooxygenase (COX). In general, all NSAIDs work to relieve pain and inflammation by preventing COX from catalyzing the conversion of arachidonic acid (a fatty acid released from cell membranes during injury/inflammation) to prostaglandin (PG).\(^1\) There are numerous PG subsets that are responsible for not only promoting pain and inflammation, but also maintaining blood pressure (BP), plasma volume, and renal function. Although less imperative in normal healthy individuals, PGs are critical in maintaining homeostasis during altered cardiovascular states (eg, hypotension) or altered fluid regulation (eg, hypovolemia and sodium imbalance)\(^2,3\) –
common conditions experienced during physical activity. Inhibiting PGs can perpetuate altered cardiovascular and renal function, potentially leading to serious fluid and electrolyte imbalances (eg, hyponatremia, edema formation, and hypertension) or even acute renal failure.3,4

Existing literature evaluating the relationship between NSAIDs and fluid regulation during physical activity is primarily centered on observational studies examining exertional hyponatremia (defined as a plasma sodium concentration [P[Na⁺]] < 135 mmol/L).4-6 The mechanism for NSAIDs to promote hyponatremia is due to their ability to decrease urine production and increase water retention, which dilutes P[Na⁺].7 To our knowledge, the only controlled study examining this relationship is by Dumke et al.,8 who found 600 mg of ibuprofen pre- and 1200 mg during an ultra-distance marathon did not significantly alter plasma electrolytes. Controlled laboratory studies examining NSAID effects on fluid-electrolyte balance during exercise is lacking. Thus, as part of a larger study, we sought to determine acute dose effects of a commonly used over the counter NSAID, naproxen, on fluid and electrolyte measures during moderate-intense endurance exercise in a hot or ambient environment. We hypothesized naproxen would increase hydration measures, indicating further hydration (ie, euhydration or hyperhydration) compared to placebo controls. We also hypothesized naproxen would significantly decrease P[Na⁺] compared to placebo controls.

METHODS

Design

We used a double-blind, randomized and counterbalanced, cross-over design. The independent variables were placebo or naproxen and hot or ambient environment. The
dependent variables were: plasma osmolality (Posm), urine osmolality (Uosm), urine specific gravity (Usg), percent change in body mass (%BM), P[Na⁺], plasma potassium concentration (P[K⁺]), fluid volume consumed (Fvol), urine volume (Uvol), heart rate (HR), and blood pressure (BP).

**Participants**

Eleven participants (5 male, 6 female) were recruited from the local community. Participants read and signed an Institutional Review Board approved informed consent form (Appendix A) prior to participation. Potential participants completed a health and injury history questionnaire (Appendix B) and were accepted if he/she was free of cardiovascular, respiratory, and metabolic disorders; musculoskeletal disorders preventing cycling exercise; fluid and electrolyte balance disorders; and gastrointestinal and swallowing disorders. Current prescription and non-prescription medication use was also asked. Females were asked supplemental questions on menstrual cycle and birth control. Potential participants then completed a graded cycling $\dot{V}O_2\text{max}$ test (Appendix E.2) to determine qualification as moderately trained (male $\dot{V}O_2\text{max}$ between 35-40 mL/kg; female $\dot{V}O_2\text{max}$ between 32-40 mL/kg).³

**Experimental Conditions**

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). A minimum of 7 days separated each trial. A 24 hr dose (3 capsules) of placebo (cellulose) or naproxen (220 mg naproxen sodium/dose) was given to participants with instructions to take 1 capsule at 16 hrs, 8 hrs, and 0 hrs prior to data collection. Participants were given specific instructions to follow 24 hrs prior to data collection.
collection, including directions for taking each capsule according to the manufacturer’s (ie, naproxen sodium) directions. Participants then completed a 90 min cycling bout in either a hot (mean temperature = 35.7 ± 1.3°C, 53.2 ± 3.2 % relative humidity [RH]) or ambient (mean temperature = 22.7 ± 1.8°C, 52.4 ± 5.5% RH) environment. After exercise, participants rested 3 hrs in an ambient environment (23°C, 56% RH).

**Instruments and Protocols**

**Anthropometric Measures.** Weight and body fat percentage was measured using a bioelectrical impedance analysis scale (Tanita SC-331S Body Composition Monitor, Tanita Co., Tokyo, Japan). Height was self-reported.

**Health and Injury History Questionnaire (Appendix B).** The Health and Injury History Questionnaire uses the Canadian Society for Exercise Physiology, which developed the PAR-Q to identify individuals who should be seen by a medical doctor before becoming more physically active and both the American Medical Society for Sports Medicine and the American Orthopedic Society for Sports Medicine. These two organizations released pre-participation physical exam forms used to identify individuals at risk for injuries. Supplemental questions were added to identify individuals with potential medical illness or injury (eg, gastrointestinal disorders, exertional heat illness history, metabolic disorders, and daily medication use) that would exclude them from study participation.

**Cycle Exercise Protocol.** Participants completed a 90 min cycling protocol on a stationary bike (Monark Ergomedic 828E, Monark Exercise AB, Vansbro, Sweden). Prior to a 3 min warm up, participants were provided their target HR, equivalent to 70% \( \dot{V}O_2\text{max} \),\(^{10}\) to maintain during 80 min of steady state cycling. Participants were instructed to
reach the HR by the end of the 3 min warm up. At the end of 80 min, participants performed 10 min at maximum effort, with each participant given verbal encouragement by research assistants. The protocol concluded with a 5 min cool down.

**Hydration and Electrolyte Measures.** Participants were required to be euhydrated prior to beginning data collection. Immediate hydration status was assessed using Usg. Further hydration analysis was conducted using Posm, Uosm, and %BM. Euhydration was defined as Posm ≤ 290 mOsmols, Uosm < 700 mOsmols, Usg ≤ 1.020, and %BM < 1%.

We collected blood from an antecubital vein into 6ml lithium heparin vacutainer tubes at pre-, post-, and 3 hrs post-exercise. Tubes were inverted several times to mix and immediately placed on ice. Samples were centrifuged at 3000 rpm for 15 min. Plasma was pipetted into microtubes and stored at -20°C until analysis. We measured osmolality using freeze point depression (Multi-sample Osmometer model 2020, Advanced Instruments, Norwood, MA). To determine changes in P[Na⁺] and P[K⁺] we utilized ion-selective electrodes (EasyLyte® Na/K electrolyte analyzer model REF 2277, Medica, Bedford, MA). Normal P[Na⁺] was defined as > 135 mmol/L and normal P[K⁺] < 5 mmol/L.

Urine was obtained pre-, post-, and 3 hrs post-exercise. A clean catch method was used in which the participant was instructed to urinate a small amount before placing the specimen cup in midstream to collect a minimum of 1 ounce of urine. We used a handheld clinical refractometer (model REF 312, Atago Company Ltd., Tokyo, Japan) to measure Usg. Urine aliquots were stored in microtubes at -20°C until analysis for osmolality using freeze point depression.

Pre-, post-, and 3 hrs post-exercise BM was measured using a body composition analyzer (Tanita SC-331S Body Composition Monitor, Tanita Co., Tokyo, Japan).
Participants dressed in shorts and a t-shirt and voided urine before stepping on the scale. Immediately post-exercise participants were asked to towel off sweat before weighing. Participants were then allowed to change clothes and instructed to return with their “wet” clothes to be weighed. Differences in “wet” versus “dry” clothes’ weight (measured at another time) were used to adjust BM for sweat loss.

**Urine Volume.** All urine produced after pre-BM and hydration measures was collected into Uvol containers and urine cups (for Usg and Uosm measures). Total Uvol produced over the trial was measured using a graduated cylinder.

**Fluid Volume.** To maintain hydration during exercise, participants were instructed to drink 3.5 ml/kg of water every 15 minutes. Participants were allowed to consume more than the required Fvol if they desired. At the end of exercise, researchers measured total Fvol consumed. During the 3 hr rest period, participants consumed water ad libitum and researchers measured Fvol consumed during rest.

**Cardiovascular.** To ensure participants remained at safe limits during exercise, HR was continuously monitored using Polar HR monitors (Polar Electro Inc., Lake Success, NY). To determine effects from naproxen, pre-, post-, and 3 hr post-exercise HR and BP were assessed.

**Diet and Activity Logs.** To control for pre-data collection dietary and physical activity effects, participants were asked to track 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 asked to mimic diet and physical activity habits the 24 hrs prior to each trial.
Experimental Procedures (Figure 3.1)

Information Session. After consenting participants were determined free of disqualifying medical conditions and to be moderately trained, a research assistant randomly assigned an experimental condition order. Participants and primary investigators were blind to whether participants were completing a naproxen or placebo trial. Participants were familiarized with the online diet and activity log and the \( \text{VO}_{2\text{max}} \) test was used to familiarize participants to the stationary cycle. Participants were instructed to not take any analgesic or anti-inflammatory medications during the course of the study.

Pre-Data Collection. Seventy-two hrs prior to data collection, participants were sent an email with instructions for completing the diet and physical activity log. Participants were instructed to refrain from intense, vigorous exercise for 48 hrs.\(^{13}\) Twenty-four hours prior, participants were provided written and verbal instructions for taking 2 capsules (placebo or naproxen), to consume a small meal prior to reporting to the laboratory, encouraged to consume fluids to promote euhydration, refrain from all physical activity, and attempt to sleep a “normal” amount.

Data Collection Session. Upon arrival to the laboratory, participants were verbally asked if they took their 2 pills before taking the 3\(^{rd}\) pill. Participants were provided a HR strap and baseline HR and BP were assessed. Participants provided a urine sample and baseline Usg was measured to ensure euhydrated prior to BM and blood measures.

Immediately following the cycling protocol participants weighed, provided a urine sample and had post- blood collected. Participants then rested in a semi-reclined/seated position where they were allowed to consume water ad libitum and given a snack. Snacks
were based on BM and the same for each trial. At the conclusion of the 3 hr rest period, blood, urine, weight, BP, and HR were measured.

**Statistical Analysis**

IBM SPSS Statistics (version XXII; IBM Corporation, Armonk, NY) was used for all analyses. Descriptive statistics (mean and standard deviations) for all dependent variables were calculated. A one-way ANOVA was used to determine differences in demographics between genders (eg, age, height, weight). A 3 (time) x 4 (condition) repeated measures ANOVA determined differences for plasma, urine, %BM, HR, and BP. Greenhouse-Geisser corrections were used for P[Na⁺], BP, and HR because these variables violated sphericity. Post hoc analysis was conducted for significant main effects using Bonferroni corrections. We used a one-way ANOVA to determine differences between conditions and gender for Fvol consumed during exercise, total Fvol, and Uvol. To control for body mass, Fvol was corrected to ml/kg and a one-way ANOVA determined differences between conditions and gender. We used paired sample t-tests to determine differences in sweat rate and Fvol during exercise. Using G*Power (version 3.1.9.2, Heinrich Heine University, Dusseldorf, Germany), post hoc power calculation with means and variances for Usg and Uosm indicated a statistical power > 0.9. Significance level was set at $P < 0.05$ for all analyses.

**RESULTS**

We began the study with 16 participants; 2 participants dropped due to the study intensity and 3 dropped due to the time commitment. Our final sample size = 11. There was no significant difference between genders for age (mean = 27.8 $\pm$ 5.7 yrs), weight (79.1 $\pm$ 17.9 kg), $\dot{V}O_2$max (41.4 $\pm$ 5.7 mL/kg), sweat rate (0.9 $\pm$ 0.3 L), baseline resting HR (52.6
± 6.6 bpm) and baseline resting BP (116/80 mmHg). Due to lost samples, the sample size for Posm, P[Na\textsuperscript{+}], and P[K\textsuperscript{+}] ranged between conditions (Control = 5, Npx = 3-4, Heat = 4, and NpxHeat = 3-4). Diet logs indicated no significant differences between calorie, fat, carbohydrate, sodium, and protein intake between conditions. On average, male daily sodium intake was significantly greater than females (2.7 ± 0.3 g vs. 2.0 ± 0.2 g, $P = 0.038$; Appendix F, Table F.2). Physical activity level 24 hrs prior to data collection was not significantly different between conditions and remained low to sedentary.

**Cardiovascular**

There was a significant main effect for HR over time (mean pre = 66.8 ± 14.3 bpm, post = 177.4 ± 16.2 bpm, and 3 hr post = 65.5 ± 11.8 bpm, $P < 0.001$). There was no significant HR difference between conditions or genders. Similarly, a significant main effect occurred for BP over time (mean pre = 118/73 mmHg, post = 137/72 mmHg and 3 hrs = 116/77 mmHg, $P < 0.008$). There were no significant differences between conditions. We found a significant main effect for systolic BP between genders ($P = 0.005$). Post-hoc analysis showed males were significantly higher at pre-, post- and 3 hrs post-exercise ($P < 0.016$). Regarding experimental conditions (Appendix F, Figure F.6), male systolic BP was significantly higher than females post-exercise in Npx (150.8 vs 124.4 mmHg, $P = 0.017$) and NpxHeat (144.2 vs 124.0 mmHg, $P = 0.043$). At 3 hrs post-exercise, males were significantly higher within Heat (121.5 vs 111.6 mmHg, $P = 0.028$).

**Hydration**

Hydration, Fvol, and Uvol for each experimental condition are presented in Table 3.1. All participants began trials euhydrated. Overall, Posm, Uosm, and Usg significantly decreased pre- to 3 hrs post-exercise, indicating participants became more hydrated.
throughout trials. All conditions maintained an average BM loss < 1%. No significant differences were found in hydration measures between conditions at any time point. Though not significantly different, mean Uvol was lower in naproxen trials versus placebos and during heat trials versus ambient (Table 3.1). There was no significant difference in Uvol between genders (Appendix F, Table F.1).

Neither Fvol during exercise or total was significantly different between conditions. As expected, participants tended to consume more fluid during exercise in hot conditions versus ambient (Table 3.1). Mean Fvol during exercise and overall was higher in both naproxen trials compared to placebos. This trend continued when corrected for body mass. Participants’ mean Fvol during exercise (1.5 ± 0.7 L) was significantly greater than their sweat rate (0.9 ± 0.3 L, $P < 0.013$). Males consumed significantly greater Fvol during exercise and total than females (Appendix F, Table F.1). During NpxHeat males consumed significantly more during exercise than females (2.0 ± 0.6 vs. 1.3 ± 0.5 L, $P = 0.046$). When corrected for BM, Fvol in males overall and during NpxHeat was not significantly different than females. During the Npx condition overall Fvol approached significance (males = 1.9 ± 1.0 L and females = 0.9 ± 0.5 L, $P = 0.055$) and trended to be different during exercise with BM corrections (males = 20.7 ± 8.8 ml/kg and females = 12.1 ± 5.0, $P = 0.087$).

**Electrolytes**

Table 3.2 shows plasma electrolytes by experimental condition. Mean aggregate P[Na$^+$] was < 135 mmol/L at pre-, post-, and 3 hrs post-exercise. We found no statistically significant differences in P[Na$^+$] between conditions at any time. Mean P[Na$^+$] was highest during NpxHeat. There was a significant main effect for P[K$^+$] changes over time ($P = 0.02$), increasing, expectedly, pre- to post-exercise and decreasing after 3 hrs. There were
no significant differences between trials; however, both naproxen groups averaged higher than placebos post-exercise. Due to the low sample size, we were unable to determine any differences between genders.

DISCUSSION

We sought to determine whether naproxen adversely affected hydration and electrolyte measures during moderate-intense endurance cycling in either hot or ambient conditions. We based our hypotheses on previous research associating NSAIDs with promoting fluid retention through decreased Uvol and renal vasoconstriction, which dilutes electrolytes and increases blood pressure. Our results did not support our hypotheses, finding naproxen did not significantly decrease P[Na⁺], Posm, urine hydration measures or increase cardiovascular strain compared to placebos. We also found responses did not differ between either hot or ambient environmental conditions.

Fluid and Electrolyte Balance

Naproxen is highly effective at inhibiting renal PGs, resulting in decreased renal blood flow. Naproxen also suppresses the renin-angiotensin-aldosterone system, which is responsible for maintaining BP, plasma volume, and electrolyte balance. Specific renal consequences include decreased glomerular filtration rate, increased vasopressin (anti-diuretic hormone), increased sodium retention, and decreased Uvol. Independently or concurrently, these effects cause water retention and vasoconstriction. In extreme cases, cell necrosis, interstitial nephritis, and renal failure may develop. Though these conditions can become severe, milder consequences include electrolyte imbalance, edema, and hypertension.
Decreasing Uvol and increasing water retention is what makes NSAIDs a suspected contributor to hyponatremia. Despite this, few studies have examined this relationship, with most being observational studies with conflicting results.\textsuperscript{5,6,15,16} Ultra-distance triathletes using NSAIDs had significantly lower post-race P[Na\textsuperscript{+}] compared to non-NSAID users (mean = 140.2 mmol/L and 141.1 mmol/L, respectively, $P < 0.02$).\textsuperscript{5} The 6 participants who experienced hyponatremia all reported using NSAIDs.\textsuperscript{5} In 15 marathon runners hospitalized with severe hyponatremia, 28.6\% used NSAIDs.\textsuperscript{15} However, other observational studies found no relationship to hyponatremia.\textsuperscript{6,16,17} One inherent issue with existing literature is not knowing what type and how much of an NSAID someone took. All NSAIDs inhibit PGs, but their COX selectively and chemical make-up, along with an individual’s unique response, means each NSAID exerts effects differently.

Another contributing factor for developing hyponatremia is an individual consuming water (hypotonic fluid) in excess of fluid lost through sweat and respiration.\textsuperscript{4} If combined with other factors that dilute P[Na\textsuperscript{+}], the individual can be at greater risk for hyponatremia. Current recommendations advise matching individual sweat losses rather than drinking in excess or following generalized protocols. Calculating sweat loss is relatively simple ([pre-activity body weight – post-activity body weight + fluid volume consumed – urine volume]/exercise time).\textsuperscript{18} Either lack of knowledge, ability, or resources may prohibit the average person from calculating his/her sweat rate. Instead, the person may guestimate or trial and error fluid intake during endurance activity. We chose to provide a standardized water protocol (3.5 ml/kg), rather than match sweat rates, and participants were allowed to consume more if desired. In part, using a standardized intake mimicked what an individual who does not know their personal water needs may do. We
found consuming water volumes exceeding sweat loss during 90 min cycling did not significantly deplete P[Na+] and there was no effect from NSAIDs. This is likely due to the short exercise duration. Though exertional hyponatremia has occurred in football,\textsuperscript{19-21} it is typically associated with long-duration endurance exercise (\textgtr 3 hrs).\textsuperscript{4,22} Our exercise session likely did not allow enough time to significantly deplete P[Na+]. During the 3 hr rest, when participants were allowed to drink water ad libitum they maintained euhydration and plasma electrolyte balance.

One interesting note is our mean P[Na+] was < 135 mmol/L in all conditions and time points except pre- and 3 hr post-exercise NpxHeat. Plasma sodium < 135 mmol/L without signs or symptoms is considered biochemical or asymptomatic hyponatremia.\textsuperscript{22} Many individuals do not experience symptoms until P[Na+] decreases below 130 mmol/L and severe hyponatremia typically occurs below 120 mmol/L.\textsuperscript{11} Our participants maintained normal diets, consuming an average 2401.1 ± 759.2 mg of sodium daily. The low P[Na+] can be explained by participants being instructed and attempting to arrive to the laboratory hydrated and by maintaining hydration during our exercise protocol. Pre-exercise Usg, Uosm, and Posm indicated our participants were slightly hyperhydrated and became extremely hyperhydrated\textsuperscript{23} by 3 hrs post-exercise.

Also interesting was that NpxHeat averaged more fluid consumed and had the lowest Uvol. Due to our low plasma sample size and lack of significance, we have difficulty definitively explaining why this response occurred. The trend with naproxen to produce less urine while consuming more fluid suggests participants were retaining water. Since we did not see significant decreases in P[Na+], Posm, or Usg pre- to post-exercise, we presume a portion of water remained in the stomach. Intense exercise slows gastric
emptying, which prevents fluid from being absorbed and/or used to maintain physiological processes.²⁴

In theory, increased Fvol and decreased Uvol should lead to BM gains. We found no significant difference in BM, which, again, may be attributed to exercise duration. However, both the Npx and NpxHeat averaged pre- to post-exercise BM losses, while both Control and Heat gained slightly or did not change (Table 3.1). Once exercise ceased (post-to 3 hrs post-exercise) this trend flipped, with Control and Heat indicating more weight loss than Npx and NpxHeat. Once exercise ceased, the possibility exists that GI and renal function returned to normal, promoting gastric emptying and water excretion as necessary to maintain fluid-electrolyte balance. Support for this explanation is seen with the slightly higher Uvol in Control and Heat. On the other hand, naproxen’s effects continuing throughout the rest period would explain the slight weight gain and lower average Posm at 3 hrs post-exercise. This thought is an important note considering reported cases of hyponatremia developing hours after activity.¹⁹,²¹ Sustained water retention combined with continued hydration would place the person at even greater risk for diluting P[Na⁺].

Regarding potassium, our participants remained within normal P[K⁺] levels (< 5 mmol/L).¹² Potassium is tightly regulated by the kidneys, because elevated levels can cause cardiac arrhythmias and death.¹² Even during exercise, when plasma levels can quickly spike due to working muscles releasing or failing to re-uptake potassium,¹² the kidneys work efficiently to clear potassium. However, potassium will stay elevated if renal blood flow is decreased²⁵ or through decreased energy metabolism¹² and increased oxygen tension.²⁶ NSAIDs can induce hyperkalemia,²⁷ but there is little research regarding this association during exercise. The increase in our overall P[K⁺] means are similar to those
reported in existing literature.\textsuperscript{5,8,28} While not significantly different between conditions, the higher means with naproxen compared to placebos suggest there could be some effect from the NSAID. Among triathletes, NSAIDs resulted in significantly higher post-race $P[K^+]$ compared to those not using NSAIDs ($4.51 \pm 0.46$ mmol/L and $4.35 \pm 0.43$ mmol/L, respectively, $P = 0.002$).\textsuperscript{5} In contrast, Dumke et al.\textsuperscript{5} found over the counter ibuprofen did not significantly affect $P[K^+]$ in ultra-distance runners. Since type of NSAIDs were not reported, it is not possible to make comparisons among specific NSAIDs. Research has established, compared to no NSAID exposure, odds for developing hyperkalemia with ibuprofen are lower (odds ratio = 0.94) compared to naproxen (odds ratio = 1.08).\textsuperscript{27} Using a higher naproxen dose for a longer duration may induce significant $P[K^+]$ changes during exercise.

**Cardiovascular**

Our results indicate 3 220 mg naproxen doses over 24 hrs did not significantly affect pre-, post-, or 3 hr post-exercise HR or BP. Cardiovascular responses often occur concurrently with renal responses, since altering fluid-electrolyte balance can control plasma volume and BP. Disregarding renal effects, naproxen is less effective at directly altering the cardiovascular system because naproxen is more COX 2 selective.\textsuperscript{29} This naproxen effect is in comparison to aspirin, which is more selective toward COX 1.\textsuperscript{30} Further, naproxen reversibly inhibits platelets while aspirin irreversibly inhibits platelet aggregation, making aspirin highly effective at reducing cardiovascular events (ie, stroke, heart attack).\textsuperscript{30} Lack of cardiovascular effects could be attributed to naproxen itself or to our methodology. By maintaining hydration we limited cardiovascular changes that would otherwise be seen with hypohydration. We also limited our participants to healthy, young,
moderately trained individuals. The same cardiovascular results may not be seen in different populations (eg, older, poor physical conditioning, cardiovascular or renal disease).

**Gender**

Previous literature identifies females are at higher risk for hyponatremia because of smaller body weights, excessive water intake, and longer race times compared to males. Though these factors play a role, hormonal differences between genders is also an important consideration. Resting plasma vasopressin varies in females depending on menstrual phase. Vasopressin is significantly lower than males during the early follicular phase, but not during luteal. These variances may help explain why we found differences in Fvol between genders. Males consumed significantly more fluid than females both during exercise and overall. Interesting, males consumed significantly more fluid during exercise in the NpxHeat, and approached significance in Npx, compared to females. Gender differences did not occur during Control and Heat and we found no difference in females’ Fvol between conditions. Considering the change in vasopressin during menstrual phases, which we did not account for, the lack of significant findings among females could be partially explained by hormones.

Related to Fvol, thirst is listed as a potential side effect for several NSAIDs. There is extremely limited research examining NSAIDs on thirst or Fvol during exercise. Among NSAID and non-NSAID users completing an 82 km mountain run (n = 44; 36 males, 8 females), NSAID users (n = 16) consumed significantly more fluid than non-users (6.1 ± 1.5 L vs 5.1 ± 2.2 L, P = 0.08). Our results support, at least in males, naproxen increases fluid intake. We used naproxen sodium, which is bioequivalent to naproxen except for rate
of absorption. The salt of a given NSAID is commonly used because it allows the drug to dissolve faster and exert effects earlier. A 220 mg naproxen sodium dose contains approximately 20 mg of sodium. Not all salts exert the same effects and 20 mg is relatively low, but Wemple et al. found 46 mg/L of sodium chloride added to a flavored beverage significantly increased fluid intake. Naproxen sodium may stimulate thirst. Another mechanism for thirst stimulation is NSAIDs increasing vasopressin. Vasopressin is typically upregulated during dehydration in order to restore plasma volume, and its increase is positively and linearly associated with thirst.

Extensively examining cardiovascular effects between genders is difficult because we cannot compare plasma measures and did not assess hormones. We identified no differences between or within genders for HR. Blood pressure is generally higher for men than females, which is attributed to physical differences, hormones, and vascular responsiveness. Male baseline BP was slightly higher than females, but not significantly. Naproxen significantly elevated post-exercise male systolic BP compared to females. This did not occur during Control and Heat. The increased Fvol with naproxen in males could partially explain the higher systolic BP. Seemingly, males would have a higher plasma volume, which would increase BP.

Limitations and Future Research

One limitation to our study is not measuring aldosterone and vasopressin. Measuring these hormones would provide a better explanation on how naproxen influences fluid and electrolyte regulation. Measuring plasma naproxen levels would have been useful to determine the drug’s concentration at 3 hrs post-exercise. Naproxen has a long half-life compared to other NSAIDs. Therefore, we would expect naproxen to continue exerting
effects during the 3 hr rest, while other NSAIDs would not. This knowledge would be important when considering post-activity effects. Our 90 min cycling was likely too short to elicit significant P[Na+] changes, considering much of the hyponatremia research is in marathon and ultra-distance events. Due to lost samples, our sample size for plasma electrolytes and osmolality was low, preventing us from achieving statistical significance between conditions. Finally, we did not control for menstrual phase.

Future research in laboratory settings is warranted to determine the relationship between NSAID use and hydration-electrolyte balance during exercise. Because each NSAID is unique, studies should evaluate different types, dosages, and length of use. Research should evaluate longer endurance exercise bouts to elicit plasma electrolyte depletion. Shorter exercise bouts are relevant, as well, considering the potential for electrolyte depletion associated with other risk factors such as inadequate nutrition, excessive hypotonic fluid intake, and/or excessive sweat sodium loss. Using different hydration regimens (metered versus ad libitum) and fluid types (eg, carbohydrate-electrolyte beverages) is also merited. Considering the trend for increased Fvol with naproxen, evaluating vasopressin and thirst would be interesting. Along these lines, examining the salt of different NSAID types would provide meaningful information. Finally, controlled studies evaluating markers of renal function and gender differences, including differences during menstrual phases is merited.

**Conclusion**

Acute naproxen intake did not adversely affect hydration, electrolyte, or cardiovascular measures during cycling in either hot or ambient conditions. An important note is the context of our findings, and that we identified some trends. Our participants
were hydrated, moderately endurance trained, free of many confounding risk factors, and
the cycling protocol was moderate in duration (90 min). Second, NSAIDs vary in
selectivity and chemical make-up. Our participants consumed the over the counter strength,
recommended daily naproxen sodium dose.

We encourage clinicians and other personnel to consider an individual’s personal
risk factors when recommending naproxen. Finding higher post-exercise systolic BP with
naproxen in males versus females warrants consideration for potential adverse events
during exercise in males with pre-existing hypertension. Further, we found naproxen
promoted greater fluid intake in males during ambient and hot environments. Beginning
exercise slightly hyperhydrated and consuming water in excess of sweat rate during a 90
min exercise time began to show the trend for naproxen to induce water retention through
decreased Uvol. Though our participants maintained electrolyte balance, their P[Na+] was
lower than 135 mmol/L. Together, these results show the importance in considering
individuals who may have risk factors for renal and/or cardiovascular imbalances during
exercise.

