Mirabegron and Physiological Function in Cold Environments

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Abbreviations

This page is optional. The list below includes some common abbreviations. However, this list should be customized for each protocol (i.e., abbreviations not used should be removed and new abbreviations used should be added to this list).

ECG	Electrocardiogram
DEXA	Dual-energy X-ray absorptiometry
IPAQ	International Physical Activity Questionnaire
PAR-Q+	Physical Activity Readiness Questionnaire Plus

1.0 Background & Rationale

Specialized Navy personnel are required to undertake duties while immersed in cold water for prolong periods. These dives regularly require the use of external thermal protection (i.e., wet suits and dry suits). However, external thermal protection during prolonged cold-water diving is often times insufficient to maintain body temperature and thermal comfort, thereby potentially negatively impacting the safety and success of diving missions. Accordingly, developing an alternative strategy that can improve tolerance to cold-water immersion is of important interest to Navy divers and special forces.

Mirabegron (Myrbetriq®, extended-release tablet, Astellas Pharma), is a medication approved by the Food and Drug Administration for the treatment of overactive bladder; the mechanism of action is stimulation of the beta-3-adrenergic receptor (1). However, mirabegron has additional effects that include the activation of beta-3-adrenergic receptors located on brown adipose tissue (1). Brown adipose tissue is considered to be highly thermogenic and contributes to non-shivering thermogenesis (i.e. the generation of heat in the absence of movement). Recent evidence indicates that resting energy expenditure (a direct reflection of thermogenesis) and supraclavicular skin temperature (an indicator of brown adipose tissue activation) are elevated for 3-4 hours after the acute administration of mirabegron in single doses ranging from 100 mg to 200 mg (2). However, it is not known which dose of mirabegron exerts the longest lasting thermogenic effects. Importantly, plasma concentrations of mirabegron peak 3-4 hours after administration (3, 4) and the biological half-life of mirabegron is 40-50 hours (4). Thus, the thermogenic effects of mirabegron might extend past 4 hours. This is important because many cold-water dives last longer than 4 hours.

2.0 Objective(s)

2.1 Primary Objectives

Specific Aim 1:

a. Determine if acute mirabegron administration will increase thermogenesis during 6 hours of a mild cold stress challenge.

b. Determine the dose of acute mirabegron administration that will produce the greatest increases in thermogenesis during 6 hours of a mild cold stress challenge.

Specific Aim 2:

a. Determine if acute mirabegron administration will delay the fall in core temperature during a progressive cold-water immersion challenge.

b. To determine if acute mirabegron administration will delay the onset of shivering during a progressive cold-water immersion challenge.

2.2 Secondary Objective

Specific Aim 1:

Determine if core temperature, mean skin temperature (6 thermocouples), heart rate (ECG), blood pressure (brachial artery auscultation), indices of shivering (e.g., oxygen consumption, surface mechanomyography (using 3 triaxial accelerometers) and the bedside shivering assessment scale), thermal perceptions (thermal comfort and thermal sensation), and brown adipose tissue activation using infrared thermography are influenced by the dose of mirabegron 6 hours of a mild cold stress challenge.

Specific Aim 2:
Determine if the shivering inflection point during the progressive cold-water immersion challenge is shifted following mirabegron ingestion.

2.3 Tertiary/Exploratory/Correlative Objectives

Specific Aim 3:
To determine if variations in gut microbiome and their metabolites between participants explains the variability associated with different doses of acute mirabegron administration.

We will correlate measures of body composition derived from dual-energy X-ray absorptiometry (DEXA) to primary and secondary outcome measures.

3.0 Outcome Measures/Endpoints

3.1 Primary Outcome Measures

Specific Aim 1:
Thermogenesis (i.e., resting energy expenditure) and thermogenesis area under the curve during the protocol. Thermogenesis will be measured using whole body indirect calorimetry, calculated using the Weir formula (5), will be measured every 30 minutes during the 6 hour protocols. Area under the curve for thermogenesis will be calculated to reflect the cumulative effect of mirabegron over time.
Specific Aim 2:
Core temperature deflection point during the progressive cold-water immersion challenge. Core temperature will be continuously measured using rectal thermistors. The point at which core temperature begins to fall (i.e., deflection point) will be identified using Prism 8 software by plotting core temperature vs. water temperature.
Specific Aim 3:
Gut microbiota phylotypes, fecal mirabegron concentration, and serum mirabegron concentration will be measured from samples obtained during the study (serum samples: pre-mirabegron/placebo and following 6 h of mild cold exposure; fecal samples: first bowel movement following 6 h of mild cold exposure). Samples will be assessed using bacterial DNA extraction and purification, polymerase chain reaction, and MinION analysis as well as metabolomics.