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3. Garella S, Matarese RA. Renal effects of prostaglandins and clinical adverse effects


5. Wharam PC, Speedy DB, Noakes TD, Thompson JM, Reid SA, Holtzhausen LM.
NSAID use increases the risk of developing hyponatremia during an Ironman


Table 3.1. Hydration Measures, Fluid Volume, and Urine Volume for Experimental Conditions (M ± SD)

<table>
<thead>
<tr>
<th></th>
<th>Aggregate</th>
<th>Placebo</th>
<th>Npx</th>
<th>Heat</th>
<th>NpxHeat</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Posm (mOsm/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>286.6 ± 6.4a</td>
<td>285.8 ± 5.3</td>
<td>288.8 ± 4.8</td>
<td>285.5 ± 10.1</td>
<td>287.8 ± 6.6</td>
</tr>
<tr>
<td>Post</td>
<td>284.8 ± 6.7</td>
<td>288.2 ± 6.6</td>
<td>281.9 ± 3.9</td>
<td>285.9 ± 10.6</td>
<td>283.8 ± 4.9</td>
</tr>
<tr>
<td>3 hr post</td>
<td>282.4 ± 5.9</td>
<td>286.4 ± 3.3</td>
<td>277.4 ± 6.6</td>
<td>283.6 ± 6.9</td>
<td>281.1 ± 4.9</td>
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<tr>
<td><strong>Uosm (mOsm/L)b</strong></td>
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<td></td>
<td></td>
<td></td>
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<td>Pre</td>
<td>399.5 ± 172.1c</td>
<td>441.6 ± 187.5</td>
<td>431.8 ± 193.5</td>
<td>341.4 ± 131.7</td>
<td>389.9 ± 183.4</td>
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<tr>
<td>Post</td>
<td>277.3 ± 236.0</td>
<td>246.8 ± 223.7</td>
<td>244.2 ± 184.1</td>
<td>233.1 ± 213.9</td>
<td>390.2 ± 310.0</td>
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<tr>
<td>3 hr post</td>
<td>176.2 ± 132.9</td>
<td>192.4 ± 107.8</td>
<td>185.6 ± 130.4</td>
<td>201.3 ± 173.0</td>
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<td><strong>Usg</strong></td>
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<td>Pre</td>
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<td>3 hr post</td>
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<td>1.007 ± 0.005</td>
<td>1.007 ± 0.006</td>
<td>1.007 ± 0.006</td>
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<tr>
<td><strong>∆BM (%)</strong></td>
<td></td>
<td></td>
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<tr>
<td>Pre - Post</td>
<td>-0.1 ± 0.8</td>
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<td>-0.3 ± 1.1</td>
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<td>Post - 3 hr</td>
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<td>0.0 ± 1.3</td>
<td>-0.4 ± 0.5</td>
<td>-0.0 ± 0.6</td>
</tr>
<tr>
<td><strong>Uvol (ml)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Exercise</td>
<td>1012.9 ± 519.1</td>
<td>1157.3 ± 681.4</td>
<td>1075.3 ± 572.3</td>
<td>982.6 ± 508.6</td>
<td>836.6 ± 214.2</td>
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<td>Total</td>
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<td><strong>Fvol (L)</strong></td>
<td></td>
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<td></td>
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<tr>
<td>Exercise</td>
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<td>1.4 ± 0.9</td>
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<tr>
<td>Total</td>
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<td><strong>Fvol (ml/kg)</strong></td>
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<td>Exercise</td>
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<td>Total</td>
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<td>25.5 ± 13.4</td>
<td>27.3 ± 8.1</td>
<td>29.5 ± 10.6</td>
</tr>
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</table>

aSignificantly higher than 3hr post (P = 0.01, observed power = 0.08). bSignificant main effect across time for all conditions (P < 0.001). cSignificantly higher than post (P = 0.003) and 3 hr post (P < 0.001). dSignificantly lower than pre (P < 0.001) and post (P = 0.03).
<table>
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<th>Npx</th>
<th>Heat</th>
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<tr>
<td><strong>PNa⁺ (mmol/L)</strong></td>
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<tr>
<td>Pre</td>
<td>134.3 ± 4.6</td>
<td>133.5 ± 5.7</td>
<td>133.2 ± 4.5</td>
<td>133.5 ± 3.2</td>
<td>135.0 ± 2.9</td>
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<tr>
<td>Post</td>
<td>133.5 ± 3.2</td>
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</tr>
<tr>
<td>3 hr post</td>
<td>134.3 ± 3.1</td>
<td>133.1 ± 1.9</td>
<td>133.5 ± 0.3</td>
<td>132.9 ± 4.7</td>
<td>135.3 ± 2.7</td>
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<tr>
<td><strong>PK⁺ (mmol/L)ᵃ</strong></td>
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<tr>
<td>Pre</td>
<td>3.9 ± 0.4</td>
<td>3.9 ± 0.6</td>
<td>4.1 ± 0.4</td>
<td>3.9 ± 0.2</td>
<td>3.6 ± 0.1</td>
</tr>
<tr>
<td>Post</td>
<td>4.2 ± 0.4</td>
<td>4.2 ± 0.7</td>
<td>4.4 ± 0.3</td>
<td>3.9 ± 0.1</td>
<td>4.2 ± 0.2</td>
</tr>
<tr>
<td>3 hr post</td>
<td>3.9 ± 0.2</td>
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<td>3.8 ± 0.0</td>
<td>4.0 ± 0.3</td>
<td>3.9 ± 0.3</td>
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</table>

ᵃSignificant main effect across time (*P* = 0.02, observed power = 0.08).
### Figure 3.1. Experimental Procedures Schematic.

Blood measures include plasma osmolality, plasma sodium concentration, and plasma potassium concentration. Urine measures include urine osmolality and urine specific gravity.

Abbreviations: BM = body mass; BP = blood pressure; HR = heart rate.
CHAPTER 4

NAPROXEN EFFECTS ON GASTROINTESTINAL DISTRESS AND PERFORMANCE DURING CYCLING IN THE HEAT

ABSTRACT

**Purpose:** Our primary objective was to determine effects of naproxen on gastrointestinal (GI) distress symptoms and performance in hydrated humans cycling in the heat. **Methods:** We utilized a double-blind, randomized and counterbalanced, cross-over design. Participants were 11 moderately endurance trained volunteers (6 male, 5 female; age = 27.8 ± 6.5 yrs, weight = 79.1 ± 17.9 kg, height = 177 ± 9.5 cm, and VO$_{2\text{max}}$ = 41.4 ± 5.7 ml/kg). Independent variables were placebo or naproxen (one 220 mg pill every 8 hrs for 24 hrs) and hot or ambient environment. Dependent variables were max heart rate (HR), rate of perceived exertion (RPE), distance, GI symptoms, and fecal occult blood. Measures were taken pre-, during, and post-90 min cycling. Four trials: 1) placebo and ambient (Control); 2) placebo and heat (Heat); 3) naproxen and ambient (Npx); and 4) naproxen and heat (NpxHeat) were completed and separated by 7 days minimum. **Results:** No statistically significant differences in RPE, max HR, distance, fecal occult blood, or GI symptoms between conditions were found. Average max HR was higher during Npx (176.2 ± 15 bpm) than Control (175.7 ± 14.2 bpm) and NpxHeat (179.0 ± 18.0 bpm) than Heat (177.8 ± 18.2 bpm). Mean distance covered was highest during Npx (3.3 ± 0.8 miles) and lowest in NpxHeat (2.8 ± 0.8 mile). Gastrointestinal symptoms occurred during exercise in 64% of trials. Of the reported GI symptoms during ambient trials, 10% were serious compared to 7% in Heat and 1% in NpxHeat. Dizziness and headache during exercise were only reported during placebos. **Conclusion:** Acute naproxen intake did not significantly affect performance or GI distress during 90 min cycling. The potential interaction between naproxen and heat stress, as indicated by higher HR and lower distance traveled, warrants
further research. **Key Words:** NSAIDs, gastrointestinal bleeding, heart rate, perceived exertion, cycling

**INTRODUCTION**

A high prevalence of prescription and over the counter non-steroidal anti-inflammatory drug (NSAID) use among various age groups, sport types, and competition levels exists.(38,40,41) These drugs are often used prophylactically or as treatment following injury.(11,39,40) For example, individuals may take NSAIDs in order to “continue playing” or in attempt to mitigate pain and inflammation during activity. Because of their association with anti-inflammatory and analgesic effects, there is a perception NSAIDs can increase performance during physical activity.(40)

Acetaminophen has been found to enhance performance by alleviating pain,(12,23) but research on NSAIDs is limited and results are inconclusive. Existing literature primarily focuses on aspirin, males, and short, intense exercise bouts in ambient environments.(6,8,15,18) For example, using aspirin (dose = 20 mg/kg of body weight) 1 hr prior to exercise did not significantly affect power output, rate of perceived exertion (RPE), heart rate (HR), or alleviate muscle pain during a ramped maximum exertion cycling protocol.(6) Similarly, a lower aspirin dose (10 mg/kg) 1 hr prior to maximum lower extremity resistance exercises increased RPE and perceived leg pain compared to placebo.(18) Gilbert et al.(15) investigated an acute aspirin dose (975 mg 1 hr prior) and chronic (975 mg 3 times a day for 4 days) on max graded treadmill runs. Acute aspirin significantly increased RPE, while chronic significantly increased post-exercise lactate, RPE, hematocrit, and fatigue.(15) Lastly, ibuprofen (1.2 g) 1 hr prior to treadmill running to fatigue did not improve time to exhaustion or RPE and resulted in no significant change
to blood lactate or HR.(8) From these results, NSAIDs appear to have little effect and, in some instances, may degrade performance.

NSAIDs may also negatively affect performance through physiological mechanisms such as damage to the gastrointestinal (GI) tract,(22) cardiovascular system, and kidneys.(4) Severity for adverse NSAID effects varies depending on dosage, length of use, formula, and individual risk factors (eg, sensitivity to the drug, previous adverse events, and health status).(22) However, in general, NSAIDs are known to cause ulcers, perforations, and hemorrhaging along the GI tract.(14,22) Symptoms of GI distress (eg, nausea, cramping, diarrhea, pain, and bloating) can be subjective and highly variable between individuals. Further, GI distress can be caused by factors such as stress, fatigue, and diet.(16,25,28) NSAIDs also increase cardiovascular strain by inducing vasoconstriction and increasing hypertension in peripheral and renal blood vessels.(4) Independently or concurrently, these NSAID driven events could decrease performance.

Our primary aim was to determine effects of an acute over the counter dose of naproxen on GI distress symptoms and performance measures during moderate-intense endurance exercise in a hot environment. A secondary aim was to investigate potential gender differences. We hypothesized naproxen would induce significantly more GI distress and decrease performance compared to placebo controls. We also hypothesized GI distress and performance decrements would not be different between males and females.

METHODS

Participants

As part of a larger study, 11 participants (5 male, 6 female; age = 27.8 ± 6.5 yrs, weight = 79.1 ± 17.9 kg, and height = 177 ± 9.5 cm) were recruited from the university and
local community. Participants read and signed an Institutional Review Board approved informed consent form (Appendix A) prior to participation. To be included in the study participants completed a health and injury history questionnaire (Appendix B). Participants were free from cardiovascular, respiratory, and metabolic disorders; musculoskeletal disorders preventing cycling exercise; fluid and electrolyte balance disorders; and GI and swallowing disorders. Questions also asked about current medication use. Females were asked additional questions on menstrual cycle. Participants completed a graded cycling $\dot{V}O_2_{\text{max}}$ test (Appendix E.2) to determine qualification as moderately trained (male $\dot{V}O_2_{\text{max}}$ between 35-40 mL/kg; female $\dot{V}O_2_{\text{max}}$ between 32-40 mL/kg).(27)

**Experimental Conditions**

Participants completed all 4 experimental conditions in a randomized, counterbalanced order with 7 days minimum between each trial. The 4 experimental conditions were: 1) placebo and ambient (Control); 2) placebo and heat (Heat); 3) naproxen and ambient (Npx); and 4) naproxen and heat (NpxHeat). A 24 hr dose (3 capsules) of placebo (cellulose) or naproxen (220 mg naproxen sodium/capsule) was given to participants prior to exercise. Participants consumed 1 capsule at 16 hrs, 8 hrs, and 0 hrs prior to data collection. All participants were given specific take home instructions (Appendix C.4) to follow 24 hrs prior to data collection, including directions for taking each capsule according to the manufacturer’s directions. All experimental trials took place in an environmental chamber (hot = 35.7 ± 1.3°C, 53.2 ± 3.2% relative humidity) or laboratory (ambient = 22.7 ± 1.8°C, 52.4 ± 5.5% relative humidity).

**Instruments and Protocols**

**Cycle Exercise Protocol.** Participants completed a 90 min cycling protocol on a
stationary bike (Monark Ergomedic 828E, Monark Exercise AB, Vansbro, Sweden). Participants began with a 3 min warm followed by 80 min at a steady HR equivalent to 70% $\dot{V}O_{2\text{max}}$. The final 10 min were performed at max effort and the protocol concluded with a 5 min cool down.

**Rate of Perceived Exertion.** The Borg Scale measured participants’ RPE pre-, every 15 min during, and post-exercise.

**Cardiovascular.** To ensure participants remained at safe limits and assist with maintaining the target HR during 80 min steady state cycling, HR was continuously monitored and recorded every 5 min using Polar HR monitors (Polar Electro Inc., Lake Success, NY). To determine effects on performance, we measured max HR at the end of the 10 min max effort.

**Gastrointestinal Symptom Index (Appendix D).** Gastrointestinal distress was assessed using a previously designed symptom questionnaire. The index is divided into 3 sections: 1) upper abdominal problems (heart burn, reflux, belching, bloating, stomach pain/cramping, nausea, vomiting); 2) lower abdominal problems (intestinal/lower abdominal pain/cramping, flatulence, urge to defecate, side aches/stitch, loose stool, diarrhea); and 3) systemic problems (dizziness, headache, muscle cramps, urge to urinate). Symptom scores are on a 10-point scale (0 = no problems at all and 9 = the worst it has ever been). A score of > 4 is considered “serious”. Questionnaires were administered pre-, post-, and 3 hrs post-exercise. An additional questionnaire was administered at the time of the post-exercise fecal sample (~ day post).

To determine GI symptoms during exercise, a scale was developed based on the GI symptom questionnaire. Participants were verbally asked every 15 min “Are you currently
experiencing GI symptoms?” If yes, he/she was asked to verbalize the symptom and rank the severity on a 10-point scale (0 = no problems at all and 9 = the worst it has ever been).

**Fecal Occult Blood.** To determine GI bleeding, fecal occult blood (FOB) was measured using take home kits (Fisher HealthCare™ Sure-Vue™ Fecal Occult Blood Slide Tests System, Thermo Fisher Scientific Inc., Waltham, MA). For each experimental trial, participants provide the first stool sample after initiating naproxen/placebo and the first stool sample following the exercise protocol.

**Hydration Measures.** Participants were required to be euhydrated prior to beginning data collection. Hydration status was determined using urine specific gravity (Usg). Urine was obtained pre-, post-, and 3 hrs post-exercise. A handheld clinical refractometer (model REF 312, Atago Company Ltd., Tokyo, Japan) measured Usg. Euhdyration was defined as Usg ≤ 1.020.(34) To maintain hydration during exercise, participants were provided 3.5 ml/kg of water to consume every 15 min.

**Diet and Activity Logs.** To control potential effects on GI distress and performance, participants were asked to track 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 asked to mimic dietary and physical activity habits during the 24 hrs prior to each data collection session. Additionally, participants were instructed to refrain from eating red meat during the 3 days prior and 1 day after due to the potential to skew FOB tests.

**Experimental Procedures**

**Information Session.** After consenting participants were determined free of disqualifying medical conditions and verified to be moderately trained, they were randomly
assigned to an experimental condition order by a research assistant. Participants and primary investigators were blind to whether participants were completing the naproxen or placebo groups. Participants were familiarized with the online diet and activity log and the $\dot{V}O_2$max test was used to familiarize individuals to the stationary cycle. Participants were instructed to not take any pain or inflammation medications during the course of the study.

**Pre-Data Collection.** Participants were sent an email with instructions for completing the diet and physical activity log 72 hrs before data collection. Participants were instructed to refrain from intense, vigorous exercise for 48 hrs. Twenty-four hrs prior, participants were provided written instructions for taking 2 capsules (placebo or naproxen) and directions for diet, hydration, and activity. Participants were instructed to consume a small meal at least 2 hrs prior to arriving to the laboratory to ensure food passed through the stomach.

**Data Collection Session.** Upon arrival to the laboratory, participants were verbally asked if they took their 2 pills before taking the 3rd pill. Participants were instructed to provide a urine sample into a urine cup and void everything else into the toilet. Baseline Usg was measured to ensure participants were euhydrated.

Participants were provided a HR monitor and the cycling protocol began with a 3 min warm up on the bike. Participants maintained their target HR during the 80 min steady state exercise and consumed water to maintain hydration. Every 15 min a research assistant asked participants their RPE and GI symptoms. The last 10 mins of the cycling protocol was completed at max effort. Each participant was given verbal encouragement by research assistants for each trial. At the conclusion of the max effort, participants were asked RPE and GI symptoms, HR was recorded, and distance traveled during the 10 mins of max
cycling was recorded. Following a 5 min cool down on the bike, participants completed a post-GI symptom index and provided a urine sample. Participants then rested 3 hrs in a semi-reclined/seated position in an ambient environment (23°C, 56% RH). Water was consumed ad libitum and each participant was given a non-sucrose snack. At the conclusion of the rest period, urine, BP, HR, and GI symptoms were measured.

**Statistical Analysis**

IBM SPSS Statistics (version XXII; IBM Corporation, Armonk, NY) was used for all analyses. Descriptive statistics (mean and standard deviations) for all dependent variables were calculated. Demographical (eg, age, height, weight) and dietary (eg, calories and protein) differences were assessed using a one-way ANOVA. We used a one-way ANOVA to determine differences between conditions for distance, max HR, and FOB. Eight (time) x 4 (condition) repeated measures ANOVAs determined RPE differences for all data and between and within genders. Because RPE violated sphericity, Greenhouse-Geisser corrections were used. Post hoc analysis was conducted for significant main effects with Bonferroni corrections. Using G*Power (version 3.1.9.2, Heinrich Heine University, Dusseldorf, Germany),(10) post hoc power calculations with means and variances for HR and distance indicated a statistical power > 0.9. Significance level was set at $P < 0.05$ for all analyses.

Due to non-parametric data, we used Wilcoxon signed rank tests to identify differences in GI symptoms pre-, post- and 3hr post-exercise within and between conditions. Wilcoxon signed rank were also used to determine differences within gender. To reduce multiplicity, questions were sectioned into upper, lower, and systemic symptoms. Responses were averaged and analyzed. Because of the number of tests and
chances for inflation, $P$ values are considered “interesting” rather than significant.\(^{(31,32)}\)

We determined frequency of symptoms scored $> 4$ (considered “serious”) within each condition. Kruskal-Wallis analysis determined differences between genders. To control for other factors that may influence GI symptoms, Spearman’s rho correlations were run for max core temperature (data presented in Chapter 2), fluid volume consumed during exercise (data presented in Chapter 3), max HR, max RPE, and distance.

**RESULTS**

Table 4.1 contains participant demographics. There was no significant differences between age, weight, and $\dot{V}O_2^{\text{max}}$ for gender. Participants began experimental trials euhydrated (mean Usg = $1.012 \pm 0.005$) and maintained euhydration throughout exercise (1.011 $\pm$ 0.008) and recovery (1.007 $\pm$ 0.006). Diet log results indicated no significant differences between conditions for calorie, fat, protein, carbohydrate, or sodium intake. Also, no significant differences in exercise (calories burned) 24 hrs prior to data collection existed, with all participants maintaining sedentary to low activity. No significant differences existed within genders between experimental conditions. We found, overall, males consumed significantly more calories, protein, and sodium than females (Appendix F, Table F.2). Subsequent analysis identified the only significant difference between genders within conditions occurred with protein for NpxHeat (male = $65.4 \pm 2.7$ g and female = $44.7 \pm 3.2$ g, $P = 0.004$).

**Performance**

Table 4.2 shows max HR, RPE, and distance traveled during 10 min max effort for each condition. There was an overall significant main effect ($P < 0.001$) for RPE over time, but no significant differences between conditions. Within gender, RPE did not significantly differ between conditions (Appendix F, Table F.3). The only difference between gender
and conditions occurred in NpxHeat with pre-exercise males significantly lower (7 ± 1) than females (9 ± 2, \( P = 0.03 \)). Significant differences occurred between overall males and female RPEs. Females were significantly higher at 0 and 15 min cycling (10 ± 2 vs. 8 ± 2 and 11 ± 2 vs. 9 ± 3, respectively, \( P < 0.03 \)). On the other hand, males were significantly higher at 90 min (19 ± 2 vs. 18 ± 2, \( P < 0.03 \)).

Distance was not significantly different between conditions. On average, distance traveled was lower during heat trials compared to ambient (Table 4.2). Interestingly, mean distance was slightly higher for Npx compared to Control. This effect was not found in the hot environment, with NpxHeat having the lowest distance covered. There were no statistically significant difference between males and females for distance within or between conditions (Appendix F, Table F.4). Overall, we found males trended toward greater distance than females (3.2 ± 0.7 miles vs 2.8 ± 0.9 miles, \( P = 0.085 \)). Similar to overall condition results, both males and females in ambient conditions averaged greater distance compared to heat. Furthermore, the Npx condition resulted in the highest average distance for both genders (Appendix F, Table F.4).

As expected, mean max HR was higher during heat trials compared to ambient, but was not significantly different (Table 4.2). Heart rate was highest during NpxHeat and lowest in the Control trial. There were no statistically significant difference within or between genders (Appendix F, Table F.4). Females’ mean max HR was highest in NpxHeat (180.2 ± 17.2 bpm) and was higher than males for both heat trials. Male max HR was highest during the Npx condition (179.5 ± 15.1 bpm).
**GI Symptoms and FOB**

Mean and max scores for each upper, lower, and systemic GI symptom were totaled for each time point (pre- to day-post) and are presented in Table 4.3. Both Heat and NpxHeat had higher averages and max scores compared to ambient conditions. Urge to urinate was the most frequent symptom reported. Reflux/heartburn, vomiting, abdominal pain, and diarrhea were only reported during heat trials.

Appendix F, Table F.5 shows aggregate upper, lower, and systemic GI scores for each time point with Wilcoxon results. Heat resulted in higher mean upper GI symptom scores post-exercise (0.3 ± 0.3). Higher mean and serious systemic post-exercise scores occurred in Control (0.9 ± 0.9, 6% serious), Npx (0.6 ± 0.6, 6% serious), and Heat (0.7 ± 0.9, 3% serious) compared to NpxHeat (0.3 ± 0.4, 0% serious). Control continued to experience more frequent and serious lower and systemic symptoms at 3 hrs post-exercise and was greater than any other condition. Day-post results showed Heat experienced higher mean and serious scores for upper (1.3 ± 2.5, 18% serious), lower (1.9 ± 12.4, 6% serious) and systemic (1.3 ± 2.2, 5% serious). Only NpxHeat resulted in more serious lower GI symptoms day post (12%).

There were no significant differences for GI symptoms between conditions within genders. The only significant differences between genders occurred at 3 hrs within the Heat condition, $\chi^2(1) = 4.4, P = 0.036$, with a mean rank 4.5 for males and 7.8 for females. There were no positive FOB tests pre-exercise. One positive FOB test indicated GI bleeding post-exercise in the Npx condition. However, this test was likely a false positive, since it occurred in a menstruating participant.
During Exercise

Overall, GI symptoms occurred during exercise in 64% of trials. Only 1 participant experienced no GI symptoms during any trial. Eight-two percent of participants reported at least 1 symptom during Control, 73% during Npx, 45% during Heat, and 55% during NpxHeat. Mean and max GI symptom scores during exercise are presented in Table 4.4. Urge to urinate and nausea was most frequent for all conditions and highest during ambient trials. Greatest percentage of serious scores (10%) occurred for systemic symptoms, specifically urge to urinate, during Control and Npx (Appendix F, Table F.6). NpxHeat had the least systemic symptoms reported (0.0 ± 0.8, 1% serious). Dizziness and headaches were reported only during placebo conditions. Though lower GI symptoms were frequently reported during exercise for all conditions, none were classified as serious. Upper GI symptoms were commonly reported with Heat (0.6 ± 1.0, 3% serious), NpxHeat (0.3 ± 0.9 and 2%), Control (0.3 ± 0.6, 1%), and Npx (0.4 ± 0.7, 1%). Nausea and vomiting were the only reported serious upper GI symptoms. There were no significant differences for GI symptoms during exercise either within or between genders.

Correlations

We identified no significant correlations for GI symptoms to fluid volume, core temperature, max RPE, or max HR. We found a significant positive correlation between max RPE and HR for Control (ρ = 0.8, P = 0.002), Npx (ρ = 0.8, P = 0.006), and Heat (ρ = 0.6, P = 0.43), but not for NpxHeat. A significant positive correlation was found for max core temperature and HR only during Control (ρ = 0.7, P = 0.025). Distance traveled was significantly correlated to max RPE during Npx (ρ = 0.6, P = 0.044) and to core temperature during NpxHeat (ρ = 0.7, P = 0.028). During Heat, we found a significant
positive correlation between lower and upper GI symptoms ($\rho = 0.9, P = 0.001$) and lower and systemic GI symptoms ($\rho = 0.7, P = 0.03$).

We identified several significant correlations within genders. Distance and max HR in females were strongly correlated during the Control ($\rho = 1.0, P < 0.001$), but this did not occur in the males. Likewise, distance was strongly correlated to max HR ($\rho = 1.0, P < 0.001$) and RPE ($\rho = 1.0, P < 0.001$) during Npx, but this was not seen in males. During Npx, we found male RPE was negatively correlated with upper GI symptoms ($\rho = -1.0, P < 0.001$). In the Heat, female, but not male, RPE was strongly correlated to distance ($\rho = 1.0, P < 0.001$) and fluid volume ($\rho = 1.0, P = 0.005$). Finally, within NpxHeat, female distance was significantly correlated to upper GI symptoms ($\rho = 0.9, P = 0.041$) and HR ($\rho = 1.0, P = 0.005$). On the other hand, in males, core temperature was strong correlated to distance ($\rho = 0.8, P = 0.036$) and systemic GI symptoms ($\rho = 0.9, P = 0.029$).

**DISCUSSION**

Extensive evidence shows NSAIDs negatively affect the GI and cardiovascular systems,(13,17,20,22) yet there remains a high prevalence of NSAIDs use before and during exercise.(11,38-40) Often, NSAIDs are taken to prevent or alleviate pain and inflammation and, seemingly, these effects would increase performance. Ours results show a 24 hr dose of naproxen does not improve performance, as measured by RPE, distance, and max HR. We also found compared to placebos, naproxen did not significantly increase GI symptoms pre-, during, or post-exercise and these responses were not different between males and females.
Performance

Like previous studies using aspirin(6,15,18) and ibuprofen,(8) naproxen did not improve performance. However, contrary to our hypothesis, we did not find naproxen significantly decreased performance variables. A potential explanation for the lack of statistical significance is using a low dose. Variation in dosage is partly due to differences in cyclooxygenase (COX) selectivity and half-life. Compared to aspirin and ibuprofen, naproxen is more selective toward inhibiting COX 2, which is produced by tissues locally in response to damage(21) and is a key mediator of pain and inflammation.(1) Through higher selectivity, naproxen is considered a more effective anti-inflammatory and analgesic than other NSAIDs.(2) Naproxen also has a longer half-life, 10-20 hrs, meaning individuals can take less doses than aspirin (2-3 hr half-life) and ibuprofen (2-4 hr half-life).(5) With these considerations, higher aspirin and ibuprofen doses are recommended to elicit anti-inflammatory and analgesic effects, and therefore higher doses are seen in literature.(6,8,15,18) Increasing the naproxen dose (ie, prescription strength) and extending time used (ie, more than 24 hrs) could elicit different, more significant responses. This thought is supported when examining acute versus chronic aspirin use, with acute eliciting significantly increased RPE, while chronic significantly affected RPE, lactate, hematocrit, and fatigue.(15)

Our participants were euhydrated throughout exercise; therefore, increased HR was not dehydration induced, but rather associated with experimental conditions. When examining heat trials, NpxHeat averaged less miles with a higher HR and RPE compared to Heat. This same increase in cardiovascular strain was seen when we examined gender differences. Males averaged the same mileage in heat trials, but they experienced higher
max HR with naproxen. Females covered the least amount of distance and experienced their highest HR during NpxHeat. Keeping in mind NSAID effects on the cardiovascular system (vasoconstriction and increased BP) and the cardiovascular systems’ response to intense exercise under thermal stress (increased HR to maintain cardiac output),(24) our results suggest combining naproxen and heat could increase cardiovascular strain and max RPE, resulting in less distance.

Slightly different results were found among the ambient conditions. Naproxen resulted in the greatest distance covered with no change in RPE and a slightly higher HR. Both males and females averaged their greatest distance during the Npx trial compared to Control. Males experienced their highest max HR and RPE during Npx, suggesting they were cycling at a higher workload than during Control. While females averaged more mileage during Npx, their max HR was lower than any other trial. The slight differences between heat and ambient suggests environment may impact the effects of naproxen on performance. To our knowledge, examining NSAIDs on performance in the heat has not been conducted. Da Silva et al.(8) used ambient conditions (18-21°C) and since other laboratory studies do not provide environment information,(6,15,18) we assume these were also conducted in ambient conditions.

Our high correlation between max HR and max RPE in all conditions, except for NpxHeat, suggests our participants were able to accurately assess their RPE and were exhibiting max effort at 90 min. During minutes 30 to 80 of steady state, RPE did not differ between genders. This is consistent with genders working at a relative % \( \dot{V}O_2 \text{max} \) during treadmill running and cycling,(33) but contrasts Cook et al,(7) who found females reported lower RPE than males during cycling at given percentages of peak power output. Once
cycling reached peak power output, males reported significantly higher RPE than females 
(19.6 ± 0.9 and 18.7 ± 1.4, respectively, P < 0.008). The significantly higher aggregate 
values for our male participants compared to females reflects at max effort males report 
higher RPE, but when working at a relative percentage of VO₂max males and females 
respond similarly. Moreover, this was not influenced by taking naproxen or by the 
environment.

**Gastrointestinal Distress**

Intense physical exercise and heat stress can induce GI symptoms and is heavily 
influenced by factors such as previous and current medical history, medication, nutrition, 
and exercise mode. Typically, endurance exercise elicits more GI symptoms than 
an aerobic, and running generally induces greater symptoms than cycling. The 
etiology for GI distress is not well understood, but is suggested to be attributed to 
mechanical vibrations, GI ischemia-reperfusion, and inflammatory 
responses. Prevalence for GI symptoms during exercise in our study is similar to 
ultra-distance runners (60%). Type of symptoms (ie, nausea, diarrhea, and vomiting) 
was also similar to runners. However, considering the prevalence of GI bleeding during 
running, we were surprised to find a lack of positive FOB, particularly with the 
NpxHeat. We likely attenuated the incidence for GI bleeding by using a less impactful 
exercise (cycling) and a shorter, moderate-intense exercise bout.

Contrary to our hypothesis, naproxen did not significantly increase GI distress 
compared to placebo. One of the most notable findings is naproxen preventing dizziness 
and headache during exercise, but not before or after exercise, in both heat and ambient 
trials. Compared to Control, Npx experienced more upper GI symptoms during exercise
but less systemic symptoms. Both Heat and NpxHeat experienced upper GI symptoms, but NpxHeat, once again, experienced less systemic. The high percentage of serious scores in the ambient conditions compared to heat are probably due to the exercise intensity during 80 min cycling prior to max effort. Because participants cycled at a steady HR, during the heat trials HR naturally increased in response to thermal strain. This forced participants to cycle at a lower intensity to maintain target HR. Less intensity explains the lower percentage of serious scores and symptoms in the heat. Additionally, urge to urinate during heat trials was likely mitigated by increased fluid needs in the hot environment (ie, sweating). Interestingly, higher frequency and serious scores were reported day-post during Heat and NpxHeat trials compared to ambient. These data suggest lingering effects from exercise in a hot environment, which was, to some extent, alleviated by naproxen. We did not assess physiological measures day-post, but exercise and thermal stress induce inflammatory responses(35) that can last hours to days after.(26) Increased inflammation could explain higher GI symptoms day-post and is an important consideration for individuals exercising on consecutive days.

Despite slight differences in cardiovascular and performance measures between genders, we found no differences in GI symptoms. Furthermore, GI symptoms in females were not different during menstruation. In our study, GI symptoms were not correlated with the amount of fluid ingested, core temperature, max HR, or max RPE. Instead, GI symptoms were most likely induced by exercise, environment, and/or naproxen. Performance variables seemed to be influenced more by each other, particularly between RPE and HR. Correlations among genders were slightly different than overall. The only significant correlations among males occurred during Npx and NpxHeat. During Npx,
more GI symptoms resulted in lower RPE in males, presumably limiting their ability or desire to cycle at max effort. In NpxHeat, higher core temperatures were associated with longer mileage and more systemic GI symptoms. In females, longer distance during NpxHeat was related to higher GI symptoms and HR. These two findings suggest the GI symptoms during NpxHeat occurred as a result of working at max rather than GI symptoms inhibiting effort, as seen with the Npx in males.

Limitations and Future Research

As with any subjective measure, we assume participants were honest when answering the GI symptom indexes. Similarly, we did not measure $\dot{V}O_{2\text{max}}$ or power output during exercise, and therefore, must base our assumption that participants gave the same, max effort during each trial on HR and RPE. Frequency and severity for urge to urinate during and post-exercise was likely skewed by forcing participants to drink to maintain hydration, making it difficult to determine actual naproxen effects. This would be mitigated if participants drank the same fluid volume during each trial; however, participants were allowed to consume more fluid than provided. As previously mentioned, using a constant HR rather than $\dot{V}O_{2\text{max}}$ resulted in a lower exercise intensity during heat trials. Our results are applicable for individuals who exercise at a given HR (eg, as a way to maintain pace), but this methodology mitigated finding significance during experimental conditions.

Another potential limitation was using guaiac-based FOB to determine GI bleeding. By measuring heme-, FOB has high specificity for detecting blood from any portion of the GI tract (ie, stomach, small intestine, and colon). However, its sensitivity varies depending on brand. It is possible, due to low sensitivity, the FOB was not able to detect blood in our test samples, resulting in false negatives. An alternative testing option is using fecal
immunological test, which is not affected by diet and is highly sensitive to blood in stool. However, this test is less specific, only being able to detect bleeding from the colon.

There are several areas for future research to examine NSAID effects on performance and GI distress during exercise. Research should examine different dosages (eg, prescription strength) and length of use (eg, 3 days, 7 days, 14 days). It is pertinent to examine hypohydrated individuals, which would increase cardiovascular strain, GI distress, and perceived exertion in both ambient and hot conditions. Using a carbohydrate electrolyte beverage for hydration would introduce potential GI distress. Research is needed on different exercise modes, intensity, and length. Lastly, further research is warranted in both males and females to identify potential gender differences.

Conclusion

Among hydrated, moderate-endurance trained males and females, a 24 hr over the counter dose of naproxen did not improve performance during a 90 min cycling bout in either ambient or hot conditions. Additionally, participants reported upper, lower, and systemic GI symptoms during exercise, but naproxen did not increase occurrence or severity. The trend for higher HR with lower distance, as well as correlations among males and females for GI symptoms and distance in NpxHeat, suggest combining naproxen and thermal stress could be detrimental to performance. Considering prevalent NSAID use among physically active persons and known effects on the cardiovascular and GI systems, further research on health and performance measures is warranted, particularly in hot environments.
REFERENCES


<table>
<thead>
<tr>
<th></th>
<th>Aggregate</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(N = 11)</td>
<td>(N = 6)</td>
<td>(N = 5)</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>27.8 ± 5.7</td>
<td>28.7 ± 5.3</td>
<td>26.8 ± 6.5</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>79.1 ± 17.9</td>
<td>88.4 ± 14.0</td>
<td>67.9 ± 16.5</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>177.0 ± 9.5</td>
<td>183.2 ± 5.3</td>
<td>169.5 ± 7.8</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>15.2 ± 8.3</td>
<td>10.7 ± 4.0</td>
<td>20.6 ± 9.1</td>
</tr>
<tr>
<td>VO₂max (mL/kg)</td>
<td>41.4 ± 5.7</td>
<td>43.6 ± 5.3</td>
<td>38.7 ± 5.3</td>
</tr>
</tbody>
</table>

*aSignificant difference between genders (P < 0.04).*
Table 4.2. Performance Measures during Cycling for Experimental Condition (M ± SD)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Npx</th>
<th>Heat</th>
<th>NpxHeat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distance (miles)</td>
<td>3.2 ± 0.8</td>
<td>3.3 ± 0.8</td>
<td>2.9 ± 0.8</td>
<td>2.8 ± 0.8</td>
</tr>
<tr>
<td>Max HR (bpm)</td>
<td>175.7 ± 14.2</td>
<td>176.2 ± 15.0</td>
<td>177.8 ± 18.2</td>
<td>179.0 ± 18.0</td>
</tr>
<tr>
<td>RPE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>8 ± 2</td>
<td>9 ± 3</td>
<td>9 ± 3</td>
<td>8 ± 2</td>
</tr>
<tr>
<td>80 min</td>
<td>13 ± 2</td>
<td>13 ± 3</td>
<td>12 ± 2</td>
<td>12 ± 2</td>
</tr>
<tr>
<td>Post</td>
<td>19 ± 2</td>
<td>19 ± 2</td>
<td>18 ± 2</td>
<td>19 ± 2</td>
</tr>
</tbody>
</table>

No significant differences between trials.
Table 4.3. Mean and Maximum Gastrointestinal Symptom Scores for Experimental Conditions

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Control</th>
<th>Npx</th>
<th>Heat</th>
<th>NpxHeat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Max</td>
<td>M ± SD</td>
<td>Max</td>
<td>M ± SD</td>
</tr>
<tr>
<td>Upper</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reflux/Heartburn</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Belching</td>
<td>1</td>
<td>0.1 ± 0.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Bloating</td>
<td>5</td>
<td>0.04 ± 1.2</td>
<td>2</td>
<td>0.2 ± 0.4</td>
</tr>
<tr>
<td>Stomach pain</td>
<td>2</td>
<td>0.3 ± 0.6</td>
<td>2</td>
<td>0.2 ± 0.5</td>
</tr>
<tr>
<td>Vomiting</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Nausea</td>
<td>3</td>
<td>0.2 ± 0.5</td>
<td>1</td>
<td>0.0 ± 0.2</td>
</tr>
<tr>
<td>Lower</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intestinal cramps</td>
<td>2</td>
<td>0.3 ± 0.7</td>
<td>1</td>
<td>0.0 ± 0.2</td>
</tr>
<tr>
<td>Flatulence</td>
<td>3</td>
<td>0.3 ± 0.8</td>
<td>2</td>
<td>0.1 ± 0.5</td>
</tr>
<tr>
<td>Urge to defecate</td>
<td>5</td>
<td>0.4 ± 1.0</td>
<td>4</td>
<td>0.2 ± 0.7</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Loose stool/Diarrhea</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Systemic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dizziness</td>
<td>3</td>
<td>0.1 ± 0.5</td>
<td>2</td>
<td>0.1 ± 0.3</td>
</tr>
<tr>
<td>Headache</td>
<td>5</td>
<td>0.6 ± 1.0</td>
<td>2</td>
<td>0.1 ± 0.4</td>
</tr>
<tr>
<td>Muscle cramps</td>
<td>3</td>
<td>0.1 ± 0.4</td>
<td>1</td>
<td>0.0 ± 0.2</td>
</tr>
<tr>
<td>Urge to urinate</td>
<td>8</td>
<td>0.8 ± 1.9</td>
<td>7</td>
<td>0.9 ± 1.7</td>
</tr>
</tbody>
</table>

Based on aggregate scores for all symptoms reported at pre-, post-, 3 hrs post-, and day post-exercise.
Table 4.4. Mean and Maximum Gastrointestinal Symptom Scores for Experimental Conditions during Exercise

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Control</th>
<th>Npx</th>
<th>Heat</th>
<th>NpxHeat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td>M ± SD</td>
<td>Max</td>
<td>M ± SD</td>
</tr>
<tr>
<td>Upper</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reflux/Heartburn</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>0.0 ± 0.2</td>
</tr>
<tr>
<td>Belching</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Bloating</td>
<td>-</td>
<td>-</td>
<td>3</td>
<td>0.1 ± 0.3</td>
</tr>
<tr>
<td>Stomach pain</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>0.1 ± 0.4</td>
</tr>
<tr>
<td>Vomiting</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Nausea</td>
<td>8</td>
<td>0.2 ± 0.9</td>
<td>8</td>
<td>0.1 ± 0.8</td>
</tr>
<tr>
<td>Lower</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intestinal cramps</td>
<td>3</td>
<td>0.3 ± 0.9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Flatulence</td>
<td>1</td>
<td>0.0 ± 0.1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Urge to defecate</td>
<td>-</td>
<td>-</td>
<td>4</td>
<td>0.1 ± 0.4</td>
</tr>
<tr>
<td>Abdominal pain</td>
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<td>Loose stool/Diarrhea</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Systemic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dizziness</td>
<td>4</td>
<td>0.1 ± 0.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Headache</td>
<td>4</td>
<td>0.2 ± 0.9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Muscle cramps</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Urge to urinate</td>
<td>8</td>
<td>0.8 ± 1.9</td>
<td>8</td>
<td>0.9 ± 1.9</td>
</tr>
</tbody>
</table>
CHAPTER 5

PROPOSAL
INTRODUCTION

During intense exercise, especially in hot, humid environments, a number of body systems must work concurrently to cool the core, maintain muscle function, and maintain vital cardiovascular function. The cardiovascular system’s response to exercise and thermal stress depends on the intensity of each, but in general responds by increasing peripheral blood flow and shunting blood away from the gut toward working muscles. Peripheral vasodilation allows the release of metabolic heat produced during exercise, and shunting blood to working muscles allows the individual to maintain the given activity. As exercise and thermal load increase, the system must prioritize whether to maintain blood pressure (BP), skin blood flow, or muscle function. The cardiovascular system can temporarily maintain BP, and overall cardiac output, by increasing heart rate (HR). However, maximum exertion and thermal overload, especially if combined with dehydration, can exceed this ability. Upon cardiovascular compromise, and with the gastrointestinal (GI) tract in a state of ischemia from decreased blood flow, core temperature (Tc) will increase and systemic inflammatory response (SIR) occurs.

Prevalence of previous illness and GI distress during exercise and thermal stress suggests the GI tract and immune system impact the development of exertional heat illness (EHI), especially exertional heat stroke (EHS). Symptoms of EHS (vomiting, dehydration, multi-system organ failure, etc.) are similar to SIR and may be driven by GI distress. Gastrointestinal barrier dysfunction leads to the entry of bacterial lipopolysaccharide (LPS) into the circulation (known as endotoxemia), release of inflammatory cytokines, and a number of other factors that compromise the immune system. Consequently, SIR and thermal stress intolerance occurs, which dramatically increases the risk for EHS and death.
STATEMENT OF THE PROBLEM

Extensive scientific literature exits examining potential risk factors,\(^6,7\) ways to prevent,\(^8,9\) and ways to treat EHS.\(^10,11\) Unfortunately, preventable deaths from EHS still occur.\(^12\) One potential contributing factor to EHS development may be non-steroidal anti-inflammatory drugs (NSAIDs). Research has shown NSAIDs increase GI permeability during exercise in thermoneutral environments,\(^13-15\) cause GI distress\(^16,17\) and induce inflammatory responses.\(^18\) Despite knowing GI distress and inflammation are associated with EHS and NSAIDs, limited research has evaluated NSAIDs’ effects on Tc during exercise under thermal stress.

SPECIFIC AIMS AND HYPOTHESIS

**Overall Hypothesis.** Naproxen (NSAID) will increase GI permeability and inflammation during exercise in the heat, and will be associated with increased GI distress and thermoregulatory strain (Figure 5.1). This overall hypothesis is tested under three specific aims.

**Specific Aim 1.** Determine the effects of naproxen on the GI tract and inflammation during exercise in the heat.

**Hypothesis 1.1.** GI tract permeability is greater in participants taking naproxen compared to placebo controls during exercise in the heat.

**Hypothesis 1.2.** GI distress, indicated by GI symptoms (eg, nausea, cramping) and occult fecal blood, is greater in participants taking naproxen compared to placebo controls during exercise in the heat.
Hypothesis 1.3. Plasma concentration of inflammatory cytokines (TNF-α and IL-6) is increased in participants taking naproxen compared to placebo controls during exercise in the heat.

Specific Aim 2. Determine effects of naproxen on thermoregulation and performance during exercise in heat.

Hypothesis 2.1. Participants taking naproxen will experience a faster rise in Tc during exercise in the heat compared to placebo controls.

Hypothesis 2.2. Cardiovascular strain (HR, BP, fluid imbalance) is greater in participants taking naproxen compared to placebo controls during exercise in the heat.

Hypothesis 3.2. Distance covered during exercise in the heat is lower in participants taking naproxen compared to placebo controls.

Specific Aim 3. Determine the relationship between naproxen and the GI tract and inflammation on thermoregulation during exercise in the heat.

Hypothesis 3.1. Participants taking naproxen will have an increased Tc due to increased GI permeability and inflammatory cytokines.

LITERATURE REVIEW

GASTROINTESTINAL TRACT

Anatomy & Physiology of Gastrointestinal Tract

The GI tract is a long, continuous, muscular tube made up of the mouth, pharynx, esophagus, stomach, small intestine, and large intestine. Three parts make up the small intestine: duodenum, jejunum, and ileum. The large intestine is made up of 8 parts: transverse colon, descending colon, ascending colon, cecum, sigmoid colon, rectum,
appendix, and anal canal. The GI tract is lined by an epithelial barrier made up of 4 layers, from deep to superficial: mucosa, submucosa, muscularis externa, and serosa.\(^\text{19}\)

**Stomach**

The stomach is a storage area and site of chemical breakdown of food products. When the stomach is empty, mucosa and submucosa layers fold inward to form rugae,\(^\text{19}\) allowing the stomach to expand as food is ingested. The stomach is divided into four major regions - cardia, fundus, body, and pylorus. Closest to the heart, the cardia region contains the gastroesophageal sphincter, which controls food entering the stomach or prevents stomach contents from entering the esophagus.\(^\text{19}\) The fundus is the dome shaped region, extending slightly superior and lateral to the cardia, resting against the diaphragm. The largest region, the body, curves into the pylorus. At the distal portion of the pylorus is the pyloric sphincter, which controls stomach contents entering the small intestine.\(^\text{19}\) Although made up of the same epithelial and muscular layers, the 4 stomach regions vary slightly in their function during digestion.\(^\text{20}\) As food moves in to the cardia region, there is only rippling of tissue layers. However, the muscularis layer of the body and pylorus regions are much thicker and therefore more powerful. Subsequently, food products in these regions are aggressively mixed with the gastric juice to produce chyme.\(^\text{19,20}\) Converting solid food products to chyme, a creamy paste,\(^\text{19}\) allows for absorption of nutrients and elimination of byproducts through the GI tract.

Gastric emptying, the time from food entering stomach to entering small intestine, typically takes 4 hours.\(^\text{19}\) However, this time is heavily influenced by type of food, amount of fluid, and individual health factors. Gastric emptying is faster with greater amounts of
fluid or carbohydrates. On the other hand, gastric emptying time slows down with more solid or fatty meals.$^{19}$

The stomach contains a number of defense features against bacteria and other pathogens. A large collection of lymph nodes are responsible for fighting pathogens within the peritoneal cavity and intraperitoneal organs.$^{19}$ Gastric acid is the stomach’s key defense. With a pH around 2, gastric acid is designed to control the amount of bacteria present in the stomach and digest proteins.$^{19}$

**Small Intestine**

Three unique structure modifications of small intestine epithelium allows for increased absorption. Together, the mucosa and submucosa layers are permanently folded into circular folds, or plicae circulares. These folds slow the movement of digestive product to allow for full absorption. On the outside of the mucosa cells are villi, fingerlike projections made of epithelial cells. Villi absorb nutrients into the blood stream through their dense capillary bed. Microvilli are located on the plasma membrane of mucosa cells and contain enzymes that complete digestion of carbohydrates and proteins.$^{19}$

Like the stomach, the small intestine contains a number of protective factors. Between villi are crypts of Lieberkühn. Within crypts are Paneth cells that release lysozyme, an antibacterial enzyme that protects the small intestine from bacteria.$^{19}$ Another protection against bacteria comes from Peyer’s patches located in submucosa and immunocytes.$^{19,21}$ The small intestine contains intestinal juice. However, unlike gastric juice, intestinal juice has a relatively neutral pH, is primarily made up of water and mucus, and does not contain many digestive enzymes. The primary function of intestinal juice is to protect the epithelial lining from pathogens.$^{19}$
**Large Intestine**

Notable differences occur in the large intestine layers. Because most nutrient absorption takes place in the small intestine, the simple columnar epithelium contains no folds, villi, or digestive enzyme secretions. Large intestine characteristics include a thick mucosa layer, large number of goblet cells, and a large number of deep crypts. Mucosa is especially important to protect the wall against passing feces and acids/gases from bacteria.\(^{19}\)

Most bacteria that enter the large intestine have been killed by highly acidic gastric juice or small intestine lysozymes. However, a number of bacteria enter the large intestine and colonize; this is known as the bacterial flora. Bacteria are naturally occurring, playing several imperative roles in maintaining human health. For instance, the bacteria flora assists in mucosal homeostasis, repair, and glucose transporter induction; induced capillary formation; innate immunity induction;\(^{22}\) and is useful in synthesizing some important vitamins (B complex and K). Large intestine bacteria are responsible for fermenting indigestible carbohydrates. A major consequence to this fermentation is the release of acids and gases such a carbon dioxide, hydrogen, nitrogen, and methane. Some of these gases are odorless, but dimethyl sulfide is extremely odorous. On average, the large intestine produces about 500ml of gas a day and this number increases with high carbohydrate rich foods (eg, beans).\(^{19}\)

**Blood Flow**

Gastrointestinal blood supply comes from hepatic, splenic, and left gastric branches of the celiac trunk. Under normal resting conditions, the GI tract receives approximately 20-25% of cardiac output, increasing with meal consumption and decreasing with intense
exercise. In addition to food and exercise, other factors such as age, temperature (environment or body), training status, and disease affect GI tract blood flow. In general, blood flow is regulated by the autonomic nervous system’s parasympathetic and sympathetic divisions.

**Parasympathetic Nervous Division**

Parasympathetic nerve fibers originate from the cranial and sacral spine. The vagus nerve accounts for approximately 80-90% of cranial parasympathetic fibers, innervating the heart, lungs, and majority of visceral organs, including the GI tract (liver, gallbladder, stomach, pancreas, small intestine, and proximal large intestine). At rest and during digestion, the parasympathetic nervous division slows HR and respiration and dilates blood vessels to the GI tract to allow relaxation and stimulate food digestion. At onset of exercise, and during low to moderate intensity exercise, parasympathetic nerves are inhibited to allow HR to increase. When an individual approaches maximum intensity exercise, parasympathetic nerves are further inhibited and sympathetic nerves are directly stimulated.

**Sympathetic Nervous Division**

Much more complex, the sympathetic nervous division is commonly referred to as the “fight or flight” response. Sympathetic fibers originate in the thoracic and lumbar spine and innervate the heart, lungs, all visceral organs, all arteries and veins, and skin. The sympathetic division stimulates release of norepinephrine and epinephrine to increase HR, BP, and respiration, and vasoconstrict cutaneous and visceral blood vessels to shunt blood to working muscles and limit nonessential functions (eg, digestion). During extreme
intense exercise, visceral blood flow may decrease up to 80%, placing these organs at risk for ischemic injury.

Other Regulators of Blood Flow

The greatest control of blood flow during exercise comes from central command, where the cerebral cortex controls anticipatory responses to exercise as well as responses during activity through the medulla and sympathetic-parasympathetic nerves. Baroreceptor and mechanoreceptors, located in blood vessels, respond to changes in mechanical pressure. Muscle chemoreceptors respond to changes in oxygen, carbon dioxide, hydrogen levels, and other metabolites from muscle activity. Other responses include vasoconstriction of blood vessels in inactive muscles and GI blood vessels and vasodilation in active muscles. Central command responses are faster than sympathetic responses (sympathetic stimulation typically does not take place until moderate to intense exercise) and are involved with emotional responses to exercise or stress.

Other hormonal regulators of blood flow include angiotensin II, aldosterone, vasopressin, and atrial natriuretic peptide. Often working collectively, these hormones exert their effects by either acting on the heart, blood vessels, or kidneys. The renin-angiotensin-aldosterone mechanism occurs in response to sympathetic nerve activity. During hypovolemia or hypotension, renin, an enzyme released by juxtaglomerular cells, promotes production of angiotensin I. Angiotensin-converting enzyme converts angiotensin I to angiotensin II, causing vasoconstriction and subsequent increase in BP. Also stimulated is aldosterone secretion from the adrenal cortex. Aldosterone stimulates reabsorption of sodium in the kidneys, subsequently pulling water with it and increasing both blood volume and pressure. Vasopressin is a hormone released by the posterior
pituitary in response to hypovolemia, hypotension, or increased plasma osmolality (ie, high plasma sodium concentration). In order to reestablish vascular homeostasis, vasopressin acts on the kidneys to promote water reabsorption. Thus, it is alternatively known as antidiuretic hormone.\textsuperscript{1,25} Lastly, atrial natriuretic hormone responds to atrial stretching, often from increased BP. Acting as a protective response and “fine tuning” BP, atrial natriuretic hormone inhibits vasopressin, aldosterone, renin, and sodium reabsorption in the kidneys.\textsuperscript{1,25}

\textbf{Gastrointestinal Barrier}

Overall purpose of the GI barrier is to prevent foreign substances such as food product, acids, and toxins from passing from the intestine into the blood stream.\textsuperscript{26} Barrier maintenance is imperative, and its function is tightly controlled. The GI barrier is comprised of 3 factors: physical, physiological, and immunological. Physical factors include the 4 epithelial tissue membranes layers. Membrane tissue integrity is primarily maintained by the apical junctional complex.\textsuperscript{19,27} Together, physiological factors (ie, mucus secretions and bicarbonate) and immunological factors (ie, macrophages) protect the tissues and entire GI tract from toxins and acid.\textsuperscript{19,28}

\textit{Tissue Membranes}

Mucosa is the innermost layer of moist epithelial membrane that lines the lumen. Major mucosa functions include secretion of mucus, digestive enzymes, and hormones (making the mucosa an endocrine organ); absorption of digested end-products into the blood stream; and protection against infectious disease.\textsuperscript{19} Stomach mucosa is exposed to extremely harsh gastric juice.\textsuperscript{19} The acidity of gastric acid is imperative in defense against excessive bacteria formation.\textsuperscript{18} However, exposure to gastric acid can cause severe
epithelial or organ tissue damage. Therefore, mucosa cells, which are rich in bicarbonate, are imperative to protect and neutralize pH at the epithelial cell surface. Any damaged cells are quickly shed and replaced by undifferential stem cells residing in gastric pits. The entire epithelium surface is completely replaced every 3-6 days.

The mucosa has 3 sublayers: lining epithelium, lamina propria, and muscularis mucosae. Lining epithelium is heavy in mucus secreting cells and bicarbonate alkaline secretions that create the protective GI barrier and allow food to pass through easier. Lamina propria, made up of loose areolar tissue, allows capillaries to reach the epithelial layer and lymph nodes to fight against bacteria and pathogens. Muscularis mucosae is a layer of smooth muscle cells that allow local movement of the mucosa.

The second tissue membrane layer, the submucosa, is made up of elastic connective tissue that allows the stomach to expand and regain its normal shape after a large meal. This layer contains blood vessels, lymphatic vessels, lymph nodes, and nerve fibers. Muscularis externa, the third tissue layer, is responsible for segmentation and peristalsis. The smooth muscle contractions and relaxations propel contents through the tract and is collectively known as GI motility. The circular layer creates sphincters throughout the tract to prevent backflow and control food passing from one section to the next. The fourth, outermost layer is the serosa, which is made up of areolar connective tissue covered by a single layer of squamous epithelial cells.

**Apical Junctional Complex**

In healthy GI barriers, epithelial cell integrity is maintained by the apical junctional complex, made up of tight junction, adherens junction, and desmosome. Tight junctions are multi-protein complexes formed by distinct contacts between two adjacent plasma
membranes. Tight junctions contain transmembrane proteins, called claudins, and peripheral proteins, called zonula occludens. Tight junctions are supported by adherens junctions and desmosomes. Adherens junctions provide strong support to membranes to allow cell-cell communication. Both tight and adherens junctions are connected to a perijunctional actomyosin ring, which controls junction position. When actomyosin is relaxed tension is reduced on the junctions, decreasing tight junction permeability. On the other hand, when actomyosin is contracted, junctions are moved, and tight junction permeability increases. Movement of tight junctions is driven by myosin light chain kinase, which phosphorylates myosin light chain in the perijunctional actomyosin ring.

Tight junctions vary along the entire GI tract, differing in their tightening ability depending on the segment function. For example, duodenum tight junctions have greater ability to tighten than those in the ilieum. This is important because the duodenum is exposed to more acid chyme leaving the stomach than the ilieum. Compared to the small intestine, the colon has greater ability to tighten. This is likely because most nutrient absorption has already occurred and cells need to be protected from the large bacterial colony.

Gastrointestinal Distress

Gastrointestinal distress can present as any number of symptoms, including abdominal pain, cramping, bleeding, dyspepsia, constipation, diarrhea, nausea, emesis, reflux, or urge to urinate or defecate. Unfortunately, etiology of these symptoms is often complex and sometimes not well understood due to a number of confounding factors within and between subject populations. Gastrointestinal distress can occur from systemic conditions or from GI damage/irritation. Systemic driven GI distress may originate from
triggers such as stress, emotions, fever, fatigue, or other diseases. Direct damage or irritation can be caused by GI disease, trauma, or even diet.

Etiology of GI Distress Symptoms

Abdominal Pain

Abdominal pain may originate from referred, mechanical, inflammatory, or ischemia-reperfusion sources. Conditions such as muscular injuries, muscle guarding, or spinal pathologies may cause referred abdominal pain. Mechanical abdominal pain may occur from stretching of an organ wall, jarring or vibrations, increased abdominal pressure, body position, or contraction of abdominal wall and diaphragm. Inflammatory pain originates from release of inflammatory mediators.

One of the primary causes of abdominal pain is GI ischemia. Appropriate blood flow is not only important in providing oxygen and nutrients to tissues, but in buffering acid exposure and healing damaged tissue. Ischemic pain occurs when blood flow to an area is decreased to the point where tissues are not supplied with appropriate oxygen and nutrients. Vascular endothelial cells are particularly vulnerable to ischemia, presenting with altered membrane potential, decreased fluidity, impaired cytoskeleton, decreased energy stores, or releasing inflammatory mediators. Reperfusion following ischemia has also been identified as a source of pain and inflammation, causing swelling, disruption of the basal membrane, leukocyte adherence, and production of reactive oxygen species (ROS).

Gastritis and Peptic Ulcers

General stomach wall inflammation is referred to as gastritis. Gastritis includes a number of conditions that typically only affect the mucosal layer and is classified as acute.
or chronic. Acute gastritis includes mucosal lining erosion. Causes are sometimes unclear but often associated with medication, alcohol, or stress. Chronic gastritis is less common and includes causes such as diabetes, autoimmune diseases, or infection by *helicobacter pylori* bacteria.\(^{19,21,34}\) Signs and symptoms of gastritis vary, some individuals are asymptomatic, but most common symptoms include abdominal pain or discomfort, nausea, vomiting, heartburn, or occult blood loss.\(^{34}\)

An ulcer occurs when erosion reaches the submucosa layer. Duodenal ulcers are more common than gastric ulcers, and, like gastritis, are most commonly caused by bacteria. Gastric ulcers are most commonly caused by NSAIDs. Other causes include alcohol, coffee, smoking, trauma (eg, burns), and increased age.\(^{19,34}\) Psychological stress is also a contributing factor, especially in individuals with recurrent ulcers.\(^{34}\) Both gastric and duodenal ulcers present similarly, with severe pain, burning, cramping or aching, distention, and nausea. Pain typically fluctuates with gastric secretions and food intake. In duodenal ulcers, pain typically occurs 1-3 hours after eating. On the other hand, eating may either relieve or exacerbate gastric ulcers.\(^{34}\) This is because presence of food allows gastric acid to target something other than the stomach lining; however, food could be the irritant causing damage. If left untreated, ulcers could lead to perforation of the stomach or intestinal wall, peritonitis, and/or severe hemorrhaging.\(^{34}\)

*Nausea and Emesis*

Nausea is stimulated from the autonomic nervous system when stomach nerve endings are irritated. Causes of nausea can range from strong emotions to toxins such as drugs or alcohol that irritate the GI tract.\(^{34}\) Emesis can occur from a number of triggers, including bacterial irritation, alcohol, and certain drugs. The medulla receives an impulse
from irritated sites that triggers abdominal wall and diaphragm contraction. Intra-abdominal pressure increases, then the cardiac sphincter closes, soft palate rises to close off the nasal passage, and contents from the stomach, and potentially duodenum, are forced upward.\textsuperscript{19}

\textit{Dyspepsia and Gastrointestinal Reflux}

Dyspepsia is general abdominal discomfort, sometimes referred to as indigestion. Dyspepsia is often caused by gastroesophageal reflux. When stomach contents move backward into the esophagus the acidity causes a burning sensation, usually located behind the sternum (heartburn). Some causes of gastroesophageal reflux include alterations in esophageal peristaltic activity; increased gastroesophageal sphincter relaxation; consumption of alcohol, spicy or highly seasoned foods; medications; stress or nervousness; and large movement (eg, bending and lifting) after a large meal.\textsuperscript{34,43,44}

\textit{Diarrhea and Constipation}

Diarrhea and constipation occur when food product passes too quickly or too slowly through the large intestine.\textsuperscript{19} If stool passes quickly water is not absorbed and stool becomes “loose”. When stool sits too long in the large intestine too much water is absorbed and stool becomes hard. Specific causes of diarrhea and constipation vary, but include disease, diet, medications, and strenuous exercise. Constipation can also cause mechanical abdominal pain.\textsuperscript{34}

\textit{GI Bleeding}

Depending on the cause, GI bleeding can be identified by either brown colored emesis, hematemesis, melena (black, tarry stool), or hematochezia (maroon colored stool).\textsuperscript{34} Severe GI bleeding during exercise is rare, but may be associated with peptic
ulcers, trauma, systemic illness, or chronic alcohol or NSAID use.\textsuperscript{34} Most often GI bleeding presents as occult blood in feces and occurs from stomach damage.\textsuperscript{35} Symptoms such as nausea, vomiting, diarrhea, and abdominal pain may be secondary to GI bleeding.\textsuperscript{34}

\textbf{Measurement Techniques}

\textit{Gastrointestinal Distress Questionnaires}

Qualitative surveys are inherently limited due to subjectivity, but are clinically useful tools to determine how someone feels. Gastrointestinal symptoms are commonly assessed prior to and after physical activity in order to determine change in GI symptoms that may be attributed to exercise or experimental conditions.\textsuperscript{36,45,46} Administering questionnaires during physical activity may be done,\textsuperscript{46-49} although this is more practical during laboratory studies.