3.2 Secondary Outcome Measures

Specific Aim 1:
Core temperature, mean skin temperature (6 thermocouples), heart rate (ECG), blood pressure (brachial artery auscultation), indices of shivering (e.g., oxygen consumption, surface mechanomyography (using 3 triaxial accelerometers) and the bedside shivering assessment scale), thermal perceptions (thermal comfort and thermal sensation), and brown adipose tissue activation using infrared thermography
Specific Aim 2:
The shivering inflection point during the progressive cold-water immersion challenge. Shivering will be assessed via oxygen consumption, surface mechanomyography (using 3 triaxial accelerometers), and the bedside shivering assessment scale. The core temperature immediately prior to the onset of shivering during the progressive cold-water challenge will be identified using Prism 8 software by plotting shivering vs. core temperature.

3.3 Tertiary/Exploratory/Correlative Outcome Measures

Specific Aim 3:
Serum mirabegron metabolites will be used to determine the mirabegron metabolism using metabolomics.

Body composition will be measured using DEXA once after informed consent during the screening visit. We will run exploratory correlational analyses among the other physiological data collected and body composition.

4.0 Eligibility Criteria

4.1 Inclusion Criteria

List the criteria:

- Men and women
- 18-40 years old
- Participate in 150 minutes or more of at least moderate intensity exercise per week during the previous 2 years

4.2 Exclusion Criteria

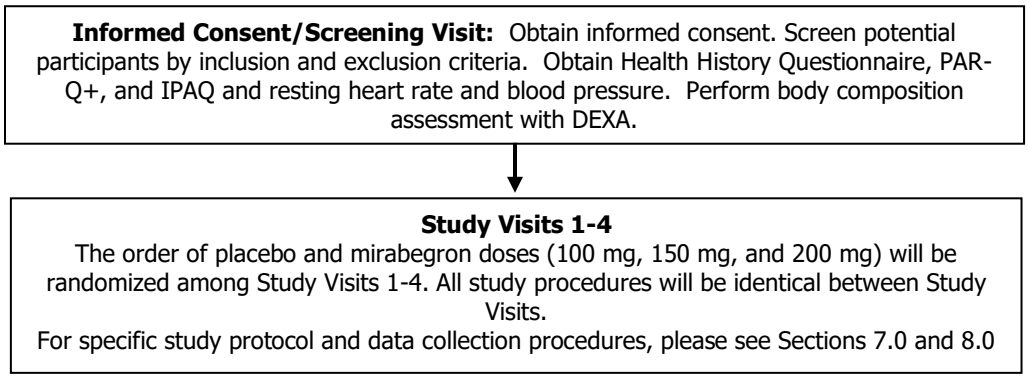
List the criteria:

- Any autonomic cardiovascular, metabolic, neurologic, endocrine, or respiratory disease
- Previously diagnosed liver and/or kidney dysfunction
- Women who are pregnant or breastfeeding
- Individuals currently taking a medication that cannot be safely discontinued for 5 biological half-lifes prior to each study visit based on consultation with the study physician. Birth control (including hormonal contraception) will be permitted despite potential to influence vasomotor activity.
    -Increases in heart rate and blood pressure are common with the higher doses of mirabegron that we will be testing. Additionally, medications that influence vasomotor activity may also influence thermoregulation. Therefore, for participant safety, we do not want to study participants that are taking a medication that might interfere with our ability to properly monitor and interpret the potential side effects of mirabegron during cold exposure.
- Current tobacco or electronic cigarette use or consistent use within the last 1 year