There are number of other challenges to existing GI symptom research. A common limitation is survey variability across studies. Some questionnaires provide specific questions to distinguish upper and lower abdominal symptoms, number of occurrences and severity,\textsuperscript{36,43} but others are less descriptive. Not identifying GI symptoms at rest (baseline) and not controlling for diet, medications, or supplement use is another common limitation. Lastly, timing of survey administration also varies, with post-activity time ranging from immediately\textsuperscript{45} to 2 hrs post.\textsuperscript{36}

\textit{Endoscopy}

Endoscopy is the gold standard to objectively quantify GI damage.\textsuperscript{50} Sometimes combined with biopsies, endoscopy allows medical providers or researchers to examine the GI lining, identify specific damage, and measure severity. Endoscopy is frequently used, but invasive and requires intensive equipment.
Occult Fecal Blood Measures

Another objective measure of GI damage is occult fecal blood. There are two major types of fecal tests – guaiac-based fecal occult blood test (g-FOBTs) and fecal immunological test (FIT). Each has unique advantages and disadvantages. Regardless of test type, kits can be self-administered and are recommended for early detection of serious GI diseases.51

The g-FOBT uses guaiac to test for heme in stool. Differences exist among test brands, some with lower sensitivity for occult blood than others.50 Advantages to g-FOBT are its specificity and ability to detect blood loss from any portion of the GI tract.51 A major disadvantage of g-FOBT is interference with certain plants and red meat. Red meat contains heme, which may alter tests results. Individuals using g-FOBT are instructed to not eat certain foods for at least 72 hrs prior to conducting the test.52

The FIT was introduced as a more selective alternative to g-FOBT. Designed to use anti-bodies to selectively detect globin, FIT identifies colon blood loss. By detecting globin rather than heme, there is no dietary interference or potential drug interactions. The major disadvantage to FIT is inability to identify small intestine or stomach bleeding.51

Since fecal occult tests are commonly used for early detection of cancer, specificity and sensitivity compared to colonoscopy is of particularly high interest. The FIT has been shown to have significantly higher sensitivity compared to g-FOBT in detecting cancer,50 with 65-90% sensitivity with one-time testing.51 On the other hand, g-FOBT has significantly higher specificity.50 Considering advantages and research outcomes, FIT is the recommended method for detecting occult fecal blood.51
**Gastrointestinal Permeability**

The apical junction complex, specifically tight junctions, closely regulates GI permeability. There are 2 primary permeability pathways: paracellular and transcellular. Paracellular permeability allows passive diffusion of large molecules, like proteins and bacteria, between adjacent cell membranes. Transcellular permeability allows active or passive diffusion of small molecules, such as ions, through specific cell membrane channels.\(^{27,53}\)

The GI tract has a usual amount of permeability for small molecules (< 150 Da) through non-mediated diffusion.\(^{53}\) Gastrointestinal permeability is considered leaky because it naturally allows a certain amount of fluid and ion exchange across the surface.\(^{27}\) On average, 8-9 L of fluid pass through the GI tract daily. Of that, only 100-200 ml is not absorbed and excreted through urine and feces. In the event of increased permeability, decreased fluid reabsorption can present clinically as diarrhea.\(^{54}\) A number of other molecules can improperly pass through the GI barrier. The immune system can typically neutralize escaped molecules unless there is a state of severe stress or disease. For example, if LPS molecules in an immunocompromised individual are allowed to pass from the GI tract to the bloodstream, local and systemic inflammation can ensue and further increase permeability. If uncontrolled, serious conditions like endotoxemia, septic shock, EHS, or death can occur.\(^{26,29}\)

**Tight Junctions**

Tight junctions are the rate-limiting step in regulating paracellular permeability.\(^{29}\) Tight junctions are dynamic, continuously undergoing remodeling and restructuring as they interact with outside stimuli. Inflammatory mediators, hormones, and pathogens
increase permeability at tight junctions through two mechanisms: 1) actin-myosin ring dysfunction through cytoskeleton disruption, and 2) direct damage to tight junction proteins. Specific mechanisms include stimulated myosin light chain kinase transcription, endocytosis or transmembrane protein cleavage, altered protein kinases and second messengers, and increased claudin expression.

Research on chronic inflammatory bowel diseases and cell cultures have associated tight junction dysfunction to elevated inflammatory cytokines and inflammatory enzymes. Pro-inflammatory cytokines alter intestinal permeability by increasing tight junction protein transcription, protein degradation, and modulating kinases and cytoskeleton. Others have been shown increased permeability by stimulating enzymes that cleave phospholipid membranes and epithelial-neutrophil interactions.

**Endotoxin**

As previously mentioned, there is an extensive bacteria flora throughout the GI tract. The outer cell membrane wall of gram-negative bacteria is predominately made up of LPS. Unlike gram-positive bacteria, the complex membrane is designed to protect against outside substances, making them more resistant to immune responses and antibiotic medications. Three components make up LPS, an O-polysaccharide chain (O-antigen), a core oligosaccharide sequence, and a lipid A region. The lipid A portion contains hydrophobic fatty acid chains and is toxic in humans. Therefore, LPS is often referred to as endotoxin and entry into the circulation as endotoxemia.

Under healthy conditions the GI barrier prevents LPS from entering the circulatory system. A certain amount of LPS in blood is normal (< 0.10 ng/ml), coming from dead bacteria and LPS shedding. The liver and immune system work together to clear LPS.
As blood filters through the liver Kupffer cells and hepatocytes neutralize LPS through endocytosis. A major immunological response comes from neutrophils releasing bactericidal/permeability-increasing proteins. They have a high affinity for endotoxin and neutralize activity by decreasing membrane integrity, altering electrochemical gradients, inducing apoptosis, and inhibiting cytokine release.

**Measurement of GI Permeability**

Compared to animal models, there are few techniques for measuring GI permeability in humans. Measures of plasma LPS provide a general indicator of GI permeability, but have inherent limitations during analysis and cannot provide specific information regarding extent and location of GI permeability. Probes such as sugars/carbohydrates, chromium-labelled ethylenediaminetetra-acetate (Cr-EDTA), and polyethylene glycol (PEG) are commonly used to determine altered permeability location.

**Sugar/Carbohydrate Probes**

A common permeability technique utilizes urine measures of non-digestible, non-metabolized probes in an orally ingested solution. Solutions typically include 3 sugars: sucrose, lactulose, and rhamnose; however, others such as mannitol, sucralose, and xylose have been used. Sucrose (molecular weight = 342 Da) is not digestible in the stomach, and under normal healthy conditions will pass into the small and large intestine where it can be absorbed. When stomach permeability increases it allows sucrose to leak out. Therefore, presence of sucrose in the urine indicates increased stomach permeability. Similarly, lactulose is not digestible in the small intestine. Due to its large molecular weight (342 Da) lactulose only passes through paracellular routes (ie, tight junctions) and its
presence in urine indicates increased small intestine permeability.\textsuperscript{28} To control for non-barrier (GI permeability) related urinary excretion, such as GI transit time, fluid distribution, and renal clearance, rhamnose (molecular weight = 164 Da) is taken with lactulose.\textsuperscript{26,28} Together, rhamnose and lactulose should be affected similarly by any non-barrier related factors. The difference between rhamnose and lactulose is attributed to absorption route, where rhamnose detects “small pore” (para- and/or transcellular) permeability and lactulose detects “large pore” (transcellular) permeability.\textsuperscript{26,28,68} Intestinal permeability is presented as a percentage, or ratio, of lactulose to rhamnose in urine.\textsuperscript{26,28}

Drawbacks vary depending on type of sugar used. Lactulose and other sugars are present in some foods, which may be excreted in urine. Sugar probes are susceptible to metabolism and degradation from bacteria, which would underestimate permeability.\textsuperscript{68} However, research has shown no significant difference in intravenous delivered lactulose recovery (92.7 ± 1.2\%) compared to Cr-EDTA (97.4 ± 0.5\%), which cannot be metabolized and is not degraded by bacteria.\textsuperscript{68} This suggests lactulose is an appropriate measure for GI permeability. Overall risk of consuming the solution is low, but some reported side effects have included minor GI disturbances.\textsuperscript{53} Low-risk in combination with efficiency makes sugar probes the standard and preferred GI permeability technique.

\textit{Chromium-labelled Ethylenediaminetetra-acetate (Cr-EDTA)}

A less commonly utilized technique, Cr-EDTA uses a γ-ray emitting isotope in an orally ingested solution. Percentage excreted in urine indicates more small intestine permeability. Advantages to Cr-EDTA is resistance to bacterial degradation.\textsuperscript{68} The major negative effect of Cr-EDTA is the small exposure to radiation, albeit minimal compared to an abdominal x-ray (0.12 mSv versus 1.4 mSv).\textsuperscript{69}
**Polyethylene Glycol (PEG)**

A mixture of polymers, PEG comes in a variety of molecular weights (400-4,000Da)\textsuperscript{28} that are orally ingested and measured for urinary concentration to determine permeability. Specificity of PEG is high due to using polymers of varying masses to mimic the mass of small molecules (eg, rhamnose), medium molecules (eg, lactuose and Cr-EDTA), and large molecules (eg, LPS). Despite high specificity, use of PEG has declined due to variability and inconsistency in GI measures. Using intravenous injected probes and comparing to Cr-EDTA, percent recovery of PEG-400 polymers was only 40.7 ± 1.8\%.\textsuperscript{68} Losses are attributed to PEG’s lipid solubility, allowing it to more easily pass through tissues and, in the case of intravenously injected probes, pass into the GI tract.\textsuperscript{70,71} Large losses of PEG polymers lead to underestimated permeability, making it less sensitive in detecting small permeability changes.\textsuperscript{68}

**Plasma Lipopolysaccharide**

Plasma LPS concentration provides a general idea of GI barrier dysfunction and is frequently used in human research to measure endotoxemia.\textsuperscript{61,72-74} *Limulus* amebocyte lysate assays are the preferred technique. They are highly sensitive and measure biological endotoxin activity.\textsuperscript{75} Unfortunately, there are several disadvantages when using this measure. First, plasma LPS levels do not identify the specific area of GI barrier dysfunction (ie, gastroduodenal, small intestine, or colon). Second, because LPS is located in the external environment, there is a risk for assay contamination during collection, handling, and storage. Third, and not well understood, LPS can be bound or cleared by a number of biological components (ie, platelets, bile salts, high-density lipoproteins, and LPS-binding
protein). Inactivation is maximum when temperatures range 37-45°C, and could lower LPS levels below the assay’s detection ability.75

INFLAMMATORY RESPONSES

Whether caused by trauma or a pathogen the body will launch an immune response to neutralize the problem. Resultant signs and symptoms such as fever, pain, lethargy, swelling, and redness are often considered negative. In actuality, inflammatory responses are vital to prevent and counter threats. Unfortunately, inflammation can be negative if not controlled (eg, autoimmune diseases and secondary hypoxic injury). Therefore, many components of the inflammatory response have both pro- and anti-inflammatory roles depending on stimuli and these responses are closely regulated in attempt to maintain homeostasis.

The two types of immunity are acquired and innate. Acquired immunity includes specific responses that have adapted due to previous exposure. These responses include humoral and cell-mediated events (antibody secretion and proliferation of antigen-specific T and B cells). Innate immunity includes all immune response that have no immunologic memory to previous pathogens.34 Innate responses include phagocytes (neutrophils, monocytes, macrophages, mast cells, and tissue dendrite cells) and basophils, eosinophils, and lymphocytes.19,34 Often working congruently with innate and required responses, other components vital during inflammation include cytokines, heat shock proteins (HSPs), ROS, and prostanoids.

Inflammatory Components

Lymphocytes
Lymphocytes play a major role in inflammatory mediation and work in close conjunction with antigen-presenting cells (APCs), such as phagocytes. After a phagocyte digests a pathogen, antigenic material is presented on the surface so it can be recognized more easily as a pathogen. Phagocytes then present the cell to lymphocytes, specifically T4 lymphocytes (cells). T cells provide cell-mediated immunity and make up between 65-85% of bloodborne lymphocytes. There are multiple subdivisions of T cells, two that are responsible for destroying pathogenic cells are T4 cells (helper T cells) and T8 cells (cytotoxic T cells).

Once a T cell is presented and/or bound to an antigen it must be stimulated to continue with cell destruction or abort. This is referred to as costimulation. While there are numerous types of signals, the major signal for continued destruction comes from cytokines. Making up 75% of all T cells, T4 cells are vital to the immune response even though they do not directly destroy a pathogenic cell. They “help” by providing costimulation signals for destruction, activating macrophages, assisting natural killer cells (NKCs), and stimulating proliferation of other T and/or B cells. Proliferation temporarily increases activated T and B cells to respond to the current pathogen attack. It also contributes to acquired immunity by forming memory T and B cells that remain in the body until the same antigen is presented again. T4 cells are further classified as either Th1 or Th2, where Th1 cells mediate aggressive cytotoxic, phagocytic inflammatory responses and Th2 cells mediate less-tissue destructive, cell mediated responses.

Cytotoxic T cells directly attack and destroy a cell. These cells bind to a presented antigen and, depending on the type of T8 cell, release a cytotoxic chemical into the target cell’s plasma membrane. The T8 cell then detaches from the target cell and continues
throughout the body looking for other pathogenic cells. Cytotoxic chemicals typically accompany perforin, a cytolytic protein that forms transmembrane pores in target cells.\textsuperscript{19} Once a pore is formed, chemicals like granzymes, a family of serine proteases, or TNFs enter and trigger apoptosis by cell lysis and/or DNA fragmentation.\textsuperscript{77,19}

Regulatory T (Treg) cells are a subset of T cells that are less understood. Known to be immunosuppressive, these cells are designed to slow down or stop immune responses by releasing anti-inflammatory cytokines,\textsuperscript{19} inhibiting T cell activation and cytokine release, inhibiting APCs,\textsuperscript{78} and upregulating Treg cell production.\textsuperscript{79} Research has shown Treg cells also contain cytotoxic properties, utilizing perforin\textsuperscript{80} and granzymes to control T cells, NKCs, and other cytotoxic cells.\textsuperscript{79} While Treg cells are considered important in preventing autoimmune reactions,\textsuperscript{19} excessive Treg production is implicated in tumor and disease progression.\textsuperscript{81}

Another type of lymphocyte, NKCs are important in initial immune response and are less specific than adaptive immune lymphocytes. As a result, NKCs are able to target many different viruses, bacteria, and tumor cells with or without an APC.\textsuperscript{19,34} Their primary function is to destroy foreign or damaged cells; this is predominately done by targeting cell membranes and releasing perforin to create cell lysis.\textsuperscript{82} In addition to direct cell destruction, NKCs promote cell death by influencing T and B cell response and producing a variety of cytokines and growth factors.\textsuperscript{83}

\textit{Cytokines}

Cytokines are chemical mediators, connecting the immune system to other body systems. Cytokines regulate responses like cell proliferation, apoptosis, chemotaxis,\textsuperscript{19,34,59} fever, leukocytosis, acute protein synthesis, muscle catabolism, and the hypothalamic-
There are several types of cytokines; interleukins (ILs) and TNFs are two families.

**Interleukin (IL)**

There are more than 30 different ILs found in humans, identified numerically and often with additional sub-classifications. Originally identified as chemical mediators produced by leukocytes ("-leukin"), in actuality T4 cells, NKCs, macrophages, endothelial cells, monocytes, and other cell types express ILs. Interleukins act as either pro- or anti-inflammatory mediators by modulating growth, differentiation, and activation.85,86

The pro-inflammatory cytokine IL-1 is primarily released by macrophages and stimulated by other cytokines (IL-4 and IL-6) and endotoxin.86 One major function of IL-1 is to co-stimulate T cells to release IL-2, a growth factor that activates NKCs and enhances proliferation of B cells, T cells, and macrophages.19,86 Other IL-1 functions are fever induction by increasing the hypothalamus’ set-point temperature,19,34 stimulating PG production,34 hypotension during septic shock, and inducing sleep and lethargy.86

A prominent pro-inflammatory mediator during early immune responses,85 IL-6 is primarily induced by monocytes and other cytokines (IL-1 and TNFs).87 IL-6 is multifunctional, enhancing T cell proliferation and activity, B cell differentiation and antibody production, macrophage differentiation, and positive acute-phase protein synthesis.86,87 IL-6 also acts as a thrombopoietin, promoting platelet production,87 and, along with IL-1, is responsible for fever and IL-2 expression.86

An anti-inflammatory mediator, IL-10 is primarily expressed by monocytes, T cells, and B cells.88 IL-10 is effective at inhibiting APCs89 and T4 cell, phagocyte, and NKC mediated expression of IL-1β, IL-6, and TNF-α.85,86 The IL-10 family includes IL-
IL-22 is suggested to protect GI barrier integrity by promoting cell proliferation and increasing expression of anti-microbial molecules and mucins.\textsuperscript{40}

\textit{Tumor Necrosis Factor (TNF)}

The most prominent TNF member is the pro-inflammatory cytokine TNF-\(\alpha\), also referred to as cachectin. These cytokines are derived from neutrophils, phagocytes, T cells, endothelial cells, and mast cells.\textsuperscript{55,86} TNF-\(\beta\) is primarily produced from lymphocytes\textsuperscript{86} and has historically been referred to as lymphotoxin. TNFs have direct cytotoxic effects and promote destruction of target cells, particularly tumor cells, by activating T cells and phagocytes.\textsuperscript{19,86}

Research has identified a clear relationship between TNF-\(\alpha\) and severe cachexia in chronic infections and cancer.\textsuperscript{86} Along with IL-1, TNF-\(\alpha\) contributes to hemorrhagic necrosis,\textsuperscript{87} vascular endothelial cell lysis,\textsuperscript{91} and stimulates IL-6 expression.\textsuperscript{87} TNF-\(\alpha\) is highly induced by endotoxin,\textsuperscript{92} causing increased tight junction permeability by stimulating myosin light chain kinase\textsuperscript{27} and increasing vascular permeability. As a result, TNF-\(\alpha\) is a key mediator in septic shock.\textsuperscript{92}

\textit{Heat Shock Protein (HSP)}

Heat shock proteins are molecular chaperones that assist in proper folding during protein synthesis.\textsuperscript{60} There are many different HSP families numbered according to their molecular weight.\textsuperscript{19} Majority of HSPs are constitutive\textsuperscript{60} and found abundant throughout the body.\textsuperscript{93} A few HSPs are inducible, protecting cells exposed to stressors such as hyperthermia, hypothermia, inflammation, trauma, energy depletion, hypoxia, or toxins.\textsuperscript{19,94,95,96}
Understanding exact HSP mechanisms is complicated, particularly since they have many functions and protect different cell areas. One suggested induction mechanism is in response to stress signals from abnormally folded and denatured proteins.\textsuperscript{97} Other research suggests HSPs are induced by increased reliance on anaerobic metabolism following mitochondria damage\textsuperscript{98} or altered cytoskeleton structure resulting in inhibited mitochondria function.\textsuperscript{99} Still yet, another mechanism is in response to inhibited RNA processing following cell nuclei thermal stress.\textsuperscript{100} Regardless of the exact mechanism, HSP induction is associated with an increased demand for protein synthesis.

Primary functions of HSPs are to prevent denaturation and/or allow damaged cells to undergo apoptosis rather than necrosis by maintaining mitochondrial function.\textsuperscript{101} Apoptosis is a controlled event and requires ATP. If mitochondria are damaged and cells undergo necrosis, leaked cell contents may perpetuate or trigger inflammatory responses.\textsuperscript{101} More recent research suggests HSPs have a variety of pro- and anti-inflammatory functions during immune responses. HSPs may facilitate APCs,\textsuperscript{102} improve tolerance to\textsuperscript{103,104} and inhibit expression of TNF-\(\alpha\) and IL-1,\textsuperscript{105,106} and promote NKC activity.\textsuperscript{96}

Another protective function of HSPs is development of thermotolerance. Increased expression and accumulation of HSPs following thermal stress allows cells to survive subsequent stress by protecting against protein denaturation. Thermotolerance is temporary, lasting 3-5 days, and dependent on initial temperature and duration of exposure.\textsuperscript{107} Considered one of the most highly inducible,\textsuperscript{108} HSP70 is preferentially induced during thermal stress and protects against ROS and cytokine driven apoptosis.\textsuperscript{94,101,109,110}
Reactive Oxygen Species (ROS)

Reactive oxygen species are free radicals (eg, superoxide, hydrogen peroxide, and hydroxyl) released as byproducts of cell and body processes (eg, aerobic metabolism). Reactive oxygen species are free radicals (eg, superoxide, hydrogen peroxide, and hydroxyl) released as byproducts of cell and body processes (eg, aerobic metabolism). These free radicals are extremely reactive to cells and easily pull electrons from molecules. Several mechanisms increase ROS production. During hypoxia the imbalance between inputting electrons for fuel oxidation and transferring electrons to molecular oxygen results in ROS formation. Other mechanisms for ROS release are ischemia-reperfusion injury, phagocytosis, and arachidonic acid metabolism.

Balance between positive and negative ROS effects is complex and depends on other inflammatory responses. A primary function of ROS is regulation of stress proteins. Acting like second-messengers, they trigger activation of other pathways and factors. Some ROSs are cytoprotective, upregulating HSPs by causing a cell to express a stress response or inducing some protein denaturation. Other ROSs provide defense against pathogens and toxins.

Despite their protective anti-inflammatory effects, ROS also promotes pro-inflammatory responses by creating oxidative stress and cell damage. A primary target for ROS damage is lipid membranes. For example, ROSs stimulate phospholipase A2, which breaks down phospholipid membranes by hydrolyzing fatty acids. ROSs have also been shown to increase cytokine gene expression. Excessive ROS accumulation can result in cytotoxic effects to enzymes, amino acids, DNA, and mitochondria.

Cyclooxygenase (COX)

Trauma and other inflammatory stimuli activate phospholipase A2, which releases arachidonic acid, a crucial fatty acid incorporated in the phospholipid membrane.
structure. Arachidonic acid is the precursor for production of 3 eicosanoids: prostaglandin (PG), thromboxane (TX), and leukotriene.⁶⁰ Leukotriene production is catalyzed by lipooxygenase, which is not inhibited by NSAIDs¹¹³ and therefore will not be further discussed in this literature review. To produce TXs and PGs (collectively known as prostanoids), arachidonic acid must be converted to the prostaglandin endoperoxides PGG₂ and PGH₂.¹¹⁴ These conversions are catalyzed by PGH synthases, more commonly referred to as cyclooxygenase (COX).¹¹⁵

There are two well established COX derivatives in humans, COX-1 and COX-2. Both derivatives are responsible for converting arachidonic acid to TXs and PGs,¹¹⁶ but vary in their functions. Inherently located in most tissues, COX-1 is responsible for production of prostanoids that regulate gastric mucus secretions, platelet aggregation, and renal blood flow.²⁵,¹¹⁶ Present in small amounts naturally, COX-2 is induced locally by inflammatory mediators (eg, cytokines and endotoxin)¹¹⁵,⁷⁶ and mediates inflammation, pain, and fever.²⁵,⁶⁰,¹¹⁶

**Prostanoids**

All prostanoids are derived from prostanoic acid, and most commonly by arachidonic acid. Prostaglandins possess a 5-member carbon ring, with different types designated by letters A-K in accordance with the nature and position of substituents.¹¹⁷ In humans, the most common biologically active PGs are dienoic (designated by a subscript 2).¹¹⁸ These PGs include PGE₂, PGD₂, PGF₂,⁶⁰ PGI₂ (prostacyclin), and PGA₂ and PGJ₂ (cyclopentenones).¹¹⁵

In general, PGs are essential to maintaining GI mucosal barrier integrity, vascular tone, and renal function. They also are responsible for pain, inflammation, and implicated
in certain chronic diseases. Hormonal stimuli, such as angiotensin II,\textsuperscript{119} vasopressin,\textsuperscript{120} or bradykinin,\textsuperscript{121} is typically necessary for most PG synthesis, but some PGs can be induced by cell-cell interactions.\textsuperscript{117} In order to exert their effects, PGs must be actively transported across membranes. However, because they are readily deactivated, they exert only local effects.\textsuperscript{117} Non-cyclopentenone PGs act through binding to specific transmembrane G protein receptors. For example, PGE\textsubscript{2} receptors are designated E prostanoid receptor (EP) 1-4. Once bound and depending on the desired action, effects may include up or down regulation of second messengers cyclic adenosine monophosphate (cAMP) or calcium, altered membrane potential, or activation of specific protein kinases.\textsuperscript{117,122}

Identifiable by an oxygen bridge between carbon 6 and 9,\textsuperscript{117} PGI is important in maintaining cardiovascular function.\textsuperscript{122} Endothelial and vascular smooth muscles cells constitutively express PGI.\textsuperscript{117} Prostacyclin is a potent vasodilator and inhibitor of platelet aggregation, leukocyte adhesion, and vascular proliferation.\textsuperscript{114,123} Its efficacy led to development of PGI as a pharmacological treatment in a number of cardiovascular and renal diseases.\textsuperscript{114}

Derived from PGE and PGD respectively, PGA and PGJ contain a unique cyclopentenone ring structure. Cyclopentenone PGs contain an α,β–unsaturated carbonyl group that can react with glutathione (GSH) or other target cell proteins.\textsuperscript{117} Glutathione is an anti-oxidant that protects cells against ROS and other toxins. Cyclopentenone PGs are highly active; by reacting with GSH, they elicit a number of physiological effects other PGs cannot. For example, PGA and PGJ have been shown to induce HSP74 by inhibiting cell proliferation.\textsuperscript{124} They can also induce apoptosis in tumor cells\textsuperscript{125} and inhibit DNA and RNA viruses.\textsuperscript{126}
Instead of a 5 carbon ring, TXs have a heterocyclic ring.\textsuperscript{117} Thromboxanes with unstable bicyclic oxygenated rings are designated TXA, and those with stable oxane rings TXB.\textsuperscript{117} Like PGs, subscript numerals distinguish the number of double bonds\textsuperscript{117} and they exert their effects through G protein receptors.\textsuperscript{122} All prostanoids have relatively short half-lives. However, TXs are extremely unstable, with 20-30 sec half-lives.\textsuperscript{117,122} In comparison, under normal blood pH and temperature the half-life for PGI is 2-3 min.\textsuperscript{127} Though TXs elicit inflammatory effects, TXA\textsubscript{2} is predominately produced from COX-1 in platelets. Along with PGIs, TXs play a major, albeit opposing, role in vascular function.\textsuperscript{117}

**Functions of Prostanoids**

**Gastric Mucosal Protection**

Throughout the GI tract COX-1 is expressed constitutively and produces cytoprotective PGE\textsubscript{2} and PGI\textsubscript{2}. Damage or irritation to gastric mucosa epithelial lining up-regulates COX-2 expression,\textsuperscript{128} and subsequently PGE\textsubscript{2} and PGI\textsubscript{2} as a defensive, anti-inflammatory response.\textsuperscript{18} Research on the exact protective mechanisms has predominately focused on animal and cell cultures. Additionally, like PGI analogues developed for cardiovascular disease treatment, the effectiveness of PGE in protecting the GI mucosa has led to use of PGE analogues in studies. Existing research concludes a multifaceted protection by maintaining blood flow, inhibiting cell apoptosis, reducing acid secretion, promoting mucus and bicarbonate secretion, inhibiting ROSs, inhibiting GI hypermotility, and maintaining tight junctions.

*Increased Epithelial Blood Flow*

Vasodilation by PGE\textsubscript{2} and PGI\textsubscript{2} increases blood flow, which prevents cell necrosis by increasing platelets and promoting angiogenesis and cell proliferation. In addition,
preserving adequate GI blood flow maintains relatively neutral pH even during times of high acid secretion. Importance of blood flow is evident in GI diseases or conditions where blood flow is impaired. In patients with portal hypertension there is a higher risk of developing GI ulcers and bleeding. This risk is associated with decreased PG, which was shown in rodents with reduced portal blood flow from stomach cirrhosis. Cirrhotic rodents exposed to a mucosal irritant had a significantly blunted blood flow compared to controls (2 ± 8% vs 51 ± 5%, \( P < .001 \)). However, PGE administration significantly increased blood flow in cirrhotic rodents compared to controls (83 ± 12% vs. 22 ± 8%, \( P < .01 \)).

**Apoptosis Inhibition**

Ability for PGs to prevent mucosal cell apoptosis has been shown *in vitro*. Isolated guinea pig gastric mucosal cells placed under maturation-dependent stress were used to mimic high turnover rate seen *in vivo* with acid exposure and damage. When compared to control cells, PGE2 inhibited spontaneous cell apoptosis and significantly increased cell viability (\( P < .01 \)). Apoptosis inhibition occurred even after DNA fragmentation occurred by protecting mitochondria in a rapid, yet transient and dose dependent manner. Similarly, when cells were stressed by 4% ethanol, PGE2 significantly increased cell viability (\( P < .01 \)) compared to control cells.

**Inhibited Gastric Acid Secretion**

Although somewhat controversial, PGI2 and PGE2 inhibit gastric acid secretion. Healthy males orally administered PGE2 showed significantly reduced gastric acid secretion by approximately 40% compared to control (\( P < .05 \)). These results are supported by studies linking acid secretion and gastric ulcer development. A 12 week pharmacological study compared no treatment, a proton-pump inhibitor (known acid
suppresser), and a PGE$_1$ analogue on GI ulcer development in 458 patients with previous gastric ulcer history. After 12 weeks of NSAID use, 93% of patients using PGE were ulcer free compared to only 51% of control and 81% of proton-pump inhibitor participant. Of those who developed ulcers, time to occurrence was significantly prolonged with PGE ($P < .001$) compared to no treatment and was not significantly different than the proton-pump inhibitor.$^{133}$

One suggested mechanism of gastric acid inhibition is increased mucosal blood flow, but others believe PGs elicit non-blood flow mediated inhibition. Intravenous PGE$_2$ and PGI$_2$ in dogs significantly inhibited acid secretion ($P < .05$). PGI$_2$ resulted in increased mucus blood flow while PGE$_2$ decreased blood flow.$^{134}$ Even stronger support for PGE acid secretion inhibition comes from *in vitro* studies where blood flow is not a factor.$^{135}$ This suggests PGE$_2$ and PGI$_2$ reduce acid secretion by directly inhibit mucus secretory cells. These mechanisms are not clearly understood, but include altered intracellular cAMP$^{135,136}$ and changes in cell membrane bound phospholipids that alter hydrogen, potassium, and ATPase activity.$^{137}$

Other studies have been unable to demonstrate significant reduction in acid secretion.$^{138,139}$ Lack of agreement may be attributed to different measurement techniques or methods such as dose or duration of use. Type of PG used also explains conflicting results. Unlike endogenous PGE$_2$, intravenous or orally administered PGs analogues are not rapidly metabolized and can exert longer lasting effects. This idea is supported by a study in healthy males comparing PGE$_2$ to different doses of PGE$_2$ analogues. No significant reduction in gastric acid secretion occurred with natural PGE$_2$, but both
analogs resulted in significant decreases directly proportionate to dosage and lasting 3 hours after administration.\textsuperscript{140}

\textit{Increased Bicarbonate and Mucus Secretions}

Both \textit{in vivo} and \textit{in vitro} studies have demonstrated upregulating PGE\textsubscript{2} production increases bicarbonate and mucus secretions to protect the GI barrier. Canines administered intravenous PGE\textsubscript{2} resulted in significantly increased bicarbonate production compared to control (mean = 64.5 μmol/hr vs 34.0 μmol/hr, \(P < .05\)).\textsuperscript{141} These results are similar to studies on mucus secretion in amphibian cells,\textsuperscript{142} rabbit gastric epithelial cells,\textsuperscript{143} and rodents.\textsuperscript{144} In 6 healthy humans administered varying doses of a PG analogue, mucus secretion also significantly increased (\(P < .01\)) in direct proportion to administered doses.\textsuperscript{138}

Despite being abundant in gastric epithelium, research has been unable to identify endogenous PGI\textsubscript{2} as a stimulator of bicarbonate secretion. One suggested reason for this is the location of epithelial producing PGI\textsubscript{2} cells. Unlike PGE\textsubscript{2} cells, which are on the surface near bicarbonate secreting cells, PGI\textsubscript{2} cells are located deeper. Considering location and a short half-life, PGI\textsubscript{2} may not have enough time to elicit effects on bicarbonate cells, and assumingly mucus cells, before being degraded.\textsuperscript{145}

\textit{Decreased GI Motility, Neutrophil Chemotaxis, and ROS}

Many protective mucosa effects are interrelated. This is particularly apparent when discussing GI hypermotility, neutrophil chemotaxis and ROSs inhibition. Hypermotility results in a number of GI disturbances and is associated with increased risk for lesions along mucosal folds.\textsuperscript{146,147} Repeated and excessive GI contraction and relaxation also leads to microcirculatory disturbances, with consequences similar to ischemic-reperfusion
injury. Lastly, hypermotility stimulates neutrophil chemotaxis and neutrophil adherence to vascular endothelium. When neutrophils increase at sites of inflammation they interact and adhere to plasma membranes of endothelial cells and release ROS. If neutrophils accumulate in microvasculature, blood vessel occlusion can potentiate ischemic conditions.

Exogenous PGs are potent inhibitors of hypermotility, neutrophils and ROS. Studies have demonstrated these effects using rodents with indomethacin induced GI mucosa lesions. Compared to control, PGE$_2$ significantly inhibited lesion occurrence by 83.6% ($P < .05$), and this was partly attributed to significant decreases in neutrophil chemotaxis and GI motility (83% and 81.3% respectively, $P < .05$). In a similar study, results showed pre-treatment with PGE$_2$ significantly inhibited lesion formation (90.6%, $P < .05$) and GI motility. Using isolated neutrophils, release of ROS was significantly inhibited by PGE$_2$ ($P < .05$) and, like many other protective effects, was dose dependent. This study also found PGD$_2$, but not PGI$_2$, was a significant and more potent inhibitor of ROS release ($P < .05$). Ischemia and reperfusion are potent producers of ROS and decreases GSH availability, which counters ROS.

**Decreased GI Permeability**

Decreased blood flow and ROS exposure can open tight junctions by damaging cell membranes and promoting necrosis. As a result, GI permeability increases and a cascade of events occurs, including cytokine release, endotoxin leakage, and monocyte activation. Ability for PGs to protect GI mucosal integrity inherently assists in maintaining GI permeability. However, rather than preventing increased permeability PGs appear to maintain permeability after damage ensues. Some studies suggest PGs stimulate growth
factors that promote epithelial cell repair. Others state permeability is maintained by contracting small intestine villi. Following 15 min of PGE$_2$ exposure, villi height and crypt depth significantly decreased as result of villi smooth muscle contraction ($P < .05$). However, negligible effects occurred after 45 min of exposure. More recent studies allude to PG restoring barrier function through closing down tight junctions. An *in vitro* study used porcine ileum to examine GI permeability following induced epithelial damage. Both PGE$_2$ and PGI$_2$ contributed to barrier recovery by collapsing tight junctions and tightening lateral membranes of crypt and villi to contiguous cells and basal membranes. Another interesting and important finding from this study is the comparison of endogenous and exogenous PG. Exogenous PGs significantly decreased permeability earlier compared to endogenous (60 min versus 120 min, $P < .01$). However, by 180 min following injury there was no significant difference, suggesting both methods are effective at restoring GI barrier function. In a similar study design, PGI$_2$ and PGE$_2$ acted synergistically to close tight junctions by increasing intracellular cAMP and calcium signals at the cytoskeleton.

**Vascular Tone and Renal Function**

Prostanoids play little role in vascular maintenance in normal healthy individuals. On the other hand, they are critical in maintaining homeostasis during altered cardiovascular states (eg, hypotension and heart failure) or altered fluid regulation (eg, renal disease, hypovolemia, and sodium imbalance). Prostanoids predominately responsible for vascular maintenance include PGI$_2$, TXA$_2$, and PGE$_2$.

Balance between COX-2 driven PGI$_2$ and PGE$_2$ production plays a significant role in cardiovascular diseases. Ischemia-reperfusion injury during a cardiac event increases PGI$_2$ and PGE$_2$ expression, which act in a protective manner to promote vasodilation
and inhibit platelet aggregation and vascular proliferation.\textsuperscript{117} Up-regulation of \textit{COX-2} following endothelial cell damage stimulates \textit{PGI_2} in attempt to inhibit artherogenesis. In contrast, \textit{PGE_2} stimulates artherogenesis due to its strong pro-inflammatory effects.\textsuperscript{160} The strongest opposition to \textit{PGI_2} is \textit{TXA_2}, which is predominately driven by \textit{COX-1} and promotes platelet aggregation, vasoconstriction, and vascular proliferation.\textsuperscript{117}

The complex relationship between \textit{PGE_2} and \textit{PGI_2} on kidney function can be summarized by three interrelated actions: 1) increased renal blood flow and natriuresis, 2) vasopressin inhibition, and 3) renin-angiotensin-aldosterone system stimulation. Produced by medullary interstitial cells, papillary collecting tubules, and glomeruli,\textsuperscript{161,162} renal PGs respond to hypertension, hypertonicity, and ischemia by stimulating vasodilation and increasing renal blood flow.\textsuperscript{118,163} Increased \textit{PGE_2} production has also been shown to decrease renal tubular pressure by inhibiting sodium reabsorption through altered cation transport.\textsuperscript{164} Prostaglandins, particularly \textit{PGE_2}, inhibit vasopressin by altering cAMP activity\textsuperscript{165} and through negative feedback.\textsuperscript{120} Thirdly, both \textit{PGE_2} and \textit{PGI_2} can stimulate renin and angiotensin II release to cause vasoconstriction and increase BP.\textsuperscript{118}

\textit{Inflammation and Pain}

Pain sensation begins with peripheral nociceptors in damaged tissue transmitting external stimuli (eg, extreme heat, toxins, or mechanical stress) into electrical energy to be carried along afferent nerves to the spinal cord. Damaged tissues also release neurotransmitters, particularly substance P or glutamate. Concurrently, \textit{IL-1\textbeta}, \textit{TNF-\alpha}, \textit{PGE_2}, \textit{PGI_2}, bradykinin, and numerous other chemical mediators can be released and potentiate inflammatory pain.\textsuperscript{39}
Increased COX-2 expression during injury stimulates PG production, preferentially producing PGE\textsubscript{2} and PGI\textsubscript{2}. These two PGs are responsible for fever, swelling, redness, and pain – the cardinal signs of inflammation. Prostaglandins do not directly cause pain but contribute to local and disperse inflammatory pain by increasing afferent nerve ending sensitivity to bradykinin, stimulating calcium and sodium channels, and inhibiting potassium channels. In the spinal cord, PGs stimulate glutamate and substance P release and activate dorsal horn neurons. Coupling mediators (ie, PGs with bradykinin or bradykinin with substance P) results in an additive effect on vasodilation, increased vascular permeability, and pain.

Prostanoids regulate inflammation and pain processes in a number of other ways. Both PGE\textsubscript{2} and PGI\textsubscript{2} inhibit T cell signaling and differentiation, shifting differentiation from Th1 to Th2. Anti-inflammatory PGE\textsubscript{2} functions include decreased lymphocyte proliferation and bradykinin stimulation, which is known to protect neurons and inhibit cytokine release. In addition to its potent changes in vascular tone, TXA\textsubscript{2} stimulates lymphocyte activation and proliferation. Lastly, cyclopentenone PGs can induce apoptosis by increasing HSP 70 transcription and suppress gene expression of macrophage induced TNF-\(\alpha\), COX-2, and PGE\textsubscript{2}. Each prostanoid plays a unique role in the body, but in general their response to disease or injury is a vital attempt to maintain homeostasis. While inhibiting prostanoids may decrease some of the perceived negative effects (eg, fever, pain, inflammation), inhibition also diminishes any positive, protective effects.
Exercise Induced GI Distress and Inflammation

Intense physical exercise induces a number of GI and acute inflammatory responses. Some underlying factors that potentiate these responses during exercise are nutrition, medical history, medication, hormones, dehydration, and Tc. Etiologies for GI dysfunction and inflammation are complex, and largely influenced by type, duration, and intensity of exercise.

Type of Exercise

GI Distress

Specific GI symptoms vary across studies, but long distance endurance exercise is typically associated with more GI distress than anaerobic, short distance events. Among 30 Ironman triathletes (29 males, 1 female, mean age = 33.0 ± 6.0 yrs) completing a GI symptom questionnaire post-event, 93% reported experiencing some type of distress and 2 participants were unable to complete the race due to severe GI distress. Most common complaints were flatulence (38%, n = 11), belching (35%, n = 10), general stomach problems (31%, n = 9), and bloating (24%, n = 7). In a sample of 125 runners (68 males, 57 females, mean age = 37 ± 9 yrs) completing the Marine Corps Marathon, there was a significant increase from hemoccult-negative stool pre-race to hemoccult-positive stool post-race (n = 29, \( P < .001 \)). Other reported symptoms included abdominal cramps (n = 21), diarrhea (n = 8), and vomiting (n = 1) during or post-race. Similar results were found in 15 ultra-distance runners. Sixty percent (n = 9) reported GI distress, predominately nausea (89%), abdominal cramps (44%), diarrhea (44%), and vomiting (22%).

Several studies have compared GI symptom occurrence, duration, and intensity between running and cycling, finding running causes more symptoms than cycling. During
a 180min cycle and running protocol in moderate temperature, 95% of participants (n = 21/22) reported GI symptoms during exercise. Running resulted in significantly more symptoms than cycling ($P < .05$), specifically belching, heartburn, cramping, and side aches.\textsuperscript{172} A similar run-cycle-run protocol in 7 well trained male athletes resulted in significantly more reflux episodes during running than cycling.\textsuperscript{43} Gastrointestinal motility was significantly higher with cycling compared to rest (6.1 versus 4.7, $P = .025$) and carbohydrate beverage transit time progressively increased across rest, cycling, and running (138min, 193min, 240min, respectively, $P = .03$).\textsuperscript{173}

\textit{Inflammation}

Inflammatory responses during exercise include increased circulating pro-inflammatory cytokines (IL-1$\beta$, IL-6, and TNF-$\alpha$), PGE$_2$, HSP70, and increased neutrophil and lymphocyte (T4 cell, T8 cell, and NKC) chemotaxis.\textsuperscript{174-176} Plasma IL-6 levels rise relatively quickly during intense exercise, peaking immediately after. In contrast, TNF-$\alpha$ responses are slower and peak hours post-exercise.\textsuperscript{174} In 9 sedentary males completing 45 minutes of downhill running IL-1$\beta$, PGE$_2$, and neutrophil chemotaxis significantly increased after exercise.\textsuperscript{177,178} No increase in IL-6 and TNF-$\alpha$ was found,\textsuperscript{177} which may be attributed to a short duration and lower exercise intensity. In 16 male marathon runners (mean age = 30.5 $\pm$ 1.9 yrs, $\dot{V}O_{2\text{max}} = 43.6 \pm 2.1$ ml/kg/min) IL-6 significantly increased baseline to post-race (1.5 $\pm$ 0.7 pg/ml to 94.4 $\pm$ 12.6 pg/ml, $P < .0001$). Pre- to post-race IL-1$\beta$ (0.61 $\pm$ 0.24 pg/ml to 0.92 $\pm$ 0.26 pg/ml, $P < .005$) and TNF-$\alpha$ (0.96 $\pm$ 0.09 pg/ml to 2.43 $\pm$ 0.37 pg/ml, $P < .0001$) also significantly increased.\textsuperscript{176} Nine male Ironman triathletes competing in moderate temperatures (mean = 23.3 $\pm$ 1.9°C) had significantly increased plasma HSP70 and IL-6 post-race ($P < .001$).\textsuperscript{175}
Potential Etiologies

Research is inconclusive regarding exact etiology of exercise induced GI and inflammatory responses. In part this is due to varying methodologies among controlled laboratory studies and the limitations of field studies. Potential etiologies include damage caused by mechanical vibrations, ischemia-reperfusion injury, and increased GI permeability with LPS translocation, but a combination of these factors is likely.

Gastrointestinal dysfunction during physical activity may be caused by mechanical vibrations and increased abdominal pressure. There is limited research in this area and the existing literature has several limitations. Using accelerometers on 6 well trained participants (3 males, 3 females) during separate cycling and running bouts resulted in significantly greater output during running (mean counts/min = 859.5 ± 130.1 versus 425.8 ± 149.5, \( P < .0001 \)). Differences within activity affect vibrations as well. Running shoe components and surface type can increase or decrease vibrations transferred to the GI. Cycling position, such as standing, sitting, and leaning can also affect vibrations.

A profound shunting of blood during intense exercise results in local and systemic inflammatory responses. Ischemia occurs when splanchnic blood flow decreases more than 50% and is often considered the primary cause of GI damage. Epithelial cells are particularly vulnerable. In hypoxic states they produce ROS and cytokines, attract neutrophils, and experience altered basal membrane attachment. Responses to GI ischemia-reperfusion impairs tight junctions, ultimately increasing permeability and LPS translocation.

Intense exercise during field and controlled laboratory studies increases GI permeability. Percent recovery of lactulose/rhamnose was significantly higher
during a 60 min run at 80% \( \dot{V}O_{2\text{max}} \) than lower intensities and rest \((P < .05)\). With hydration controlled in a thermoneutral environment, 10 participants (9 males, 1 female) completed a rest, cycling, and running protocol. Small intestine permeability was significantly higher during running (mean lactulose/rhamnose ratio = 0.04 ± 0.02; \( P < .01 \)) compared to cycling (0.03 ± 0.02) and rest (0.02 ± 0.02).

Similar results are indicated with plasma LPS measures. During cycling to exhaustion in 10 males plasma LPS significantly increased pre- to post-exercise (0.14 to 0.24 Eu/ml, \( P < .01 \)). Higher incidence of vomiting, diarrhea, and nausea were reported in marathon runners with elevated LPS measures (0.33 ± 0.04 ng/ml) compared to those with low LPS (0.08 ± 0.01 ng/ml, \( P < .001 \)). In attempt to identify if GI distress is associated with ischemia driven endotoxemia and inflammatory cytokines, blood measures were taken 24 hours pre-Ironman, immediately post-race, and 1, 2, and 15-20 hrs post-race. A significant increase from pre- to 1 hour post was found for mean LPS and IL-6 concentration (0 pg/ml to 4 pg/ml, and 0 pg/ml to 40 pg/ml, respectively, \( P < .05 \)). No GI symptoms were correlated with LPS concentration. However, some symptoms were significantly, albeit weakly, correlated to IL-6 (vomiting \( r^2 = 0.268 \) and diarrhea \( r^2 = 0.504 \), \( P < .05 \)).

**Heat Stress Induced GI Distress and Inflammation**

The greatest GI response to non-exertional thermal stress is impaired barrier function and subsequent LPS translocation. In 12 primates subjected to passive hyperthermia (41 ± 0.3°C, 100% relative humidity), blood LPS measures significantly increased from 0.06 ± 0.01 ng/ml to 0.32 ± 0.03 ng/ml (\( P < .01 \)). In non-EHS patients (mean Tc = 42.1 ± 0.2°C), significant decreases pre- to post-cooling were found for mean
plasma TNF-α (247.4 ± 41.2 pg/ml to 133 ± 27.9 pg/ml, $P < .05$) and LPS (12.26 ± 1.86 ng/ml to 6.12 ± 1.18 ng/ml, $P < .05$). Raising Caco-2 human intestinal epithelial cell temperature from 37°C to 41°C cultures resulted in a strong, linear, inverse relationship ($r = 0.96$) between temperature and transepithelial resistance, a measure of tight junction integrity. These results, along with HSP research suggest thermal stress induced permeability changes occur at tight junctions. Following 2hrs of heat exposure (Caco-2 cell temperature = 41°C) HSP70 expression peaked 4-8hrs post and tight junction protein expression decreased. When HSP was inhibited paracellular permeability was significantly greater ($P < .001$) than no HSP inhibition.

A number of other inflammatory mediators are released during passive hyperthermia. In particular, elevated IL-6 (220 ± 44 pg/ml) was identified in 18 non-EHS patients with a mean Tc = 41.04 ± 0.2°C. Another study showed pre-cooling IL-6 concentration and severity of passive heat stroke was significantly correlated ($r = 0.516, P < .03$). In patients experiencing non-EHS, TNF-α significantly decreased pre- to post-cooling (247.4 ± 41.2 pg/ml to 133 ± 27.9 pg/ml, $P < .05$) and remained above control values 31.4 ± 8.4 pg/ml. Working synergistically, TNF-α and IL-6 may exacerbate hyperthermia. If combined with exercise, GI and inflammatory responses during thermal stress may lead to serious, potentially lethal conditions of endoxtemia, SIR, or EHS.

**EXERTIONAL HEAT STROKE**

Individuals may experience a number of symptoms when heat dissipation mechanisms are compromised during intense exercise in extreme thermal environments. Initially, minor symptoms such as dizziness, lightheadedness, or fainting may occur, which are typical of heat syncope. Heat syncope occurs due to decreased brain blood flow as a
result of peripheral vasodilation, postural blood pooling, diminished venous return, cardiac output reduction, and/or cardiac ischemia.\textsuperscript{9,188} A more serious EHI, heat exhaustion can occur as the cardiovascular fails and plasma volume decreases. Heat exhaustion symptoms include dizziness and lightheadedness, as well as fatigue, headache, normal-to-elevated Tc ($< 40^\circ C$), nausea, vomiting, intestinal cramps, tachycardia, and/or hyperventilation. Complete thermoregulatory and cardiovascular system failure results in EHS.\textsuperscript{4} Excessive metabolic heat production dangerously increases Tc, and combined with decreased brain blood flow, the central nervous system is impaired and organ damage ensues.

**Uniqueness and Individuality of Exertional Heat Stroke Cases**

Exertional heat stroke is identified by 2 key characteristics: 1) a Tc $\geq 40^\circ C$ and 2) alterations in central nervous system function.\textsuperscript{9} It is important to note that $40^\circ C$ is not a “golden number”. It remains unclear why some individuals can participate in intense activity with Tc above $40^\circ C$ without any EHS symptoms and other individuals experience EHS below $40^\circ C$. Some variability can be explained by physical conditioning, acclimatization, body mass, hydration, equipment or clothing, and sleep deprivation. These factors are well understood in regard to physiological strain and disseminating metabolic heat. Untrained, unacclimatized individuals with larger body-surfaces have decreased thermal tolerance.\textsuperscript{189} Dehydration impairs cardiovascular function, increasing Tc and perceptual thermal strain measures.\textsuperscript{190} Equipment and clothing creates a microenvironment that limits heat dissipation\textsuperscript{189}

Previous illness and medication use are known risk factors for EHS, but less understood because controlled laboratory studies in human subjects are not available. Insight into the impact these factors play in EHS primarily comes from case and
observational studies. A retrospective assessment of 38 EHS deaths among American college and high school football players identified 1 player who experienced a GI virus the day before and 1 who had a history of taking ephedra.\textsuperscript{191} A 19yr old male participating in intense exercise in the heat experienced multiple heat-related incidences. The first episode was precipitated by gastroenteritis.\textsuperscript{192} Clinical observations of 36 male EHS patients found 1 experienced a fever the week prior, 6 presented with GI distress within 3 days of the event, and 1 had EHS 3 days earlier. In addition to the 6 with previous GI distress, 16 patients experienced GI distress during the EHS.\textsuperscript{193} In one of the most publicized EHS deaths in American football, the athlete experienced GI distress ("upset stomach" and vomiting) the day prior to and day of his death.\textsuperscript{194}

**Etiology: Models of Exertional Heat Stroke**

The hypothalamus is considered the body’s thermostat. Individual baseline Tc may vary slightly, but the hypothalamus attempts to maintain Tc at about 37 ± 1°C. Unlike a thermostat, the hypothalamus cannot turn off heat and can only initiate responses designed to mitigate rising Tc. Hypothalamic driven mechanisms for heat loss include stimulating evaporative heat loss and cutaneous blood vessel vasodilation.\textsuperscript{1} Inability for the hypothalamus and thermoregulatory responses to rid heat gain can dangerously increase Tc.

Heat transfer occurs by movement from warmer to cooler objects/environments. Sun exposure creates radiation heat gain. Radiation heat loss occurs when the body is warmer than surrounding air.\textsuperscript{19} Conduction involves movement of heat energy with two objects in contact with one another. For example, warm skin in contact with cold water allows heat to dissipate from the body to the water. Similarly, convection is heat exchange
between moving air/fluids, and is impacted by movement speed. Using the previous conduction example, when no movement occurs the water in contact with the skin will become warm and the skin will cease to dissipate heat. Moving the water across the skin will allow for cooler water to pass by, promoting additional heat dissipation. Less heat is lost with slower movement. Evaporation occurs through respiration and sweating and is the major defense against heat gain.¹

**Early Theory of Exertional Heat Stroke**

Exertional heat stroke is typically thought to occur due to metabolic heat gain from working muscles and inability to dissipate heat to the surrounding environment.¹ During fever, infection, or passive hyperthermia elevated Tc increases metabolic rate. However, during intense physical exercise metabolic heat from chemical and mechanical actions raises Tc.¹⁹ In effort to promote heat loss the hypothalamus induces skin capillary vasodilation to deliver blood throughout the circulatory.¹⁹⁵ This also increases the demand to dissipate heat through evaporation, conduction, and convection.⁴

High environmental temperature and humidity are major limiting factors for effective heat loss. The greatest EHS risk occurs when wet bulb globe temperatures exceed 28°C,¹⁸⁹ but has been reported in cooler environments (6 – 9.5°C).¹⁹⁶ Through convection and conduction, the body will gain heat when environmental temperature is higher than body temperature. This requires a high demand in evaporative heat loss, seen with elevated sweat rates proportionate to elevated temperatures. In the presence of high humidity evaporative sweat loss is limited.¹ The combination of metabolic heat production during intense exercise and inability to dissipate heat exceeds the body’s thermoregulatory response. As a result, Tc increases and an individual is susceptible to experiencing an EHS.
**Dual Model of Exertional Heat Stroke**

More recently, the prevalence of previous illness and GI distress in EHS cases has led some to speculate that the GI tract and immune system are key underlying mechanisms. These general similarities have led researchers to examine a closely intertwined dual model for developing EHS. Both models are largely influenced by GI and immune responses due to prolonged exposure to intense exercise or hyperthermia during exercise.\(^5\)

**First Model of Exertional Heat Stroke: Hyperthermia during Exercise**

The first EHS model is due to exercise and thermal stress. An increase in heat storage causes GI distress and increased GI permeability.\(^5\) Exacerbated by GI ischemia and inflammatory cytokines, increased permeability allows LPS to escape into blood circulation.\(^5,197\) Exercise can continue as long as circulating LPS is cleaned up through previously mentioned immune and liver responses. However, once the liver is overwhelmed, endotoxemia occurs and additional pyrogenic cytokines are released.\(^5\) At higher temperatures, such as with intense exercise, both pro- and anti-inflammatory cytokines are released. A somewhat futile immune system results, with the body producing anti-inflammatory cytokines to counteract endotoxin and pyrogenic cytokines. Unfortunately, the pro-inflammatory cytokines (IL-6 and TNF-\(\alpha\)) are necessary to neutralize endotoxemia.\(^187\) Cytokines also promote necrosis, vasodilation, acidosis, decreased plasma volume, increased vascular permeability, and fever.\(^185,193,198\) The end result is SIR, multi-organ failure, and/or EHS.\(^5\)

**Second Model of Exertional Heat Stroke: Prolonged Exposure to Intense Exercise**

The second model states regardless of environmental conditions, prolonged intense exercise is immunosuppressive. Down-regulation of Th1, up-regulation of Th2, and
decreased NKCs contribute to blunted immune responses. In the event a pathogen is introduced, a sub-clinical infection develops\textsuperscript{5} where the individual may or may not experience symptoms.\textsuperscript{192} At this point, the individual attempts another bout of intense exercise, potentially in a thermal or thermoneutral environment. The compromised immune system and increased cytokines limits the liver’s ability to neutralize circulating LPS. Like the first model, if uncontrolled, endotoxemia, SIR, or EHS may occur.\textsuperscript{5}

An example of this model may be observed in individuals participating in successive intense exercise bouts (eg, pre-season football practices). The individual’s immune system is disturbed after the first intense exercise bout. On the following day, the individual attempts another bout of intense physical activity. However, GI damage and a compromised immune system decreases his/her thermal and work capacity. Unable to mitigate LPS, and with elevated pyrogenic cytokines, endotoxemia develops, $T_c$ rises, and the person experiences an EHS.

It is possible to develop EHS without experiencing endotoxemia. Animal studies have shown blocking LPS protects against heat stroke until a $T_c > 43.8^\circ$C.\textsuperscript{199,200} Extremely high temperatures denature proteins and cause cell necrosis.\textsuperscript{5} Since EHS does not always occur in hot environmental temperatures and some individuals can exercise at $40^\circ$C with no EHS symptoms, the dual model provides insight into underlying GI and inflammatory factors that contribute to EHS. Furthermore, risk factors (eg, previous illness, medication, sleep disturbances, and nutrition) that alter GI integrity and immune responses play roles in the dual model.
NON-STEROIDAL ANTI-INFLAMMATORY DRUGS (NSAIDs)

Non-steroidal anti-inflammatory drugs (NSAIDs) include a large variety of prescription and non-prescription medications including acetylsalicylic acid (aspirin), ibuprofen (eg, Advil, Motrin), indomethacin (eg, Indocin), naproxen (eg, Aleve, Naprosyn), and numerous others. Prevalence of NSAID use among the general public is high, spending billions on over-the-counter pain medication.\textsuperscript{201} Consumer reports and advertisers suggest ibuprofen and naproxen are safe, affordable, and effective pain medications,\textsuperscript{202} which is supported by 2013 revenue reports including both Advil (ibuprofen) and Aleve (naproxen sodium) in the top 10 over-the-counter brands.\textsuperscript{203}

Prevalence of medication use among elite athletes has been well documented due to World Anti-Doping Agency and International Olympic Committee drug policies. Medication, particularly NSAIDs, is a concern across all age groups, sport type, and competition level. Physically active persons use NSAIDs prophylactically or following onset of inflammation, pain, and/or fever.\textsuperscript{204-208} Despite an injury, athletes may choose to use NSAIDs in order to continue participation or because they perceive it will help their performance.\textsuperscript{208} Among 3,887 elite international track and field athletes completing a 7 day recall of medication use, 3,237 reported taking medications, with COX-1 inhibitors the most common (27.3%, n = 782).\textsuperscript{206} In a similar study, of 912 elite athletes across 34 sports completing 3 day recall doping control forms, 24.7% (n = 225) reported NSAID use.\textsuperscript{207} Of particular interest, 22% (n = 50) reported taking multiple NSAIDs at one time.\textsuperscript{207} Combining NSAIDs is not advised, particularly due to potential adverse drug reactions\textsuperscript{209} and increased risk of GI damage.\textsuperscript{210}
Similar prevalence of NSAID use has been reported in recreational athletes. Of 333 ultra-distance triathletes, 100 (30%) reported taking NSAIDs within the 24 hours prior to the race. Less research has been conducted among youth and team sport athletes. However, of the available studies, prevalence of use is similar or even higher than in adults. Of 604 high school football players reporting medication use over the past 3 months, 75% (n = 452) reported using NSAIDs related to their sport participation. Two hundred thirty-two independently administered NSAIDs (i.e. no adult supervision) and only 51 perceived any disadvantages to taking NSAIDs. Although this was a limited population, studies among the general population suggest adolescents often self-administer NSAIDs and are unaware of potentially toxic side effects.

NSAIDs are perceived as performance enhancing because they mitigate pain and inflammation. Differences between medication type, dosage, and exercise mode in existing literature make it difficult to conclusively state whether NSAIDs do or do not enhance performance. However, aspirin does not alleviate exercise induced muscle pain, and may degrade endurance exercise performance by increasing lactate production and decreasing time to fatigue. Results are similar in upper and lower extremity resistance exercise, finding aspirin increased rate of perceived exertion (RPE) and perceived leg pain versus a placebo. Further supporting the lack of performance enhancing effects, compared to placebo, aspirin had little effect on respiratory variables (respiratory rate, oxygen consumption, carbon dioxide, etc.), HR, anaerobic threshold onset, and work load.

**Mechanisms of Action**

All NSAIDs work to reduce inflammation and pain, but their mechanisms of action varies depending on chemical structure. Consequently, their effectiveness as anti-
inflammatory and anti-pain medications, along with their side effects, also varies greatly. The plant based salicylic acid (acetylsalicylic acid is a derivative) has been used for thousands of years to reduce inflammation and pain, long before its mechanism of action was understood. It was not until the 1970s researchers identified aspirin and all other NSAIDs’ mechanism of action – inactivation of COX.²²⁰,²²¹ By inhibiting COX, and therefore PG synthesis, NSAIDs decrease inflammatory responses and inflammatory induced pain. An NSAID’s mechanism of action can be further defined as either “non-selective” or “selective”, where non-selective inhibit both COX-1 and COX-2 and selective target COX-2 only.²²²

The most common way to determine a drug’s selectivity is to measure the half maximal inhibitory concentration (IC₅₀). This value represents the drug concentration needed to inhibit a substance or function by 50%.²²³ A ratio of COX-1 to COX-2 inhibition is determined by dividing the IC₅₀ value for COX-2 in to COX-1. It is important to note that drug selectivity is a continuous variable; there is no absolute cut-off value to classify selective versus non-selective.²²³,²²⁴ Utilizing IC₅₀ ratios may allow for more standardized comparison and classification²²⁰ within a study in regards to effectiveness and side effects. A drug is generally considered to be more COX-2 selective if it has a lower IC₅₀ ratio. Unfortunately, within and between studies there is variability in using whole blood or cell cultures, assays, incubation time, and substrate concentration.²²⁵,²²⁶ Direct comparison across studies is not recommended. Instead, ranking NSAID selectivity relative to IC₅₀ ratios (eg, most COX-1 selective to least) is commonly seen. While this allows for some generalization across studies, when reviewing rankings one would also see large variability in IC₅₀ ratios for a given drug.
Non-Selective COX Inhibitors

Majority of NSAIDs, particularly those available over-the-counter, are non-selective COX inhibitors. Due to different chemical compositions, inhibition of COX-1 and COX-2 is not equal and each NSAID has its own selectivity toward one COX variant or the other. A “non-selective” NSAID may be more COX-1 selective, while still inhibiting COX-2 to a certain percent. Choosing one NSAID over another is often dependent on desired therapeutic effect and patient response to a given drug.

Acetylsalicylic Acid (Aspirin)

Acetylsalicylic acid is the most widely used and researched NSAID, partly because it is oldest and inexpensive. Its COX inhibition is heavily time dependent, with rapid liver enzymatic activity resulting in a relatively short 2-3 hour half-life. Acetylsalicylic acid is indicated for mild to moderate pain, fever, and reducing risk of cardiac events. Its ability to irreversibly inhibit platelet driven TXA2 production by almost 95% with low-doses (30 mg per day) make it highly effective at preventing blood clots and reducing risk of heart attacks and strokes. Due to greater selectivity toward COX-1 than COX-2, much higher doses (320 mg per day) are recommended to achieve anti-inflammatory effects. Research using recommended over-the-counter doses has been unable to identify substantial anti-inflammatory effects. Following endotoxin injection, oral ingestion of 1000 mg resulted in no change in circulating TNF-α and IL-6 versus a placebo in resting patients.