5.0 Study Design

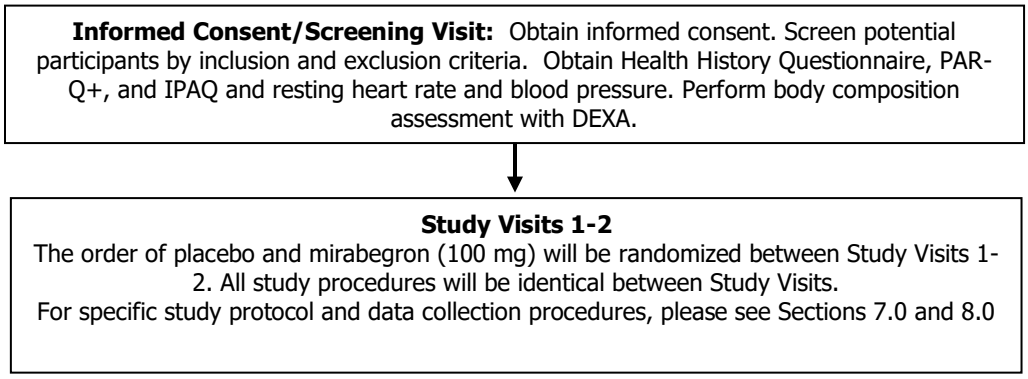
Specific Aim 1: This is a randomized, double-blind, placebo-controlled, cross-over experimental design. Participants will be asked to complete 5 visits to the laboratory; one informed consent/screening visit and 4 study visits. During the informed consent/screening visit, participants will complete the informed consent, eligibility criteria will be presented, resting blood pressure and heart rate (derived from the blood pressure measurement) will be measured, and participants will complete the following questionnaires: iPAQ, PAR-Q, and Health

History Questionnaire. During the study visits, participants will undergo baseline measurements (described in detail in Section 7.0) followed by ingesting either a placebo or 3 different doses of mirabegron (100 mg, 150 mg, or 200 mg). Mirabegron will be prescribed by our study physician, Dr. Andrew Watters. Dr. Watters is a physician in the Department of Emergency Medicine at Indiana University Health and he is a Fellow in Wilderness Medicine through the Wilderness Medical Society as well as a member of the Indiana Task Force 1 (part of the FEMA Urban Search and Rescue System. Dr. Watters will determine if it is safe for a potential participant to stop their medication for 5 half-lifes during screening. Dr. Watters will also review health history, resting heart rate and blood pressure measurements, and contraindications for mirabegron use to determine eligibility. A second set of baseline measurements will be made prior to participants entering our whole-body indirect calorimeter set at 20°C (68°F) for 6 hours. Every 30 minutes in the chamber, participants will be asked to assume the supine position to record resting energy expenditure (i.e., thermogenesis) and other physiological variables (described in detail in Section 7.0). This design will allow us to determine which dose of mirabegron produces the greatest increase in thermogenesis.



Specific Aim 2: This is a randomized, double-blind, placebo-controlled, cross-over experimental design. Participants will be asked to complete 3 visits to the laboratory; one informed consent/screening visit and 2 study visits. During the informed consent/screening visit, participants will complete the informed consent, eligibility criteria will be presented, resting blood pressure and heart rate (derived from the blood pressure measurement) will be measured, and participants will complete the following questionnaires: iPAQ, PAR-Q, and Health History Questionnaire. During the study visits, participants will undergo baseline measurements (described in detail in Section 7.0) followed by ingesting either a placebo or one dose of mirabegron (100 mg) prior to entering our water immersion tank. Mirabegron will be prescribed by our study physician, Dr. Andrew Watters (Department of Emergency Medicine, Indiana University Health). The dose of mirabegron that will be used for Specific Aim 2 is dependent on our findings from Specific Aim 1. The dose of mirabegron that elicits the greatest increase in thermogenesis in Specific Aim 1 will be used for Specific Aim 2. A second set of baseline measurements will be made and then the water immersion tank will then be rapidly filled with 35°C (95°F) water. The temperature of the water will be progressively lowered by ~12°C every 60 minutes until the water temperature reaches 10°C (50°F) or until the participant can no longer tolerate the cold or if rectal temperature reaches 35.5°C (95.9°F). Participants will be continuously monitored for rectal temperature, indices of shivering, skin temperature, heart

rate, blood pressure, and thermal perceptions (described in detail in Section 7.0). This design will allow us to determine if mirabegron improves cold water immersion tolerance.



Specific Aim 3: This aim will be addressed using the study design and visits used for Specific Aim 1 (a randomized, double-blind, placebo-controlled, cross-over experimental design). During these study visits, we will collect whole blood at pre-mirabegron/placebo and 15 minutes post the 6 h protocol. The whole blood will be allowed to clot and we will obtain serum samples for analyses. Participants will also be given fecal sample collection kits that they will take home to be used to collect fecal samples from their first bowel movement following each study visit. The serum and fecal samples will be sent to a colleague at Rutgers University for analyses. All samples will be de-identified prior to being sent to Rutgers University.