Ibuprofen

Ibuprofen is another commonly used NSAID and the first non-aspirin over-the-counter NSAID. Similar to acetylsalicylic acid, ibuprofen is indicated for mild to
moderate pain, fever, and inflammatory relief and has a relatively short half-life of 2-4 hours.\textsuperscript{227} Ibuprofen is considered equipotent against both COX derivatives.\textsuperscript{233} Extensive research has been conducted on inflammatory effects during various exercise bouts\textsuperscript{234,235} or following endotoxin injection.\textsuperscript{236-238} Results are conflicting, with some studies showing ibuprofen has significant\textsuperscript{235-238} or no effect on inflammatory markers.\textsuperscript{234,235}

Contradictions in exercise studies can be partially explained by interpersonal differences of participants (eg, history of medication use and illness, age, acclimatization, and conditioning) and varying methodology (eg, exercise duration, intensity, environment, and medication dosage). A one-time 400 mg dose prior to 1500 m run in unknown environmental conditions showed no significant difference in TNF-\(\alpha\), IL-6, or IL-1\(\beta\) pre-, immediately post-, or 24 hours post.\textsuperscript{234} On the other hand, 29 runners completing a 160km ultramarathon in moderate temperatures (10\degree C-25\degree C, 56\% relative humidity) were given 600 mg of ibuprofen the afternoon prior to the race and another 1,200mg on race day (one 200 mg tablet every 4 hours). Compared to no medication, most inflammatory markers, including IL-6, significantly increased pre- to post-race; however, there was no significant change in TNF-\(\alpha\).\textsuperscript{235}

Results from endotoxin injection studies at rest are slightly more consistent. As expected, ibuprofen blunts Tc rise following endotoxin challenge, but has been shown to increase inflammatory markers. Compared to control, two 800mg ibuprofen doses pre-endotoxin injection significantly increased IL-6 (27 ± 12 ng/ml vs. 113 ± 66 ng/ml, \(P = .05\), respectively) and TNF-\(\alpha\) (369 ± 44 pg/ml vs 627 ± 136 pg/ml, \(P = .003\), respectively).\textsuperscript{237} Similar results were found after subjects were given three 800mg doses (1.5 hours pre-, at the time of, and 3hrs after endotoxin challenge).\textsuperscript{238}
**Indomethacin**

Indomethacin is typically used to control moderate pain in patients who do not respond to other, less toxic NSAIDs. Absorption is variable depending on administration route, and average half-life is 4-6 hours.\(^{227}\) Like other non-aspirin NSAIDs, it is less effective at reducing cardiovascular events.\(^{239}\) However, it is a potent inhibitor of COX-1\(^{240}\) making it useful as an anti-inflammatory agent. In 10 recreational athletes, indomethacin significantly blunted post-exercise NKC suppression, IL-6 expression, and TNF-α expression (\(P < .05\)).\(^{241,242}\)

**Naproxen**

Naproxen is a relatively newer drug with less selectivity toward COX-1 than aspirin or indomethacin.\(^{243,222}\) Naproxen is available in either free acid or sodium form.\(^{244}\) Naproxen sodium is commonly seen as Aleve® or naprosyn. The two forms are bioequivalent on all levels except rate of absorption. Gastric juice rapidly dissolves naproxen sodium, resulting in high plasma concentrations within 1 hr compared to 2 hrs with naproxen. The earlier onset of symptom relief with naproxen sodium is beneficial for acute pain.\(^{244}\) Over-the-counter doses (200 mg or 400 mg) are effective for mild to moderate pain, fever, and inflammation. Compared to other NSAIDs, naproxen has a long half-life of 10 to 20 hours.\(^{227}\) While a patient may need to take aspirin or ibuprofen every 4 hours to maintain therapeutic effects, patients using naproxen are instructed to take 1 dose every 8 hours and to not exceed 600 mg per day.\(^{227}\)

In regards to therapeutic effects, 500 mg of naproxen sodium twice a day has been shown to inhibit platelet COX-1 activity close to 95%. However, platelet inhibition is reversible and inconsistencies between subjects make it a less than ideal choice as a cardio-
protective medication.\textsuperscript{245,246} Naproxen significantly inhibits PGE\textsubscript{2} and PGI\textsubscript{2},\textsuperscript{247} making it useful in a variety of inflammatory and pain diseases.\textsuperscript{244}

**Selective COX-2 Inhibitors**

Three popular selective COX-2 inhibitors, also known as coxibs, are rofecoxib (Vioxx), valdecoxib (Bextra), and celecoxib (Celebrex). Meloxicam (Mobic) was not originally designed to be a COX-2 inhibitor, but research has shown it to be very COX-2 specific.\textsuperscript{248} With little inhibition of COX-1, these NSAIDs are more effective as anti-inflammatories while reducing toxic GI side effects.\textsuperscript{222}

**Adverse Effects of NSAIDs**

**Gastrointestinal Effects**

Risk and prevalence of adverse GI effects from NSAIDs is well established among published cases, multicenter case-control studies, and randomized control trials.\textsuperscript{17,69,243,249-251} Time from initial NSAID use to onset of GI distress varies from days to several years\textsuperscript{252} and risk increases with higher doses, longer use, longer half-life, slow-release formulation, greater COX-1 selectivity, and previous adverse events.\textsuperscript{243} In general, NSAIDs, particularly non-selective, cause adverse effects by inhibiting COX-1 and, therefore, GI protective PG effects – increased mucus and bicarbonate secretions, increased blood flow, decreased acid secretion, TJ maintenance, etc. Selective COX-2 inhibitors were developed to create a NSAID that reduced GI effects by preserving COX-1. However, research has shown both COX derivatives play a role in GI protection\textsuperscript{253} and NSAIDs may directly induce GI distress through non-PG mechanisms.\textsuperscript{16}
Gastrointestinal Damage

Specific GI damage includes colitis and ulcerative colitis; peptic ulcers; small and large intestine perforation, hemorrhaging and abscesses; and small intestine strictures.\textsuperscript{252,254} The relationship between the spectrum of COX inhibition and relative risk (RR) for GI damage has been illustrated in a meta-analysis comparing common NSAIDs and doses. Lowest risk was found for the more COX-2 selective celecoxib (RR = 1.42, 95% CI 0.85 – 2.37) and ibuprofen (RR = 2.69, 95% CI 2.17 – 3.33). The highest risk was found in the greater COX-1 specific ketorolac (Toradol; RR = 14.54, 95% CI 5.87 – 36.04).

Interestingly, compared to no use, NSAIDs inhibiting both COX derivatives by greater than 80% resulted in a higher GI damage risk; this included naproxen (RR = 5.63, 95% CI 3.83 – 8.28) and indomethacin (RR = 5.40, 95% CI 4.16 – 7.00).\textsuperscript{243} In a comparison of GI toxicity among 8,076 rheumatoid arthritis patients, naproxen use elicited significantly more upper GI damage (ulcers, obstruction, perforation, and bleeding) versus rofecoxib (121 vs 56 events, \(P < .001\)).\textsuperscript{249} Thirty-six healthy participants (24 males, 12 females, mean age = 48 yrs) with no previous NSAID intolerance were used to compare 500 mg naproxen versus 100 mg nimesulide (a selective COX-2 inhibitor) administered twice a day for two weeks. Naproxen significantly inhibited PGE\(_2\) and PGI\(_2\) (\(P < .001\)) and caused stomach damage (hemorrhage, lesions, and ulcers) in 24 participants compared to 2 in the nimesulide group.\textsuperscript{250}

There is a lack of research on lower GI damage and results are less consistent regarding selectivity. In 34,701 rheumatoid and osteoarthritis patients receiving either diclofenac (non-selective) or etoricoxib, bleeding, perforation, obstruction, diverticulitis, and ulcers were reported, but there was no significant difference between treatment
groups.\textsuperscript{255} One the other hand, relative risk for developing serious lower GI damage was significantly greater with naproxen use compared to rofecoxib (0.46, $P = .032$).\textsuperscript{256} A 43 year old female patient with low back pain regularly taking diclofenac (Voltaren) and intermittently using ibuprofen and indomethacin reported to a hospital with hematochezia. Examination and biopsy revealed ulcers along the ascending colon, cecum, and ileum. Two months after cessation of NSAIDs a follow-up colonoscopy revealed normal mucosa.\textsuperscript{257} A concurrent inflammatory response may exacerbate GI damage. Intestinal edema, lymphocyte and neutrophil accumulation\textsuperscript{257} and increased cytokines is associated with gastric lesions. Oral administration of indomethacin and aspirin in rats significantly increased TNF-\(\alpha\) and gastric lesion scores compared to control ($P < .001$). Pre-treatment of a TNF-\(\alpha\) inhibitor significantly decreased indomethacin induced gastric lesions ($P < .05$).\textsuperscript{258}

**Gastrointestinal Permeability**

Several randomized, blinded control trials have shown different NSAIDs increase GI permeability within hours and after single doses. In 14 healthy participants, GI permeability, measured using Cr-EDTA, significantly and linearly increased with potency after 2 single doses of aspirin ($2.3 \pm 0.3\%$, $P < .05$), ibuprofen ($2.9 \pm 1.2\%$, $P < .001$), or indomethacin ($4.7 \pm 1.3\%$, $P < .001$) compared to baseline ($1.9 \pm 0.5\%$).\textsuperscript{259} Nineteen healthy females were used to determine differences in permeability following naproxen, meloxicam, indomethacin, or celecoxib. Compared to no NSAID, only naproxen resulted in significantly higher gastroduodenal permeability (median sucrose excretion = 78.4 mg [51.4 – 105.4 95% CI] versus 107 mg [82.9 – 138.5 95% CI], $P < .05$). In regards to small intestine permeability, naproxen significantly increased the lactulose/mannitol ratio (0.032
versus 0.022 [baseline], \( P < .05 \) while celecoxib had no significant effect.\(^{251}\) In 8 participants (6 males, 2 females; mean age = 22.5 ± 2.5 yrs; mean \( \dot{V}O_{2max} = 61.8 \pm 5.7 \) ml/kg/min) running 60 min in a thermoneutral environment, aspirin and ibuprofen significantly increased gastroduodenal permeability compared to placebo (% sucrose secretion = 0.37, 0.22, and 0.09, respectively, \( P < .05 \)). Only aspirin significantly increased small intestine permeability versus placebo (lactulose/rhamnose ratio = 0.09 versus 0.065, \( P < .05 \)).\(^{14}\) An attempt to determine aspirin dosage needed to induce GI permeability found 3 doses of 325 mg significantly increased gastroduodenal permeability (\( P < .05 \)).\(^{260}\) Overall, research supports acute, over-the-counter NSAID doses alter GI permeability.

Some question has been raised against the generally accepted theory that NSAIDs induce permeability changes due to PG inhibition. Using 7 participants to determine if increased GI permeability was driven by COX inhibition, co-administration of PGE\(_2\) and indomethacin did not result in significant differences in excreted Cr-EDTA compared to indomethacin alone (6.6 ± 4.4% and 4.6 ± 1.5%, respectively).\(^{259}\) Similar results were found among 40 healthy participants administered indomethacin, indomethacin and exogenous PGE\(_2\), or indomethacin and metronidazole (an antibiotic). Co-administration of PGE\(_2\) failed to prevent increased GI permeability. On the other hand, metronidazole significantly inhibited GI permeability compared to indomethacin alone (\( P < .05 \)).\(^{261}\) Inability of PG to emolliate NSAID induced GI damage does not rule this mechanism out, particularly considering the limitations to exogenous PG use and other studies finding PG co-administration reduces GI permeability.\(^{262}\) However, it does allude to non-PG mediated mechanisms.
The GI mucosa is hydrophobic, preventing potentially harmful molecules from passing through. Altering hydrophobicity can increase GI permeability. Compared to saline, aspirin significantly decreased mucosa hydrophobicity among wild-type and COX-1 deficient mice \((P < .05)\). This was associated with significantly higher gastric lesion scores \((P < .05)\). Further support comes from a group of researchers showing aspirin, ibuprofen, and naproxen interact with phosphatidylcholines (a group of constituent phospholipids in cell membranes). This interaction disrupts TJs and creates unstable pores by altering hydrophobicity, fluidity, and cell structure on mucosa surface and at the lipid bilayer.

Other suggested mechanisms are less researched and focus around NSAIDs reducing ATP production. The ability for metronidazole to maintain GI permeability in the aforementioned study suggests an inflammatory mediated mechanism. By reducing ATP, presumably through glycolysis and tricarboxylic acid cycle inhibition, there is a disruption of the cytoskeleton and loss of TJ regulation, increase in ROS production, and protein degradation. Results on glucose supplementation maintaining permeability are conflicting. Co-administration of an indomethacin, glucose, and citrate formula blunted permeability changes compared to indomethacin. In contrast, no difference in permeability occurred with 1300 mg of aspirin and replenishing glucose through a carbohydrate beverage.

**Cardiovascular Effects**

As discussed earlier, PGs play an important role in maintaining cardiovascular function, producing prostanoids to maintain BP and blood volume. Selective COX-2 inhibitors have greater cardiac risks. Like GI effects, degree of selectivity plays a role on
occurrence of cardiac events, meaning even non-selective NSAIDs cause adverse cardiac effects. Aspirin is cardioprotective, preventing blood clots by irreversibly inhibiting TXA₂, but aspirin is also linked to increased bleeding time. Both selective and non-selective NSAIDs have been shown to increase myocardial infarction and stroke risk. Lastly, inhibiting COX driven vasodilation decreases skin blood flow, which limits the body’s ability to dissipate heat during exercise or thermal strain.

**Renal Effects**

In addition to altering renal function through cardiovascular effects, NSAIDs directly impact kidney function by inhibiting renal PGs, which maintain renal blood flow. Serious side effects include papillary necrosis and interstitial nephritis. Less serious side effects are fluid-electrolyte imbalances such as hyponatremia (plasma sodium concentration \( P_{[Na^+] < 135 \text{ mmol/L}} \) and renal hypertension. If coupled with volume depletion, a potential risk during exercise, these effects can be exacerbated and lead to acute renal failure. By inhibiting PGs, NSAIDs suppress the renin-angiotensin-aldosterone system, decrease glomerular filtration rate, increase vasopressin, and increase sodium retention. As a result, water is retained and vasoconstriction occurs, leading to edema and hypertension.

Decreased urine production with increased water retention from NSAID use is considered a contributing factor for hyponatremia. Thirty percent of ultradistance triathletes \( n = 100/333 \) reported using NSAIDs and had significantly lower post-race plasma sodium compared to athletes not using NSAIDs \( \text{mean} = 140.2 \text{ mmol/L versus 141.1 mmol/L, } P < .02 \). The 6 participants who experienced hyponatremia during the race all took NSAIDs \( \chi^2 = 14.24, P = .0002 \). Out of 60 hospitalized marathon runners, 15
experienced severe hyponatremia. Percent NSAID use was significantly higher compared to normonatremic runners (28.6% versus 4.6%, \( P = .010 \)).\(^{273}\) Other observational studies have not identified a relationship between NSAIDs and hyponatremia.\(^{274,275}\) Controlled laboratory studies are lacking and observational studies have inherent limitations. Nevertheless, it is known NSAIDs adversely affect fluid-electrolyte balance. Additional cardiovascular and thermoregulatory strain limits ability to dissipate heat and increases EHI risk.

**NSAIDs: A Risk Factor for Exertional Heat Stroke?**

Due to their anti-pyretic effects, taking NSAIDs during exercise and/or thermal stress has been speculated to lower Tc, but current literature is limited and inconclusive. After taking 7,800 mg of sodium salicylate or a placebo, 12 trained males walked for 100 min at 3.8 mph in a hot, dry environment (48.9°C dry bulb, 26.7°C wet bulb) and 16 in a hot, wet environment (33.3°C dry bulb, 30.6°C wet bulb). No significant differences in Tc were recorded between placebo and sodium salicylate in either environmental condition. Though not significant, Tc with salicylate began to increase compared to placebo at 40 min (hot, dry) and 30 min (hot, wet), continuing until the exercise protocol ended.\(^{276}\) A possible explanation for lack of significance between Tc could be the exercise protocol intensity. Mean HR was not significantly different between control (152 bpm) and NSAID (159 bpm) and below the established cut-off of 180 bpm to cease walking.\(^{276}\) An earlier study administered acetylsalicylic acid in either 1 dose, 48 hours, or 7 days on 10 males (age range = 23 – 26 yrs). Participants completed a short 20 min walk at 3.5 mph and 8.6% grade in a temperate environment (22.2°C, 50-55% RH). Resting, exercise, or recovery Tc was not significantly different across experimental groups and did not rise above 38°C.\(^{277}\)
Ten male participants taking 6 days of rofecoxib or placebo completed an exercise protocol at 75% $\dot{V}O_{2\text{max}}$ in temperate conditions (28°C, 50% RH). No significant difference was found in Tc between experimental conditions during the first 45 minutes of exercise (running). Experimental participants exhibited significantly lower Tc during the last 45 minutes of cycling (mean difference = 0.33 ± 0.26°C, $P < .05$) and throughout a 60 min recovery (mean difference = 0.34 ± 0.26°C, $P < .01$). Overall, TNF-α significantly increased pre- to post-exercise (2.96 ± 2.07 to 4.17 ± 3.25 pg/ml, $P = .03$), but no difference was found between groups. Change in skin blood flow from rest to cycling was significantly higher in the experimental group versus placebo ($P = .01$). Together, these results suggest rofecoxib blunts Tc rise by increasing skin blood flow. This is in contrast to a recent study using aspirin. In a randomized, double-blind, crossover study ($n = 14$, 7 males, 7 females, mean age = 55 ± 1 yr), the effects of 7 days of low dose (81mg) aspirin on Tc, skin blood flow, thermal sensation, and hydration measures were compared to placebo and PlavixR (a prescription anti-platelet medication). Participants rested 40 min in a thermal environment (30°C dry bulb, 22°C wet bulb, 40% RH) then biked 2 hours at 60% $\dot{V}O_{2\text{peak}}$. Following rest, Tc was significantly higher for aspirin compared to placebo ($P < .05$) and remained significantly higher throughout exercise ($P < .001$). Rate of temperature rise, thermal strain, HR, and plasma volume were not significantly different between experimental groups, but skin blood flow was significantly decreased with aspirin use ($P < .05$). Attenuating skin blood flow, thus limiting heat dissipation, is elevates Tc.

**SUMMARY AND CONCLUSION**

Literature supports a significant role of the GI tract and immune system in development of EHS. Together or alone, intense exercise and thermal stress increase GI
permeability, LPS translocation, and inflammatory responses. The result is increased Tc and EHS risk. Several risk factors may accelerate EHS development, but NSAIDs are currently not considered a risk factor.

Adverse NSAID effects share a number of components with the dual EHS model – GI damage, increased GI permeability, and inflammatory responses. Therefore, it can be inferred that NSAIDs use could exacerbate EHS risk via these shared mechanisms. There is limited research on NSAIDs and Tc during exercise and thermal stress. Existing literature typically fails to measure inflammatory markers and GI damage, varies from low to high intensity exercise, and varies from temperate to hot environments. Furthermore, using ibuprofen or acetylsalicylate is practical, but neither drug is a potent COX inhibitor. Naproxen is a commonly used, potent over-the-counter medication, but because it is relatively newer there is less research on its effects.

Physical conditioning, acclimatization, electrolyte and fluid balance, and other intrapersonal factors may impact Tc during activity. Considering existing literature, there is a clinically relevant question as to whether NSAIDs should also be considered a risk factor for EHS. Unfortunately, there is an overall lack of well-developed, controlled studies with intense exercise in thermal environments. Future research is needed to examine how NSAIDs may negatively impact thermoregulation through effects on the GI tract and immune system.

METHODS

The purpose of this study is to determine the effects of over-the-counter naproxen on Tc, GI permeability, GI distress, and inflammatory markers in hydrated, cycling adults in a thermal environment.
Research Design

We will utilize a double-blind, randomized, cross-over design to determine the effects of naproxen on the dependent variables Tc, TNF-α, IL-6, GI permeability, GI distress symptoms, occult fecal blood loss, P_{[Na^+]}, HR, BP, and performance (distance covered during a 10min time trial). Independent variables are naproxen (220mg naproxen/dose), placebo (0mg naproxen/dose), thermal environment (35°C, 30% humidity), and thermoneutral environment (15°C, 30% humidity). All participants will attend an informational session and complete the STEEP test to estimate \( \dot{V}O_{2\text{max}} \). Participants will be randomly assigned to 1 of 4 groups: 1) placebo and thermoneutral (Control); 2) placebo and heat stress (PHeat); 3) naproxen and thermoneutral (Npx); and 4) naproxen and heat stress (NpxHeat) in a counterbalanced order with a minimum of 7 days between each data collection session. Dependent measures will be taken pre-, during, and post- a 90 min cycling protocol.

Participants

A convenient sample of participants (n ≈ 20; age = 18-38 yrs) from the University of South Carolina and surrounding community will be used to participate in this study. To be included in the study participants must be moderately trained (male \( \dot{V}O_{2\text{max}} \) between 35-40 mL/kg; female \( \dot{V}O_{2\text{max}} \) between 32-40 mL/kg), non-heat acclimatized, in good general health and will be accepted based upon the absence of cardiovascular, respiratory, and metabolic disorders; musculoskeletal disorders preventing cycling exercise; fluid or electrolyte balance disorders; and GI or swallowing disorders. Participants will be screened using a Health and Injury History Questionnaire and read and sign an Institutional Review Board approved Informed Consent Form prior to participation.
**Experimental Conditions**

*Control Condition*

Participants in the Control condition will report to the laboratory 24 hrs prior to data collection and will be given 3 placebo capsules (glucose) and a Take Home Instruction sheet. Participants will complete a 90 min cycle trial in a thermoneutral environment (15°C, 30% humidity).

*Placebo and Heat Stress (PHeat) Condition*

Participants in the PHeat condition will report to the laboratory 24 hrs prior to data collection and will be given 3 placebo capsules (glucose) and a Take Home Instruction sheet. Participant will complete a 90 min cycle trial in a thermal environment (35°C, 30% humidity).

*Naproxen (Npx) Condition*

Participants in the Npx condition will report to the laboratory 24 hrs prior to data collection and will be given 3 naproxen capsules (220mg naproxen/dose) and a Take Home Instruction sheet. Participants will complete a 90 min cycle trial in a thermoneutral environment (15°C, 30% humidity).

*Naproxen and Heat Stress (NpxHeat)*

Participants in the NpxHeat condition will report to the laboratory 24 hrs prior to data collection and will be given 3 naproxen capsules (220mg naproxen/dose) and a Take Home Instruction sheet. Participant will complete a 90 min cycle trial in a thermal environment (35°C, 30% humidity).
Instruments and Protocols

Health and Injury History Questionnaire

The Health and Injury History Questionnaire references the Canadian Society for Exercise Physiology, which developed the PAR-Q to identify individuals who should be seen by a medical doctor before becoming more physically active, and both the American Medical Society for Sports Medicine and the American Orthopedic Society for Sports Medicine. These two organizations have released pre-participation physical exam forms used to identify individuals at risk for injuries. Supplemental questions have been added to identify individuals with potential medical illness or injury (eg, GI disorders, history of EHS, metabolic disorders, daily NSAID use) that would exclude them from study participation.

Dosage for Experimental Conditions

A 24 hr dosage (placebo or naproxen) will be administered per the manufacturer’s recommendations of 1 capsule every 8 hrs, with the final dosage being taken upon arrival to the laboratory. A total of 3 capsules is consumed per the experimental protocol. Each dosage will be taken with 1 8oz glass of water and not with food, as this may alter the effectiveness of the dose. Previous studies have utilized NSAIDs to examine the effects of a 24 hrs dose on GI permeability and our total 24 hr dosage (660 mg) is comparable to previous research.

Take Home Instruction Packets

Upon arrival to the laboratory 24 hrs prior to data collection, participants will be given a Pre-Data Collection Take Home Packet containing: 3 capsules (placebo or naproxen) with directions for taking capsules, a fecal occult blood test kit with directions,
and a pre-data collection GI symptoms survey. After completing the data collection trial, participants will be given a Post-Data Collection Take Home Packet containing: directions for collecting the last fecal stool sample, a GI symptom survey to complete when collecting the last fecal sample, and directions on returning the kit and survey to the researchers.

**STEEP Test**

The STEEP Test, adapted from the Bruce Protocol for cyclist, will estimate \( \dot{V}O_2_{\text{max}} \). The test will begin at a low intensity and progressively increase the work load 15% every 1 minute. A \( \dot{V}O_2_{\text{max}} \) between 40-50 mL/kg in males and 32-40 mL/kg in females will be considered “trained” and qualify for participation in the study. Results from \( \dot{V}O_2_{\text{max}} \) and HR will also be utilized to determine appropriate resistance for each participant to maintain a steady state of 70% \( \dot{V}O_2_{\text{max}} \) during cycling exercise trial.

**Cycle Exercise Trial**

Participants will complete a 90 min cycle trial at a steady state of 70% \( \dot{V}O_2_{\text{max}} \) for 80 min. The final 10 min of the trial will be completed at maximum effort. Distance covered during the 10 min will be utilized to determine performance. Cycling was chosen as the form of exercise due to the lesser impact on the GI tract compared to running. Any changes in the dependent variables is better explained from the effects of naproxen or environment and not as responses to running. The exercise trial will begin with a 3 min warm and be conducted in an environmental chamber.

**Inflammatory Cytokines**

An intravenous catheter will be placed in the cubital vein prior to exercise. The catheter is secured so that only one needle stick is required and blood is collected pre-, during, and immediately post-exercise. A total of 6 ml per blood draw will be collected.
into an ethylenediaminetetraacetic acid (EDTA) vacutainer tube and inverted several times to mix. EDTA tubes are the preferred choice to prevent coagulation of the blood sample prior to analysis. TNF-α and IL-6 is assessed using enzyme linked immunosorbent assay (ELISA) using high sensitivity kits (BD Biosciences TNF-α and IL-6 ELISA kits, BD Biosciences, San Diego, CA).

**Gastrointestinal Permeability**

To determine GI permeability, participants will arrive to the laboratory, void their bladder and then ingest 150 ml of a solution containing 5 g sucrose, 5 g lactulose, and 2 g mannitol (Sigma-Aldrich Co. LLC, St. Louis, MO). Urinary excretion of sugars will be determined by urine sugar concentration, total urine volume, and dose of sugar ingested. Urine samples will be prepared and analyzed for percent excretion of sucrose, lactulose, and mannitol using gas chromatography with mass spectrometry. Sucrose will be used to determine gastroduodenal permeability, while the ratio of lactulose/mannitol is used to determine small intestine permeability.

**Fecal Occult Blood Test**

To determine GI bleeding fecal occult blood is measured using take home kits (Fisher HealthCare™ Sure-Vue™ Fecal Occult Blood Slide Tests System, Thermo Fisher Scientific Inc., Waltham, MA). Kits include triple slide monitors, collection tissues, applicators, test instructions, and a mailing envelope. For each experimental trial, participants will provide the first stool sample after initiating NSAID/placebo administration and the first stool sample following the exercise protocol. Participants are instructed to document time of stool sample collection.
Hydration and Sodium Balance

Participants will be required to be euhydrated prior to beginning data collection. Hydration status is assessed by Posm, urine specific gravity (Usg), and body mass (BM). Euhydration will be defined as a Posm ≤ 290 mOsmols, Usg ≤ 1.020, and/or %BM < 1% change from baseline BM.288

Blood is obtained pre-, during, and immediately post-exercise. Blood samples are collected into a 6ml EDTA vacutainer tube, centrifuged at 3000 rpm for 15 min, plasma pipetted into microtubes, and stored at -20°C until analysis. Osmolality is measured using a freeze point depression (Multi-sample Osmometer model 2020, Advanced Instruments, Norwood, MA). Plasma osmolality is considered one of the most valid and reliable measures of acute hydration status changes289 and is used to ensure participants are in a hydrated state throughout each trial.

Urine is obtained pre- and post-exercise to obtain immediate estimates of hydration status. Participants must be in a euhydrated state prior to completing the data collection trial. A clean catch method will be used in which the participant is instructed to urinate a small amount before placing the specimen cup in midstream to collect a minimum of 1 ounce of urine. Usg is measured using a handheld clinical refractometer (model REF 312, Atago Company Ltd., Tokyo, Japan). Designed for clinical use, the clinical refractometer measures total concentration of liquid compared to water. The clinical refractometer is calibrated before and after each Usg measurement using two drops of distilled water on the prism, holding the instrument toward light, and adjusting the turning screw to correspond the light and dark boundary at 1.000. Following calibration, two drops of specimen are pipetted onto the dry prism, the refractometer is held to the light, and the Usg is recorded.290
A Tanita body composition analyzer (model TBF-300, Tanita Corporation, Arlington Heights, IL) is used to measure BM pre-, immediately post-exercise, and 3 hrs post-exercise. Participants are asked to void urine before stepping on scale, dress in shorts and t-shirt, and towel off any sweat. Baseline BM measures are taken at the information session, where participants will provide a urine sample to determine euhydration (Usg < 1.020).

To determine changes in $P_{[Na^+]}$ we will utilize ion-selective electrodes (EasyLyte® Na/K electrolyte analyzer model REF 2277, Medica, Bedford, MA). Plasma will be obtained using an intravenous catheter placed in the cubital vein. Blood will be centrifuged, plasma pipetted into microtubes and stored at -20°C until analysis. All samples will be discarded after analysis.

**Core Body Temperature**

$T_c$ is recorded continuously throughout the exercise trial using rectal temperature (model 4600, YSI Precision Temperature Group, Dayton, OH). Participants are not allowed to exceed a $T_c > 40°C/104°F$. Rectal temperature is accepted as a valid and reliable measure of $T_c$,291 frequently used as a criterion measure of $T_c$ during exercise under thermal stress,292,293 and is used in studies examining GI permeability and aspirin.14,15

**Gastrointestinal Symptom Index**

Gastrointestinal distress will be assessed using a symptom questionnaire adopted from previous research.45,294 The index is divided into 3 sections: 1) upper abdominal problems (heart burn, reflux, belching, bloating, stomach pain/cramping, nausea, vomiting); 2) lower abdominal problems (intestinal/lower abdominal pain/cramping, flatulence, urge to defecate, side aches/stitch, loose stool, diarrhea); and 3) systemic
problems (dizziness, headache, muscle cramps, urge to urinate). Symptoms are scored on a 10-point scale (0 = no problems at all and 9 = the worst it has ever been). A score of > 4 is considered “serious”. Questionnaires will be administered pre-, immediately post-, and 2 hrs post-exercise. To determine GI symptoms during exercise, participants are verbally asked about current GI symptoms and severity every 15 min.

*Rate of Perceived Exertion*

The Borg Scale is used to measure the participants’ rate of RPE pre-, every 15 min during, and immediately post-exercise.

*Cardiovascular*

To ensure participants remain at safe limits and determine any effects from NSAID use, HR is continuously monitored using Polar HR monitors (Polar Electro Inc., Lake Success, NY). In addition, BP is measured every 15 min to ensure participants maintain a normal BP response throughout the trial.

*Diet and Activity Logs*

Participants will be required to track diet and activity for 3 days prior to and 1 day after data collection using an online nutrition software (ESHA Research, Salem, OR). Participants are asked to refrain from red meat for 3 days prior and 1 day after data collection to limit false positive fecal occult blood tests. Logs are also used to help participants maintain similar dietary and activity habits during the 24 hrs prior to data collection.
Experimental Procedures (Figure 5.2)

Information Session

Consenting participants will sign the informed consent form and complete the Health and Injury Questionnaire. If participant has no disqualifying conditions (i.e. metabolic, cardiovascular, respiratory, and/or musculoskeletal disorders; current pain medication or NSAID use, etc.) he/she will complete the STEEP test to measure \( \dot{V}O_{2\text{max}} \). Participants are randomly assigned to 1 of 4 groups, with all 4 groups being completed in a randomized cross-over design. Both the participant and primary investigator are blind to whether the participant is completing the Npx or placebo group. Take Home Instructions are reviewed with the participant at the end of a session and prior to leaving the laboratory. Participants are not allowed to take any other medications for pain or inflammation during the course of the study. Participants are instructed to refrain from intense, vigorous exercise 2 days (48 hrs) prior to data collection, no exercise 24 hrs prior to data collection, and will complete an online dietary and activity log from 72 hrs prior to and 24 hrs post data collection.\(^{172}\)

Data Collection Session

Participants will report to the laboratory 24 hrs prior to data collection to obtain 3 pills (Npx or placebo), fecal occult blood tests, and Take Home Instructions. Participants will consume the 3 pills over the 24 hr period, drinking 1 8oz glass of water with each dose and not with food. At least 2 hrs prior to arriving for data collection, participants should consume a small meal and are instructed to consume the same meal prior to each trial. All data collection sessions are conducted at the same time of day for each participant and separated by a minimum of 7 days.
Upon arrival to the laboratory, participants will consume the 3rd pill. A member of the research team will measure hydration status to determine if the participant is in a euhydrated state. Participants are provided a rectal thermometer probe and a private area to insert the probe and a baseline Tc will be taken. A venous catheter is inserted into the cubital vein and 2 6ml vials of blood will be collected for baseline measures. Participants will complete a 3 min warm up on the bike prior to starting the 90 min cycle protocol. Participants will consume 3.5ml/kg of water every 15 min to maintain hydration status. Tc and HR will be monitored continuously. RPE and BP will be measured every 15 min. Two 6ml vials of blood will be taken pre-, 80 min, immediately post-cycle, and 3 hours post-cycle. At the conclusion of the cycling protocol, participants will weigh, provide a urine sample, and complete the GI symptom index questionnaire. Participants will then rest for 3 hrs in semi-reclined/seated position in a thermoneutral environment. Participants will consume 3.5ml/kg of water every 15 min to maintain hydration status. Blood, urine, weight, and GI symptom measures are taken at the conclusion of 3 hrs rest. The cycling protocol will cease if one of the following criteria is met: 1) participant completes the 90 min cycle protocol; 2) participant’s Tc = 40°C; 3) participant experiences severe GI distress (eg, vomiting); or 4) participant requests to stop the trial.

**Statistical Analysis**

SPSS statistical software (version XIIV; SPSS Inc, Chicago, IL) is used for all analyses. Descriptive statistics (mean and standard deviations) for all dependent variables is calculated. Repeated measures ANOVA is used to determine the differences of all dependent variables during exercise between the 4 groups. Significance level is set at $P <$
.05 for all analyses. Power analysis, *a priori*, calculated a participant number of 20 to have a power of 0.90, which is comparable to previously published studies.  

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176


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Figure 5.1. Working Model for Overall Study.
NSAIDs will exacerbate responses seen during exercise and no thermal stress, increasing Tc through immune, GI, and cardiovascular pathways.
### Information
- Informed Consent Form
- Explain study procedures
- Health and Injury History Questionnaire
- STEEP test
- Random group assignment
- Take Home

### 3 Days – 1 Day Prior to Data Collection
- Begin diet and physical activity log 3 days prior
- Report to lab 1 day prior to pick up
- Take Home Instructions
- 3 pills
- Fecal occult

### Data Collection (4 Trials)

<table>
<thead>
<tr>
<th>Arrive to Lab</th>
<th>Cycling Trial</th>
<th>Immediately Post-Exercise</th>
<th>3hrs Post-</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Confirm 2 pills taken</td>
<td>- Continuous Tc and HR</td>
<td>- Blood</td>
<td>- 3.5 ml/kg water every 15 min</td>
</tr>
<tr>
<td>- Give 3rd pill</td>
<td>- BP, RPE, and GI symptoms every 15 min</td>
<td>- Urine</td>
<td>- Tc every 5 min</td>
</tr>
<tr>
<td>- Void bladder, ingest sugar solution</td>
<td>- 3.5 kg/mL water every 15 min</td>
<td>- BM</td>
<td>- Post</td>
</tr>
<tr>
<td>- Baseline</td>
<td>- Blood at 80 min</td>
<td>- Tc</td>
<td>- Blood</td>
</tr>
<tr>
<td>- Blood</td>
<td>- Urine</td>
<td>- BP, HR, RPE</td>
<td>- Urine</td>
</tr>
<tr>
<td>- Urine</td>
<td>- HR, BP</td>
<td>- GI symptoms</td>
<td>- BM, BP, HR</td>
</tr>
<tr>
<td>- Tc</td>
<td>- GI symptoms</td>
<td></td>
<td>- GI symptoms</td>
</tr>
</tbody>
</table>

### Figure 5.2. Experimental Procedures Schematic.
Includes information session and data collection. Blood measures include plasma osmolality, plasma sodium concentration, and cytokines TNF-α and IL-6. Urine measures include sugar excretion and urine specific gravity. Abbreviations: BM = body mass; BP = blood pressure; HR = heart rate; GI = gastrointestinal; RPE = rate of perceived exertion; Tc = core temperature.


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Dokladny K, Moseley PL, Ma TY. Physiologically relevant increase in temperature causes an increase in intestinal epithelial tight junction permeability. Am J Physiol Gastrointest Liver Physiol. 2006;290(2):G204-212.


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APPENDIX A. INFORMED CONSENT FORM

Title of the Study: Non-steroidal Anti-inflammatory Drugs on Gastrointestinal Permeability during Thermal Stress in Exercising Humans

Investigators:  Dawn M. Emerson, MS, ATC; J. Mark Davis, PhD; Toni M. Torres-McGehee, PhD, ATC; Stephen Chen, PhD

The purpose of this study is to determine the effects of the non-steroidal anti-inflammatory drug (NSAID) Naproxen on core body temperature (Tc), gastrointestinal (GI) permeability, inflammatory markers, and fluid regulation during exercise in a thermal environment. The study is being conducted to fulfill partial requirements for a Doctorate degree in exercise science - applied physiology. You are being invited to participate in this study because you are between the ages of 18 and 38yrs, endurance trained, and are in good general health. The study will take place at the Exercise Science Laboratories at the University of South Carolina’s Arnold School of Public Health. If you agree to participate, you will be participating with approximately 20 other people.

STUDY PARTICIPATION
After signing the informed consent form, potential participants will complete a Health and Injury History Questionnaire. Any potential participant with a health or safety concern will be required to have his/her questionnaire reviewed by the physician overseeing this study to determine inclusion or exclusion into the study.

Upon acceptance into the study, participants will complete a cycle test to measure VO2max. Participants will then be randomly assigned to complete four trials: control (C), placebo and heat stress (PHeat), Naproxen (Npx), and Naproxen and heat stress (NpxHeat). You will complete all four conditions in a random order with a minimum of 7 days in between each trial. You will be unaware of whether you are taking a placebo or Naproxen. During the course of this study, you will be asked to maintain normal levels of physical activity, normal state of hydration, and avoid changes in caffeine consumption and dehydration-inducing substances such as alcohol. The study will require you to perform a 90 minute cycle time trial and allow Tc, GI permeability, inflammatory markers, and hydration measurements before, during, and after the cycle bout. You will be provided fluids to consume throughout the sessions in order to maintain your hydration. The time commitment for each trial will be approximately 6 hours each time. Total time to complete all trials will be 24 hours.
CONDITIONS

Control
You will be given 3 placebo (no medicine) capsules with Take Home Instructions instructing you when to take each capsule. Upon arriving to the lab you will provide hydration measures, ensure the temperature sensor is working, and have a flexible catheter inserted into a vein in your arm for blood measures. You will complete a 90-minute cycle protocol in a cool environment (15°C/59°F, 30% humidity).

PHeat
You will be given 3 placebo (no medicine) capsules with Take Home Instructions instructing you when to take each capsule. Upon arriving to the lab you will provide hydration measures, ensure the temperature sensor is working, and have a flexible catheter inserted into a vein in your arm for blood measures. You will complete a 90-minute cycle protocol in a hot, humid environment (35°C/95°F, 30% humidity).

Npx
You will be given 3 over-the-counter doses of Naproxen with Take Home Instructions instructing you when to take each capsule. Upon arriving to the lab you will provide hydration measures, ensure the temperature sensor is working, and have a flexible catheter inserted into a vein in your arm for blood measures. You will complete a 90-minute cycle protocol in a cool environment (15°C/59°F, 30% humidity).

NpxHeat
You will be given 3 over-the-counter doses of Naproxen with Take Home Instructions instructing you when to take each capsule. Upon arriving to the lab you will provide hydration measures, ensure the temperature sensor is working, and have a flexible catheter inserted into a vein in your arm for blood measures. You will complete a 90-minute cycle protocol in a hot, humid environment (35°C/95°F, 30% humidity).

INSTRUMENTS AND PROTOCOLS

STEEP Test
Used to measure VO2max, this cycle test progressively increases in difficulty and respiratory variables are measured throughout. Participants with a VO2max between 50-60 ml/kg/min for males and 48-58 ml/kg/min for females will be considered endurance trained and qualify to participate in the study.

Naproxen
Naproxen (commonly known as Aleve), is an over-the-counter available NSAID. You will receive a 24-hour dosage (3 pills, 220mg per pill) with instructions on what time to take the pills (1 pill every 8 hours).

Placebo
During the placebo trials, you will be provided 3 pills that contain no medicine and are made to resemble the Naproxen. You will take 1 pill, every 8 hours.
Temperature Measures
Temperature will be continuously monitored using a flexible rectal thermometer. Thermometers are attached to a cable which inserts into a machine that records temperature. After inserting the thermometer you will not need to remove it until completion of the session. Minimal discomfort should be felt during exercise.

Blood and Urine Measures
To obtain the blood we will draw blood from a vein in your arm prior to the cycling exercise. A flexible catheter will be used so only one needle stick is required and you will complete the exercises. For each blood draw, we will take 1 tube (1.2 tsp). Approximately 9 blood draws will be done during one data collection session for a total of 5 tbsp (1/6th of what you would donate to the American Red Cross).

To obtain urine samples, participants will be provided a cup, and will be able to use a private restroom to collect the urine sample. You will be instructed to completely void your bladder into the cup during each urine collection. The cup will be labeled with your participant number.

GI Permeability, GI Bleeding, and Inflammatory Markers
Gastrointestinal permeability will be determined by the presence of ingestible sugar probes in urine samples. Upon arriving to the laboratory you will be given 150ml (about ¾C) of a sugar-water solution to consume. You will then provide urine samples throughout the session for a total of a 5 hour period.

To determine GI bleeding we will measure blood using take-home kits. Prior to each session you will be provide a kit, including instructions, and asked to collect the first stool sample after taking the first pill (Naproxen or placebo). You will then be asked to take the first stool sample following the exercise session. You will then bring the kit to the laboratory. Only your participant number will be used on take-home kits.

Inflammation will be determined by the presence of the inflammatory markers TNF-α and IL-6 in your blood sample.

Hydration and Electrolyte Measures
We will determine hydration status and electrolyte balance through urine and blood measures. Hydration measures will include P_{osm}, urine osmolality (U_{osm}), urine specific gravity (U_{SG}) and percent change in body mass (%BM). Electrolytes will be measured from P_{[Na^+] and potassium (P_{[K^+]})}.

Borg Scale for Rate of Perceived Exertion
We will measure your rate of perceived exertion before, every 15 minutes during, and after exercise.
Heart Rate and Blood Pressure
To ensure participants remain at safe limits and determine any effects from NSAID use, we will continuously measure heart rate. Blood pressure will be measured before, every 15 minutes during, and after exercise.

Gastrointestinal Symptom Index
GI distress before and after exercise will be assessed using a symptom questionnaire. The index is divided into 3 sections: 1) upper abdominal problems, 2) lower abdominal problems, and 3) systemic problems.

Cycle Time Trial
A 90-minute cycle time trial will be used to determine the participant's performance. Participants will cycle at a steady 70% VO2max for 80 minutes. Any participant that cannot complete this will be disqualified from the study. The last 10 minutes of the cycle trial will be a maximum effort for distance.

Diet and Activity Logs
You will be asked to complete an online form to track your diet and activity for 3 days before and 1 day after each data collection session. You will be given a login and password for your personal account.

SECURITY OF PERSONAL INFORMATION
Participation will be confidential. To assure your confidentiality, all information pertaining to this study and participation will be stored in a secure and locked cabinet. Additionally, a code number will be assigned to each participant at the beginning of the project. This number will be used on project records rather than your name, and no one other than the researchers will be able to link your information with your name. Data from all participants will be analyzed together. All blood and urine samples will be discarded after analysis. All data will be stored on a secure, password protected computer and only researchers will have access to the data. The results of this study may be reported in professional journals or presented at meetings; however, you will not be identified.

RISKS
There is a moderate risk for developing adverse effects due to NSAID use. Potential risks and side effects include: ulcers, bleeding, or holes, which may develop without warning, in the stomach or intestine; constipation; diarrhea; gas; sores in mouth; excessive thirst; headache; dizziness; lightheadedness; drowsiness; difficulty falling asleep or staying asleep; burning or tingling in the arms or legs; cold symptoms; ringing in the ears; hearing problems. Serious side effects include: changes in vision; feeling that the tablet is stuck in your throat; unexplained weight gain; sore throat, fever, chills, and other signs of infection; blisters; rash; skin reddening; itching; hives; swelling of the eyes, face, lips, tongue, throat, arms, hands, feet, ankles, or lower legs; difficulty breathing or swallowing; hoarseness; excessive tiredness; pain in the upper right part of the stomach; nausea; loss of appetite; yellowing of the skin or eyes; flu-like symptoms; bruises or purple blotches under the skin; pale skin; fast heartbeat; cloudy, discolored, or bloody urine; back pain; difficult or painful
urination. In the event you experience these symptoms, you may request to stop or the researchers will stop your trial.

There is a low probability that you experience a more serious risk during this study. Serious risks include, but are not limited to, heat illness and cardiac events. There is a risk of developing an exertional heat illness (for example, heat exhaustion and heat stroke) while exercising in a thermal environment. In the event that you experience symptoms of heat exhaustion, (nausea, vomiting, dizziness, etc.) you may request to stop or the researchers will stop your trial. In the case that you are suffering from a heat stroke, your trial will be immediately stopped and ice/cold water immersion will be available. Ice/cold water immersion is the best method available to quickly decrease the body’s Tc to a safe limit. The probability of developing a heat stroke during this study is minimal. We will continuously monitor Tc and cease the cycling protocol if and when you reach a Tc of 40°C (104°F) and/or when you begin to experience any of the symptoms mentioned above.

As with any exercise, there is a small risk of a cardiac event due to an undetected cardiac condition. Heart rate will be monitored continuously and blood pressure will be monitored every 15 minutes to help ensure your safety. All investigators are American Red Cross AED, CPR Professional Rescuer and First Aid certified.

Other risks of this study are general risks associated with exercise, such as muscle or tendon strains. Cycling is a low impact exercise and should minimize the risk of developing a muscle or tendon injury. There is no known risk for consuming the sugar solution.

Risks of drawing blood include temporary discomfort from the needle stick, bruising, tenderness and infection. Fainting could also occur. OSHA guidelines will be followed to minimizing these risks. Investigators will abide by all OSHA guidelines and will be able to act if there is an emergency or other medical issue during the study.

**PARTICIPANT SAFETY**

In the event you experience a heat stroke there will be a cold/ice water tub available for immersion. Ice water immersion is the fastest and safest method to cool the body to a safe Tc. In the event that you experience a sudden stop in your heart, there is access to an Automated External Defibrillator (AED), room 316 of the Public Health Research Center. All investigators are trained to use an AED and in CPR. If you experience any medical issues such as a heat illness or cardiac event as a participant in this study, the investigators will provide appropriate immediate care and you will be referred for additional medical treatment if necessary. However, in the event you suffer from a research related injury, there will be no financial compensation from the investigators or the University of South Carolina to assist with medical fees.

The investigators will terminate your participation in the study, without your consent, if you suffer any symptoms or conditions that the investigators believe make it inadvisable for you to continue in the study. In the case of a trial being terminated due to adverse symptoms an incident report will be completed and given to the physician overseeing the study. In the case of trial termination due to mild symptoms (dizziness, nausea, headache,
etc.) the participant may choose to continue the trial at another date. However, the participant cannot continue until the physician has reviewed the incident report and deems it appropriate for the participant to continue with the study. In the case of a more severe incident (for example, heat stroke) the participant will not be allowed to continue in the study.

**PARTICIPANT PAYMENT**

As a participant in this study, you will have the opportunity to earn $100.00. Participants will be paid $20.00 at the completion of each trial, for a total of $80.00 ($20.00 x four trials). Upon completion of the fourth trial, each participant will be given $20.00 if he or she follows all study instructions and provides maximum effort during all exercise trials.

**BENEFITS**

Participating in this study will increase your awareness of personal hydration behaviors. Each participant will receive a personalized hydration protocol that includes fluid and electrolyte needs during exercise. The primary investigator will also be available to discuss the individual reports and answer any specific questions the participant has in regards to fluid and electrolyte needs during exercise.

The information from this study will be helpful for competitive athletes as well as recreational athletes and athletic trainers regarding the use of NSAIDs in hot, humid environments.

**VOLUNTARINESS**

Participation is voluntary. You may decide not to participate at all, or to stop participating at any time, for any reason without negative consequences. Participants wishing to withdraw voluntarily from the study should notify the primary investigator (Dawn Emerson) by phone or email. Your participation, non-participation and/or withdrawal will not affect your grades or your relationship with your professors, college(s), or the University of South Carolina.

**QUESTIONS**

If you have any questions, please feel free to ask at any time. You should contact Dawn M. Emerson at (cell) (XXX) XXX-XXXX or (email) mintond@mailbox.sc.edu or Dr. Mark Davis at (office) (XXX) XXX-XXXX or (email) mard@mailbox.sc.edu if you have any questions or if you believe that you have suffered a research related injury.

If you have any questions about your rights as a research subject contact, Lisa Marie Johnson, IRB Manager, Office of Research Compliance, University of South Carolina, 901 Sumter Street, Byrnes 515, Columbia, SC 29208, Phone: (803) 777-7095 or LisaJ@mailbox.sc.edu. The Office of Research Compliance is an administrative office that supports the USC Institutional Review Board. The Institutional Review Board (IRB) consists of representatives from a variety of scientific disciplines, non-scientists, and community members for the primary purpose of protecting the rights and welfare of human subjects enrolled in research studies.
SIGNATURES
I have read the contents of this Consent Form. The purpose of this study, procedures to be followed, risks and benefits have been explained to me. I have been allowed to ask questions, and my questions have been answered to my satisfaction. I have been told whom to contact if I have additional questions. I agree to participate in this study, knowing that I may withdraw at any time. I have been given a copy of this Consent Form to keep for my reference.

__________________________
Signature of Participant

__________________________
Name (Please Print)

I have explained and defined in detail the research procedure in which the participant has agreed to participate and have offered him a copy of this informed consent form.

__________________________
Signature of Investigator

__________________________
Name (Please Print)
APPENDIX B. HEALTH AND INJURY HISTORY QUESTIONNAIRE

Instructions: Complete the following questions the best of your knowledge/ability. Let the investigator know if you need further explanation.

Part 1. Participant Information
Name: ________________________   Age: ______   Participant #: _____

Part 2. Physical Activity Readiness Questionnaire© (Canadian Society for Exercise Physiology)

1. Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor? □ Yes □ No
2. Do you feel pain in your chest when you do physical activity? □ Yes □ No
3. In the past month, have you had chest pain when you were not doing physical activity? □ Yes □ No
4. Do you lose your balance because of dizziness or do you ever lose consciousness? □ Yes □ No
5. Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity? □ Yes □ No
6. Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition? □ Yes □ No
7. Do you know of any other reason why you should not do physical activity? □ Yes □ No

Part 3. Medical History Explain “yes” answers below. (American Medical Society for Sports Medicine, American Orthopedic Society for Sports Medicine, American Heart Association, and American College of Sports Medicine)

8. Have you had a medical illness or injury since your last check up or sports physical? □ Yes □ No
9. Do you have an ongoing chronic illness? □ Yes □ No
10. Have you ever passed out during or after exercise? □ Yes □ No
11. Have you ever been dizzy or fainted during or after exercise? □ □

12. Have you had a medical illness or injury since your last check up or sports physical? □ □

13. Have you ever had pain, discomfort, tightness, or pressure in your chest during or after exercise? □ □

14. Do you get tired more quickly than your friends do during exercise? □ □

15. Have you ever had racing of your heart or skipped heartbeats? □ □

16. Have you had high blood pressure or high cholesterol? □ □

17. Have you ever been told you have a heart murmur? □ □

18. Has a physician ever denied or restricted your participation for sports or for any heart problems? □ □

19. Have you ever had a head injury or concussion? □ □

20. Have you ever been knocked out, become unconscious, or lost your memory? □ □

21. Have you ever had an unexplained seizure? □ □

22. Have you ever had numbness or tingling in your arms, hands, legs, or feet? □ □

23. Have you ever become ill from exercising in the heat? □ □

24. Do you cough, wheeze, or have trouble breathing during or after activity? □ □

25. Do you have diabetes? □ □

26. Do you have asthma or other lung disease? □ □

27. Do you smoke? □ □

28. Do you have or think you may have any bleeding disorders? □ □

29. Have you had any other problems with injury, surgery, pain or swelling in muscles, tendons, bones, or joints? □ □

If yes to question 29, check appropriate blank and explain below.
30. Do you have, or think you may have, any of the following conditions? Check all that apply.

- Nausea, vomiting, or other gastrointestinal disorders
- Obstructive disease of the gastrointestinal tract, such as diverticulitis or inflammatory bowel disease
- A history of disorders or impairment swallowing or of the gag reflex
- Any gastrointestinal surgery

Explain “Yes” answers here:

31. Do you get frequent muscle cramps when exercising? □ Yes □ No

32. Do you or someone in your family have sickle cell trait or disease? □ Yes □ No

33. Have you ever experienced an exertional heat stroke or other significant heat illness? □ Yes □ No

34. Are you currently taking any prescription medications, pills, or an inhaler? For example: Albuterol, Prednisone, Naproxen, etc. □ Yes □ No

Explain “Yes” answers here:

35. Are you currently and regularly taking any non-prescription (over-the-counter) medications or pills? For example: Bayer, Prilosec, NyQuil, etc. □ Yes □ No

Explain “Yes” answers here:
36. Are you currently and regularly taking any **vitamins, supplements, or other substances**?  
For example: multi-vitamin, iron, diet pills, energy drinks, protein powder, etc.  
☐ Yes  ☐ No  
Explain “Yes” answers here:  

*FEMALES ONLY*  
37. Is your menstrual cycle regular?  
☐ Yes  ☐ No  
38. Are you on birth control?  
☐ Yes  ☐ No  
39. Are you currently on your period?  
☐ Yes  ☐ No  
40. What is the date of your last period?  
______________________________  

I hereby state that, to the best of my knowledge, my answers to the above questions are complete and correct.  

Signature of Participant        Printed Name of Participant        Date  

University of South Carolina  
Athletic Training Program  
Blatt PE Center  
Columbia, SC 29208
APPENDIX C. TAKE HOME INSTRUCTIONS

C.1. First Trial Email to Participants 4 Days Prior to Data Collection

Dear __________________,

Thank you for participating in our study.

Starting _____________ we ask you to begin recording your diet and physical activity. First, you must initiate your account.

You will need to register on FoodProdigy. Use this online system to record all your daily intake of food and drinks as well as exercise and physical activity. This will allow us to look at your current habits throughout the study and mimic these for each trial. If you wish, you can receive a more detailed report of your information at the conclusion of the study.

To get started:
1. Go to www.foodprodigyonline.com/ui/registration and type in your email address.
2. Enter a unique Password.
3. Enter this Subscription ID: _____________________
4. Go to www.foodprodigyonline.com. Use the email and password you registered with to log in to this account.

Use the FoodProdigy™ program to enter your diet and exercise information for 4 days. At the conclusion of the 4th day send your report to our co-investigator - Dr. Toni Torres-McGehee - using FoodProdigy. You will see a link that states e-mail Toni Torres-McGehee.

We recommend you save a copy of the report for yourself so you can refer back to it.

Remember things to avoid during this time:
Red meat
Prescription or non-prescription NSAIDs
Strenuous exercise

If you have any questions regarding FoodProdigy or completing the diet/activity log in general, please feel free to contact myself or Dr. Torres-McGehee.

Please to arrive to the lab on ______________ at ______________ to pick up your pre-trial information. Attached to this email are directions to the Public Health Research Center.
Contact us immediately if you must reschedule your appointment or if you have any questions or concerns.

Sincerely,
Dawn M. Emerson

Toni M. Torres-McGehee
Associate Professor/Graduate AT Program Director
University of South Carolina
Blatt PE Center 218
Columbia, SC 29208

C.2. Reminder Email to Participants to Register and Complete Diet/Activity Log

Dear ________________,

This is a reminder to register for FoodProdigy and begin today to record your daily diet and physical activity. You may stop recording _______________ and then send your report to Dr. Torres-McGehee.

Please let me know if you have any questions regarding FoodProdigy or your daily diet/activity log.

Sincerely,
Dawn M. Emerson

C.3. Subsequent Trials Email to Participants 4 Days Prior to Data Collection

Dear ________________,

Starting ______________ we ask you to begin recording your diet and physical activity.

You must remember your login information for FoodProdigy (www.foodprodigyonline.com). Use the FoodProdigy program to enter all your daily intake of food and drinks as well as exercise and physical activity for 4 days. You will need to overwrite/clear your previous entries.

At the conclusion of the 4th day send your report to our co-investigator - Dr. Toni Torres-McGehee - using FoodProdigy. You will see a link that states e-mail Toni Torres-McGehee.

Remember things to avoid during this time:

Red meat
Prescription or non-prescription NSAIDs
Strenuous exercise
If you have any questions regarding FoodProdigy or completing the diet/activity log in general, please feel free to contact myself or Dr. Torres-McGehee.

Please to arrive to the lab on ______________ at ______________ to pick up your pre-trial information.
Attached to this email are directions to the Public Health Research Center.

Contact us immediately if you must reschedule your appointment or if you have any questions or concerns.

Sincerely,
Dawn M. Emerson
Toni M. Torres-McGehee
Associate Professor/Graduate AT Program Director
University of South Carolina
Blatt PE Center 218
Columbia, SC 29208

C.4. Pre-Data Collection Take Home Instructions

Thank you for participating in our research investigation!

Arrive to the lab on _____________________________, at your scheduled time ______________.

What should I wear?
Athletic shorts, a t-shirt, socks, and a pair of athletic shoes

Optional things I can bring?
Change of clothes and a towel for after exercising
A computer, book, or other material to use while you rest

What should I eat or drink before I come?
About 2 hours before coming to the lab you should eat a small meal (bagel, peanut butter and jelly, etc.)
We also ask that you arrive hydrated by drinking water

What things should I avoid during this time?
Alcohol Non-prescription medications
Energy supplements Dehydrating behaviors (sauna, diuretics, etc.)
Exercise Changes in your sleep or eating habits

What else do I need to do?
Attached to this letter is:
1. An envelope with 2 pills
   ✓ Take 1st pill at __________
   ✓ Take 2nd pill at __________

**Directions for taking pills:**
Take each pill with a glass of water
Do NOT take with food

2. A bag with a fecal test kit
   ✓ Collect one fecal sample **before** you come to the lab

**Directions:**
Take card out of the bag and open the card.
Apply a THIN smear of specimen on ONE window.
Do NOT cover the entire window.
Close the card and place back in bag. Throw away used stick.

3. A parking pass that you MUST bring with you to park at the Public Health Research Building. Use the handicap spaces on the left side of the parking lot behind the building.

Please contact us immediately if you need to reschedule your appointment or if you have any questions or concerns.

Sincerely,
Dawn M. Emerson

**C.5. Post-Data Collection Take Home Instructions**

We have a few things for you to complete after you leave the lab today.

1. Continue recording your diet and activity using the online program until you collect your next fecal sample.

2. Using your fecal take home test kit, please collect your **first** fecal sample **after** leaving the lab

**Directions:**
Take card out of bag and open the card. Apply a THIN smear of specimen to the SECOND window.

Do NOT pull the tab or alter the card in any other way. Close the card and place back in bag.

3. At the time of your fecal sample, complete the *Gastrointestinal Symptom Index* attached to this letter.

4. Return the fecal test kit and questionnaire by placing both items in the bag. **Return the bag on _____________ at _______________. At this time you will receive your payment.**

Please contact us immediately if you need to reschedule your appointment or if you have any questions or concerns.

Thank you for your participation,
Dawn M. Emerson
APPENDIX D. GASTROINTESTINAL SYMPTOM INDEXES

D.1. GI Symptom Index during Exercise

Are you CURRENTLY experiencing GI symptoms?

<p>| | |</p>
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<tbody>
<tr>
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<td>Worst it has ever been</td>
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<table>
<thead>
<tr>
<th>Symptom</th>
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<tbody>
<tr>
<td>Reflux/Heartburn</td>
<td>Left abdominal pain/stitch</td>
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<tr>
<td>Belching</td>
<td>Right abdominal pain/stitch</td>
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<tr>
<td>Bloating</td>
<td>Loose stool</td>
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<tr>
<td>Stomach pain/cramps</td>
<td>Diarrhea</td>
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<tr>
<td>Vomiting</td>
<td>Dizziness</td>
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<tr>
<td>Nausea</td>
<td>Headache</td>
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<tr>
<td>Intestinal cramps</td>
<td>Muscle cramps</td>
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<tr>
<td>Flatulence</td>
<td>Urge to urinate</td>
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<td>Urge to defecate</td>
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</table>
D.2. GI Symptom Index Pre- and Post-Exercise

Participant Number: ____  Trial Number: ____  Date: _______________  Time: __________

Directions: Please circle the number that corresponds to symptoms you are currently experiencing.

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Worst it has ever been
APPENDIX E. DATA COLLECTION PROCEDURE CHECKLISTS

E.1. Information Session

30 minutes prior to participant arrival

☐ Set out:
  - Informed Consent Form
  - Second ICF signature sheet
  - Information Session sheet
  - Health and Injury History Questionnaire
  - Serving Size document
  - Pens
  - Computer
    ▪ Ensure internet access
    ▪ Logon to FoodProdigy
  - Stethoscope and BP cuff
  - If doing \( \dot{V}O_{2\text{max}} \) Test
    ▪ Tanita scale
    ▪ See \( \dot{V}O_{2\text{max}} \) Procedures

Participant arrival

☐ Provide participant with ICF and second signature sheet
☐ Primary investigator reviews ICF and answers any questions
☐ Gather signed participant ICF
☐ Instruct participant to complete Health and Injury History Questionnaire
☐ Review information on diet/physical activity logging, show FoodProdigy online program
  - Review serving size document and give copy
☐ Assess participant’s resting HR and BP
☐ Gather participant’s self-reported height
  - Convert to cm (1 foot = 30.48 cm)
☐ If scheduled/able conduct \( \dot{V}O_{2\text{max}} \) test
☐ If not, schedule \( \dot{V}O_{2\text{max}} \) test
☐ Gather general information about available days/times to conduct data collection
☐ Provide Take Home Instructions if applicable

E.2. \( \dot{V}O_{2\text{max}} \) Testing

(30 minutes prior to participant arrival)

☐ Ensure room is at appropriate thermoneutral temperature
☐ Obtain equipment:
- Bike
- Heart rate strap
- Heart rate monitor
- RPE scale
- Metronome
- Pen
- Tanita scale
- “Information Session” sheet
- Water bottle for after test

- Ensure Monark is working (batteries in computer), pedals, break system, etc.

**Participant Arrival**
- Ensure participant is dressed appropriately
- Verbally inquire/verify participant has not eaten immediately before coming, received an appropriate amount of sleep and has not performed strenuous exercise within 24 hours
- Request participant remove shoes and socks
  - Take weight measure
- Give participant HR strap and ensure working
- Allow participant to sit and rest while you complete the following:
- Briefly explain the purpose of the test in layman terms
  - Aerobic fitness level
  - Measurement of the maximal amount of oxygen one can consume
  - Relative measure of the amount of endurance work one can perform
  - Done in all professional endurance athletes
  - Describe range of values for $\dot{V}O_2$ max and what our criteria is
  - Encourage your subject to ask questions before you move on
- Explain the cycling protocol
  - Cycle to complete exhaustion (physical fatigue)
  - Minutes per stage
  - Incremental increases in resistance
  - Speed should be constant throughout the study (50rpm)
  - Describe the physiological measurement including heart rate and RPE
    - Rate of perceived exertion (RPE)
      - Describe this chart very clearly to participant
    - ‘During the exercise test we want you to pay close attention to how hard you feel the exercise work rate is. This feeling should reflect your total amount of exertion, and fatigue, combining all sensations and feelings of physical stress, effort and fatigue. Don’t concern yourself with factors such as leg pain, shortness of breath or exercise intensity, but try to concentrate on your total inner feeling of exertion. Try not to underestimate or overestimate your feelings of exertion. Be as accurate as you can.’ - ACSM
  - Repeat: Cycle to complete exhaustion (physical fatigue)
- Explain termination criteria
Onset of angina or angina-like symptoms
- Signs of poor perfusion: lightheadedness, ataxia, pallor, nausea, cold and clammy skin
- Failure of HR to increase with increasing exercise intensity
- Participant requests to stop
- Failure of testing equipment
- Participant cannot keep up cadence (50 RPM) during stage

- Encourage your subject to ask questions about this section before you move on
- Assess participant’s resting HR and blood pressure

Running the VO\(_2^{\text{max}}\) test
- Position participant on bike
  - Seat height (5-10 degrees knee flexion at bottom position)
  - Handle bar setting
  - Make sure participant is comfortable before starting!
  - Record corresponding seat and bar height/position
- Explain clearly to subject that he/she will follow the metronome beeps for the cadence
- Ensure bike is set to 0 kp and allow participant to warm up for 2 minutes at set cadence
- Increase resistance (kp/watts) every 2 minutes as corresponding on the Information Sheet
- Record heart rate and RPE at the end of each stage
- *You may provide encouragement to the participant, but this must be consistent between participants*

Ending VO\(_2^{\text{max}}\) Test
- Once participant reaches termination criteria, immediately record heart rate, RPE, and time
- Immediately decrease/remove resistance
- Allow participant to actively cool down for 3-5 minutes
- Monitor heart rate to ensure it decreases appropriately
- Provide water to participant

Criteria for reaching VO\(_2^{\text{max}}\)
- Maximal heart rate within 10 beats of age-predicted max heart rate
- RPE > 17

Calculate VO\(_2^{\text{max}}\) on data sheet
Must be between ~ 40-50 ml/kg/min for males based on age and 31-40 ml/kg/min for females based on age and cycling vs running*

E.3. Pre-Trial Appointment

30 minutes before participant arrival
- Prepare Pre-Trial Take Home Instructions
- Fill out report time and date
- Envelope with 2 pills
- Fill out time to take each pill
- Bag with fecal occult blood test and two sample collection sticks
- Dated parking pass

**Participant arrival**
- □ Thoroughly review Take Home instructions with participant
- □ Answer any questions

**E.4. Post-Trial Appointment**

15 minutes before participant arrival
- □ Obtain participant payment
- □ Fill out receipt book

**Participant arrival**
- □ Observe fecal occult blood test and GI Symptom Index
- □ Give participant money
- □ Have participant sign receipt book and provide participant with receipt
- □ Verify next trial appointment

**E.5. Data Collection Session**

60-90 minutes before participant arrival
- □ Verify chamber is set to appropriate temperature
  - o Thermal: 35°C/95°F, 30% RH
    - ▪ If 32.5 then set humidity to 50%
  - o Thermoneutral: 15°C/59°F, 30%RH
    - ▪ If in room 317 check temperature
- □ Set out following equipment:
  - o Blood collection
    - ▪ Label 2 pink 6ml tubes for each blood time point (8 total)
      - • Participant #, Trial #, pre
      - • Participant #, Trial #, 80 min
      - • Participant #, Trial #, post
      - • Participant #, Trial #, 3hr post
  - ▪ Styrofoam cooler
    - • Fill ¾ with ice
  - o Gloves
  - o Sugars
- Urine volume containers and 3 cups
  - Label cups:
    - Participant #, trial #, pre
    - Participant #, trial #, post
    - Participant #, trial #, 3hr post
- 3 gallon cooler
  - Fill with ice and water
- 1 water bottle with cap, 1 water bottle without cap
- Graduated cylinder for water
- 3rd pill
- White plastic container with ice for urine
- HR monitor and strap
- Stop watch
- RPE chart
- GI symptom chart
- GI Symptom Index
- Refractometers
  - 2 clinical, 1 pen, and 1 digital
- Pens and sharpies
- Clipboards
- Pre Post Data Collection sheet and Cycling Protocol sheet
- 2.0ml microtubes
  - Label 4 urine tubes
    - Participant #, Trial #
  - Label minimum 2 plasma tubes for each blood time point
    (minimum 8 total)
    - Participant #, Trial #, pre
    - Participant #, Trial #, 80 min
    - Participant #, Trial #, post
    - Participant #, Trial #, 3hr post
- Distilled water
- Disposable pipettes
- Kestrel environmental monitor
- Rectal temperature probe and reader
- Tanita scale
- Ice chest and tarp
  - Fill chest with ice and place with tarp in men’s or women’s locker room

☐ Bike
  - Calibrate Computrainer See Procedures OR
  - Calibrate Monark See Procedures
  - Set bike up to participant’s previously determined set-up
☐ Set up Kestrel in chamber (or room 317) to verify environment
☐ Calibrate refractometers
  - Clinical refractometers
- Using a disposable pipette, put 2 drops of distilled water onto prism
- Close cover plate
- Hold up to light
- Adjust the correcting screw to make light/dark boundary coincide at 1.000
  - Pen refractometer
    - Turn Pen on
    - Using a cup of distilled water, place end of pen into water
      - Ensure prism is covered by water
    - Wait for reading
    - If not 0, press ZERO to calibrate
  - Digital refractometer
    - Turn refractometer on
    - Using disposable pipette, pipette distilled water on prism
    - Wait for reading
    - If not 0, press ZERO to calibrate
- Check centrifuge and set up if necessary
- Prepare sugar solution
  - 5g lactulose, 5g sucrose, 2g mannitol powder mixed with 150ml water
- Prepare water to be consumed during exercise
  - 3.5ml/kg every 15 minutes
  - Measure from 3 gallon cooler into graduated cylinder
  - Pour into designated water bottle
  - Set aside bottle without cap to be used to refill participant bottle

**Participant arrival**
- Confirm participant
  - Consumed a light breakfast and water
  - Consumed 2 pills at assigned times
  - Dressed appropriately
  - Inquire if participant has been logging diet/activity and collected 1 fecal sample
- Primary investigator inserts venous catheter and takes 2 6ml vials for pre-trial measure
- Give participant HR strap and ensure monitor is reading HR
- Give participant 3rd pill to consume with water
- Participant complete GI Symptom Index
- Assess pre-trial HR and BP
- Provide participant with urine cup and rectal probe
  - Instruct participant to provide pre-trial urine sample into cup, and to completely void bladder the rest of the way into the toilet
  - Instruct participant to insert rectal probe approximately 10cm past the anal sphincter (to marked line on probe)
- Measure urine specific gravity using clinical refractometer to ensure participant is < 1.020
□ Measure participants pre-trial body mass
  o Shirt and shorts
  o NO shoes and socks
□ Measure pre-trial core body temperature
□ Provide participant with sugar solution and instruct to consume within 5 minutes
□ Explain fluid consumption during trial
  o “You will be provided with a designated amount of water in a bottle. We have superficially measured out this amount of water for you in order to ensure you maintain hydration. You are to consume the entire contents of the bottle, **(their specific volume)** ml, every 15 minutes. We encourage you to drink a little bit throughout the entire 15 minutes and to not wait till the end to consume the whole amount. At the end of each 15 minute increment we will provide you with more water.”
  o Answer any questions regarding this
□ Take participant into chamber and orient him/her to the bike and room
□ Explain cycle exercise
  o “Based on your VO₂max test you will need to bike at an intensity that elicits a HR at **their HR corresponding to 70% VO₂max**. We have set up the bike for you. This display shows your rpm and time. Here is the HR monitor. You will bike at this workload and HR for 80 minutes. Every 15 minutes we will ask you RPE (show scale) GI symptoms (explain scale), take BP and refill your water bottle. We will have the probe plugged into the monitor so we can continuously monitor your core temperature and we can also continuously monitor your HR. At the end of the 80 minutes we will collect 2 6ml vials of blood.”
  o Answer any questions up to this point.
  o “At the end of 80 minutes we will ask you to finish the last 10 minutes of the cycle exercise in an ‘all-out effort’. Consider it a sprint to the finish. You want to go as fast and far as you can in 10 minutes.”
  o Answer any questions up to this point.
□ Record environment on Data Collection Cycle Protocol sheet
  o Check environment post-cycle, if different record on sheet
□ Co-investigators may run analysis and spin blood during cycle protocol
  o Follow **Blood Mapping** for appropriate procedures on handling, preparing, and freezing blood and plasma samples
  o Analyze urine samples with refractometers and record on Pre Post Data Collection sheet

**Cycle Protocol**
□ Instruct participant to get on bike
  o Ensure participant is comfortable on the bike
□ Allow participant to warm-up for 3 minutes
  o Answer any questions at this point and ensure their comfort
  o Check core temperature and HR are being monitored
□ At 2.5 minutes measure time point 0

236
- Core temperature
- HR
- BP
- RPE
- GI symptoms

☐ Begin 70% VO2max/HR portion
  - 5 minutes record core temperature, HR, RPE, and GI symptoms
    ▪ Remind participate to consume fluids
  - 10 minutes record core temperature, HR
    ▪ Remind participate to consume fluids
  - 15 minutes record core temperature, HR, BP, RPE, and GI symptoms
    ▪ Refill water bottle with designated fluid volume
  - 20 minutes record core temperature, HR
    ▪ Remind participate to consume fluids
  - 25 minutes record core temperature, HR
    ▪ Remind participate to consume fluids
  - 30 minutes record core temperature, HR, BP, RPE, and GI symptoms
    ▪ Refill water bottle with designated fluid volume
  - 35 minutes record core temperature, HR
    ▪ Remind participate to consume fluids
  - 40 minutes record core temperature, HR
    ▪ Remind participate to consume fluids
  - 45 minutes record core temperature, HR, BP, RPE, and GI symptoms
    ▪ Refill water bottle with designated fluid volume
  - 50 minutes record core temperature, HR
    ▪ Remind participate to consume fluids
  - 55 minutes record core temperature, HR
    ▪ Remind participate to consume fluids
  - 60 minutes record core temperature, HR, BP, RPE, and GI symptoms
    ▪ Refill water bottle with designated fluid volume
  - 65 minutes record core temperature, HR
    ▪ Remind participate to consume fluids
  - 70 minutes record core temperature, HR
    ▪ Remind participate to consume fluids
  - 75 minutes record core temperature, HR, BP, RPE, and GI symptoms
    ▪ Refill water bottle with designated fluid volume
  - 80 minutes record core temperature, HR, RPE, and GI symptoms
    ▪ Take blood sample
      ▪ Flush catheter with saline
    ▪ Record starting distance
  - 85 minutes record core temperature, HR
  - 90 minutes record core temperature, HR, BP, RPE, and GI symptoms
    ▪ Record end distance

☐ Allow participant to actively cool down for 5 minutes
  - Take post-cycle blood measure
    ▪ Flush catheter
allow to consume fluid and refill bottle if necessary
- monitor and record HR and core temperature
- at end of 5 minutes remove from bike
- instruct participant to towel off any sweat and remove shoes and socks
- weigh participant
- allow to rest for 10 minutes
  - record HR and core temperature
  - instruct to complete post- GI Symptom Index
- provide urine cup and urine volume container
  - instruct participant to provide small sample in urine cup
  - instruct participant to completely void urine into the large urine container
  - "Please Do NOT void into the toilet/urinal"
  - place urine volume container in white tub with ice
- participant may remove rectal thermometer
  - if participant chooses to remove it, remind them a final core temperature must be taken at the end of the 3 hours
- participant may change clothes at this point
  - instruct them to take care to not pull out the catheter
  - instruct them to complete this as efficiently as possible to avoid an extended period in the locker room/restroom
- while participant is gone
  - prepare fluid to be consumed during rest (provide amount enough ½ hour)
- once participant returns
- once finished participant rests for 3 hours
  - remind participant to consume fluids regularly to promote hydration and urine volume
  - instruct participant that any time he/she needs to void urine it must be done in the urine volume container
  - he/she is permitted to work, read, or watch something to occupy themselves over the next 3 hours; however, he/she cannot leave and should not be up being active
- every 1 hour venous catheter should be flushed and water bottle refilled
- approximately ten minutes before the end of the rest
  - provide GI Symptom Index
  - review Post-Trial Take Home Instructions
    - provide GI Symptom Index
    - set up return appointment
  - obtain 2 6ml vials of blood
    - remove catheter
  - weigh
  - core temperature (unless not inserted, then must reinsert and measure at end of rest)
- at end of rest
  - obtain urine sample in urine cup and void any remaining urine into urine volume container
  - instruct participant to remove rectal probe
☐ Answer any questions and finalize session

Post-trial
☐ Centrifuge all blood
  o Follow Blood Mapping
☐ Record total urine volume
  o Pipette aliquots of urine into the 4 microtubes to be frozen
☐ Record total fluid volume consumed
☐ Ensure all blood and urine samples are in freezer and appropriately labeled
☐ Ensure all biohazard/sharps are appropriately disposed of
☐ Clean all equipment
  o Water bottles
  o Wipe down bike
  o Rectal probe
  o Counters and tables
  o Cooler and cylinder (end of day)
☐ Put away all equipment
  o Kestrel
  o Scale
☐ Ensure data sheets are all in box
☐ Turn off all equipment and lock up all labs

Miscellaneous
☐ Avoid providing food unless participant absolutely is in need of food. In these instances, attempt to provide non-sucrose containing foods

In Case of Emergency
☐ Immediately notify primary investigator if not currently involved with incident
☐ Cardiac
  o Call 911, obtain AED from room 317
  o Begin CPR
☐ Exertional heat stroke
  o Confirm heat stroke
    ▪ CNS dysfunction and core temperature above 104°F/40°C
  o Immediately remove from environmental chamber and into locker room
  o Lay out tarp and place participant on tarp
  o Turn on cold water in shower
  o Dump ice over participant
  o Monitor core temperature and HR
☐ As soon as possible, call supervising physician
  o Dr. Brian Kiesler
☐ Notify supervising faculty advisor
  o Dr. Mark Davis
☐ Complete incident report.
Figure F.1. Male Mean Core Temperature during Cycling and Rest for Experimental Conditions
No significant differences between conditions at any time point
Figure F.2. Female Mean Core Temperature during Cycling and Rest for Experimental Conditions
No significant differences between conditions at any time point.
Figure F.3. Mean Core Temperature during Cycling and Rest for Gender.

*Significant difference between gender ($P < 0.05$).
Figure F.4. Mean IL-6 Pre-, Post-, and 3 Hours Post-Cycling for Males.
N = 4. Significant main effect for each condition ($P < 0.04$). aSignificantly greater than pre ($P = 0.014$) and 3 hr post ($P = 0.038$). bSignificantly greater than pre ($P = 0.042$).
Figure F.5. Mean IL-6 Pre-, Post-, and 3 Hrs Post-Cycling for Females.
N = 3. Significant main effect for Control (P = 0.022) and Npx (P = 0.017). aSignificantly greater than 3 hr (P = 0.046). bSignificantly greater than pre (P = 0.015).
Table F.1. Fluid Volume and Urine Volume for Gender by Experimental Conditions (M ± SD)

<table>
<thead>
<tr>
<th></th>
<th>Fvol (L)</th>
<th></th>
<th>Fvol (ml/kg)</th>
<th></th>
<th>Uvol (L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Exercise</td>
<td>Total</td>
<td>Exercise</td>
<td>Total</td>
</tr>
<tr>
<td>Aggregate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>1.7 ± 0.7(^a)</td>
<td>2.5 ± 1.3(^a)</td>
<td>19.0 ± 6.5</td>
<td>27.5 ± 11.9</td>
<td>1.1 ± 0.6</td>
</tr>
<tr>
<td>Females</td>
<td>1.1 ± 0.5</td>
<td>1.7 ± 0.7</td>
<td>16.3 ± 5.9</td>
<td>24.1 ± 7.2</td>
<td>0.9 ± 0.4</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>1.3 ± 0.5</td>
<td>1.9 ± 0.7</td>
<td>14.1 ± 3.4</td>
<td>20.3 ± 5.7</td>
<td>1.2 ± 0.8</td>
</tr>
<tr>
<td>Females</td>
<td>1.0 ± 0.3</td>
<td>1.5 ± 0.6</td>
<td>14.4 ± 5.5</td>
<td>22.8 ± 8.0</td>
<td>1.1 ± 0.5</td>
</tr>
<tr>
<td>Npx</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>1.9 ± 1.0(^b)</td>
<td>2.8 ± 1.8</td>
<td>20.7 ± 8.8</td>
<td>30.8 ± 16.8</td>
<td>1.3 ± 0.6</td>
</tr>
<tr>
<td>Females</td>
<td>0.9 ± 0.5</td>
<td>1.4 ± 0.7</td>
<td>12.1 ± 5.0</td>
<td>20.1 ± 7.1</td>
<td>0.8 ± 0.5</td>
</tr>
<tr>
<td>Heat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>1.7 ± 0.7</td>
<td>2.5 ± 1.2</td>
<td>18.8 ± 5.3</td>
<td>27.4 ± 10.1</td>
<td>1.1 ± 0.6</td>
</tr>
<tr>
<td>Females</td>
<td>1.4 ± 0.4</td>
<td>1.9 ± 0.7</td>
<td>20.7 ± 4.6</td>
<td>27.2 ± 6.1</td>
<td>0.8 ± 0.3</td>
</tr>
<tr>
<td>HeatNpx</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>2.0 ± 0.6(^a)</td>
<td>3.0 ± 1.4</td>
<td>22.5 ± 5.4</td>
<td>32.0 ± 12.8</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td>Females</td>
<td>1.3 ± 0.5</td>
<td>1.9 ± 0.9</td>
<td>17.9 ± 5.9</td>
<td>26.4 ± 7.4</td>
<td>0.7 ± 0.3</td>
</tr>
</tbody>
</table>

\(^a\)Significantly higher than females \((P < 0.05)\). \(^b\)Approached significance \((P = 0.055)\).
Figure F.6 Mean Systolic BP Pre-, Post-, and 3 Hrs Post-Exercise for Experimental Conditions by Gender.

Significant main effect over time ($F_{2,40} = 37.0, P < 0.001$) for males (a) and females (b). \(^a\)Significantly higher than female Npx post ($F_{1,10} = 8.5, P = 0.017$). \(^b\)Significantly higher than female NpxHeat post ($F_{1,10} = 5.8, P = 0.043$). \(^c\)Significantly higher than female Heat 3 hrs ($F_{1,10} = 6.9, P = 0.028$).
Table F.2. Diet and Exercise Overall and for Gender (M ± SD).

<table>
<thead>
<tr>
<th></th>
<th>Aggregate (N = 22)</th>
<th>Males (N = 14)</th>
<th>Females (N = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calories (kcal)</td>
<td>1522.2 ± 388.0</td>
<td>1660.1 ± 379.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1280.9 ± 281.8</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>67.3 ± 21.6</td>
<td>75.9 ± 19.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>52.3 ± 16.6</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>48.1 ± 19.9</td>
<td>51.4 ± 22.9</td>
<td>42.3 ± 12.5</td>
</tr>
<tr>
<td>Carb (g)</td>
<td>194.5 ± 62.7</td>
<td>206.7 ± 73.0</td>
<td>173.0 ± 32.9</td>
</tr>
<tr>
<td>Sodium (mg)</td>
<td>2401.1 ± 759.2</td>
<td>2650.9 ± 853.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1964.0 ± 180.0</td>
</tr>
<tr>
<td>Exercise&lt;sup&gt;a&lt;/sup&gt;</td>
<td>260.4 ± 78.9</td>
<td>226.0 ± 29.1</td>
<td>303.5 ± 105.0</td>
</tr>
</tbody>
</table>

N = number of logs reported (22/44). <sup>a</sup>Based on calories burned during activity 24 hrs prior to data collection. All reported activity categorized as low-sedentary. <sup>b</sup>Significantly greater than females (P < 0.04).
Table F.3. Rate of Perceived Exertion during Cycling for Gender by Experimental Condition (M ± SD)

<table>
<thead>
<tr>
<th>Cycle time (min)</th>
<th>Aggregate</th>
<th>Control</th>
<th>Npx</th>
<th>Heat</th>
<th>NpxHeat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>0</td>
<td>8 ± 2(^a)</td>
<td>10 ± 2</td>
<td>8 ± 2</td>
<td>9 ± 2</td>
<td>8 ± 3</td>
</tr>
<tr>
<td>15</td>
<td>9 ± 3(^a)</td>
<td>11 ± 2</td>
<td>10 ± 3</td>
<td>12 ± 1</td>
<td>10 ± 2</td>
</tr>
<tr>
<td>30</td>
<td>10 ± 3</td>
<td>12 ± 2</td>
<td>11 ± 4</td>
<td>11 ± 2</td>
<td>11 ± 2</td>
</tr>
<tr>
<td>45</td>
<td>11 ± 3</td>
<td>12 ± 2</td>
<td>11 ± 3</td>
<td>13 ± 1</td>
<td>12 ± 2</td>
</tr>
<tr>
<td>60</td>
<td>11 ± 2</td>
<td>12 ± 2</td>
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<td>12 ± 1</td>
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<tr>
<td>75</td>
<td>12 ± 2</td>
<td>12 ± 2</td>
<td>12 ± 3</td>
<td>13 ± 2</td>
<td>12 ± 3</td>
</tr>
<tr>
<td>80</td>
<td>12 ± 2</td>
<td>13 ± 2</td>
<td>12 ± 2</td>
<td>14 ± 2</td>
<td>12 ± 3</td>
</tr>
<tr>
<td>90</td>
<td>19 ± 2(^a)</td>
<td>18 ± 2</td>
<td>19 ± 2</td>
<td>18 ± 2</td>
<td>20 ± 1</td>
</tr>
</tbody>
</table>

\(^a\)Significantly different than females (P < 0.03).
\(^b\)Significantly less than females (P = 0.03).
Table F.4. Distance and Max Heart Rate during Cycling for Gender by Experimental Condition (M ± SD)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Distance (miles)</th>
<th>Max HR (bpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aggregate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>3.2 ± 0.7</td>
<td>177.2 ± 17.1</td>
</tr>
<tr>
<td>Females</td>
<td>2.8 ± 0.9</td>
<td>177.2 ± 15.0</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>3.4 ± 0.8</td>
<td>175.0 ± 16.6</td>
</tr>
<tr>
<td>Females</td>
<td>2.9 ± 0.9</td>
<td>176.6 ± 12.7</td>
</tr>
<tr>
<td>Npx</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>3.5 ± 0.7</td>
<td>179.5 ± 15.1</td>
</tr>
<tr>
<td>Females</td>
<td>3.0 ± 1.0</td>
<td>172.2 ± 15.5</td>
</tr>
<tr>
<td>Heat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>3.0 ± 0.7</td>
<td>176.3 ± 20.2</td>
</tr>
<tr>
<td>Females</td>
<td>2.7 ± 1.0</td>
<td>179.6 ± 17.6</td>
</tr>
<tr>
<td>NpxHeat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>3.0 ± 0.8</td>
<td>178.0 ± 20.3</td>
</tr>
<tr>
<td>Females</td>
<td>2.5 ± 0.9</td>
<td>180.2 ± 17.2</td>
</tr>
</tbody>
</table>
Table F.5. Mean, Maximum, and Serious (> 4) Gastrointestinal Symptom Scores for Experimental Conditions

<table>
<thead>
<tr>
<th>Condition</th>
<th>Upper</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>Lower</th>
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<th></th>
<th></th>
<th></th>
<th>Systemic</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Max</td>
<td>Mean ± SD</td>
<td>Serious</td>
<td>Max</td>
<td>Mean ± SD</td>
<td>Serious</td>
<td>Max</td>
<td>Mean ± SD</td>
<td>Serious</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
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<td></td>
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<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>2</td>
<td>0.1 ± 0.1$^b$</td>
<td>-</td>
<td>2</td>
<td>0.0 ± 0.1</td>
<td>-</td>
<td>1</td>
<td>0.1 ± 0.1$^f$</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post</td>
<td>2</td>
<td>0.2 ± 0.2</td>
<td>-</td>
<td>2</td>
<td>0.1 ± 0.1</td>
<td>-</td>
<td>8</td>
<td>0.9 ± 0.9</td>
<td>6%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3hr Post</td>
<td>5</td>
<td>0.2 ± 0.3</td>
<td>3%</td>
<td>3</td>
<td>0.2 ± 0.2$^{d,e}$</td>
<td>-</td>
<td>5</td>
<td>0.7 ± 0.7$^j$</td>
<td>6%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day Post$^k$</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5</td>
<td>0.3 ± 1.0</td>
<td>3%</td>
<td>2</td>
<td>0.2 ± 0.5</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Npx</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>2</td>
<td>0.1 ± 0.1</td>
<td>-</td>
<td>1</td>
<td>0.0 ± 0.1</td>
<td>-</td>
<td>2</td>
<td>0.2 ± 0.3</td>
<td>-</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Post</td>
<td>2</td>
<td>0.1 ± 0.2</td>
<td>-</td>
<td>2</td>
<td>0.0 ± 0.1</td>
<td>-</td>
<td>7</td>
<td>0.6 ± 0.6$^g$</td>
<td>5%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3hr Post</td>
<td>2</td>
<td>0.0 ± 0.1</td>
<td>-</td>
<td>1</td>
<td>0.0 ± 0.1</td>
<td>-</td>
<td>2</td>
<td>0.1 ± 0.2</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Day Post$^k$</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4</td>
<td>0.2 ± 0.8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>Heat</td>
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<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>0</td>
<td>0.0 ± 0.0$^a$</td>
<td>-</td>
<td>1</td>
<td>0.0 ± 0.0</td>
<td>-</td>
<td>3</td>
<td>0.2 ± 0.3</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post</td>
<td>2</td>
<td>0.3 ± 0.3$^c$</td>
<td>-</td>
<td>2</td>
<td>0.1 ± 0.1</td>
<td>-</td>
<td>8</td>
<td>0.7 ± 0.9$^h$</td>
<td>3%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3hr Post</td>
<td>1</td>
<td>0.0 ± 0.1$^a$</td>
<td>-</td>
<td>2</td>
<td>0.1 ± 0.1</td>
<td>-</td>
<td>6</td>
<td>0.4 ± 0.6</td>
<td>3%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day Post$^k$</td>
<td>6</td>
<td>1.3 ± 2.5</td>
<td>18%</td>
<td>6</td>
<td>1.9 ± 2.4</td>
<td>6%</td>
<td>6</td>
<td>1.3 ± 2.2</td>
<td>5%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NpxHeat</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>2</td>
<td>0.1 ± 0.2</td>
<td>-</td>
<td>1</td>
<td>0.0 ± 0.0</td>
<td>-</td>
<td>5</td>
<td>0.3 ± 0.6</td>
<td>3%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post</td>
<td>2</td>
<td>0.2 ± 0.3</td>
<td>-</td>
<td>1</td>
<td>0.0 ± 0.1</td>
<td>-</td>
<td>3</td>
<td>0.3 ± 0.4$^i$</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3hr Post</td>
<td>4</td>
<td>0.1 ± 0.2</td>
<td>-</td>
<td>2</td>
<td>0.1 ± 0.1</td>
<td>-</td>
<td>3</td>
<td>0.2 ± 0.2</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day Post$^k$</td>
<td>3</td>
<td>0.1 ± 0.6</td>
<td>-</td>
<td>7</td>
<td>0.7 ± 2.1</td>
<td>12%</td>
<td>2</td>
<td>0.1 ± 0.5</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$Significantly less than Heat post ($Z = -2.3$, $P < 0.019$). $^b$Significantly greater than Heat pre ($Z = -2.1$, $P = 0.038$). $^c$Significantly greater than Npx post ($Z = -2.2$, $P = 0.027$). $^d$Significantly greater than Npx 3hr ($Z = -2.0$, $P = 0.048$), HeatNpx 3hr ($Z = -2.4$, $P = 0.014$). $^e$Significantly greater than Control pre ($Z = -2.4$, $P = 0.016$), post ($Z = -2.1$, $P = 0.039$). $^f$Significantly less than post ($Z = -2.4$, $P = 0.018$), 3hr post ($Z = -2.2$, $P = 0.026$). $^g$Significantly greater than 3hr ($Z = -2.1$, $P = 0.035$). $^h$Significantly greater than pre ($Z = -2.0$, $P = 0.042$). $^i$Significantly less than Control post ($Z = -2.0$, $P = 0.046$). $^j$Significantly greater than Npx 3hr ($Z = -2.4$, $P = 0.018$), Heat 3hr ($Z = -2.0$, $P = 0.042$). $^k$Varying sample size (n = 3-5) and not included in Wilcoxon analysis.
Table F.6. Mean and Serious (> 4) Gastrointestinal Symptom Scores for Experimental Conditions during Exercise

<table>
<thead>
<tr>
<th>Condition</th>
<th>Upper</th>
<th></th>
<th>Lower</th>
<th></th>
<th>Systemic</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Serious</td>
<td>Mean ± SD</td>
<td>Serious</td>
<td>Mean ± SD</td>
<td>Serious</td>
</tr>
<tr>
<td>Control</td>
<td>0.3 ± 0.6</td>
<td>1%</td>
<td>0.4 ± 0.8</td>
<td>-</td>
<td>0.3 ± 0.4</td>
<td>10%</td>
</tr>
<tr>
<td>Npx</td>
<td>0.4 ± 0.7</td>
<td>1%</td>
<td>0.1 ± 0.3</td>
<td>-</td>
<td>0.2 ± 0.3</td>
<td>10%</td>
</tr>
<tr>
<td>Heat</td>
<td>0.6 ± 1.0</td>
<td>3%</td>
<td>0.3 ± 0.6</td>
<td>-</td>
<td>0.1 ± 0.3</td>
<td>7%</td>
</tr>
<tr>
<td>NpxHeat</td>
<td>0.3 ± 0.9</td>
<td>2%</td>
<td>0.3 ± 0.7</td>
<td>-</td>
<td>0.0 ± 0.8a</td>
<td>1%</td>
</tr>
</tbody>
</table>

*aSignificantly less than Control (Z = -2.0, P = 0.046).