6.0 Enrollment/Randomization

We plan to recruit 60 total participants for this project (30 for Specific Aim 1 and 30 for Specific Aim 2). Participants who partake in Specific Aim 1 will also be used for Specific Aim 3 and can participate in Specific Aim 2. Two informed consent forms will be used for Specific Aim 1 & 3 (Mirabegron and Thermogenesis in Cold Air) and Specific Aim 2 (Mirabegron and Thermogenesis in Cold Water).

Participants will be recruited from the IU student body and local citizen population through flyers placed around campus, announcements made to classes of students, and word of mouth. Participants who are interested will contact the principal investigator or IRB approved personnel by email or by phone. This population may include IU students and individuals outside the IU community.

Potential participants will be given the basic information about the study and schedule an appointment for screening if they indicate that they would be interested in participating. Participants will be provided with detailed description of study procedures prior to study enrollment. Participants will be asked to read the consent form at this time and encouraged to ask any questions. Participants can then: 1) sign the consent form; 2) take it home for further consideration; or 3) decide not to participate. Participants will be screened once written informed consent has been given.

Both Specific Aim 1 (& 3) and Specific Aim 2 study visits will be completed in a randomized order. Randomization of the order of the study visits will be completed by a research technician using a

randomization list that will be created by a trained statistician external to the research team using R (R Foundation for Statistical Computing, Vienna, Austria) and our subjects will be randomized by our research technician.

7.0 Study Procedures

All of the below mentioned procedures are being conducted for purposes of the research.

- Height: Body height will be measured using a stadiometer.
- Weight: Weight will be measured using a scale in a private room.
- Pregnancy test: To ensure female participants are not pregnant they will undertake a urine pregnancy test during screening and before each study visit.
- Urine Specific Gravity: A sample of urine will be used to assess urine specific gravity using a refractometer.
- Heart rate: Heart rate will be obtained using a 3-lead electrocardiogram.
- Arm Blood Pressure: Blood pressure will be monitored using a cuff placed on the upper arm that is inflated and deflated periodically. Blood pressure measurements will be taken at several different time points. Heart rate will also be derived from this measure during the informed consent/screening visit. Each measurement will last approximately 30 seconds.
- Arterial Oxygen Saturation: Pulse oximetry will be used at the forehead to obtain continuous arterial oxygen saturation values.
- Partial Pressure of End Tidal CO_2: A nafion tube will be connected to a small port in the mouthpiece to measure end tidal partial pressure of CO_2.
- Ventilation: A pneumotach connected to a mouthpiece or facemask will be used to measure the flow of inspired and expired air. Expired gases will be sampled using oxygen and carbon dioxide sensors to determine oxygen consumption and carbon dioxide production.
- Core temperature: Participants will self-insert a single use rectal temperature probe in a private room. A small (~3 mm in diameter), single use, lubricated, flexible probe will be inserted by the participant to a pre-marked depth of ~10 cm.
- Skin Temperature: Fourteen iButtons will be adhered to the skin using adhesive tape on the calf, shin, front thigh, back thigh, foot, chest, upper back, lower back, forearm, hand, forehead, fingertip, toetip, and abdomen area to measure skin temperature.
- Perceptual Scales: Participants will complete Likert scales to assess their thermal comfort (1 = comfortable, 4 = very uncomfortable) and thermal sensation (1 = cold, 4 = neutral, 7 = hot).

- Bedside Shivering Scale: Two investigators will subjectively assess shivering by the participants using a Likert scale (0 = none, 3 = Severe) and the scores will be averaged.

- Surface Mechanomyography: Three triaxial accelerometers adhered to the skin using adhesive tape on the chest, upper back, and thigh will be used to assess shivering.

- Resting Energy Expenditure (thermogenesis): Expired oxygen, carbon dioxide, and ventilation will be collected from the whole-body calorimetry and used to calculate thermogenesis using the abbreviated Weir formula (5).

- International Physical Activity Questionnaire: Participants will complete this form to self-report and quantify their level of physical activity.

- Health history and demographics questionnaire: Participants will complete a health history questionnaire to ensure they meet the inclusion and exclusion criteria.

- Health history update questionnaire: Upon each visit to the laboratory participants will complete the heath history update questionnaire to ensure no changes in their health history has occurred.

- Infrared Thermography Images: An infrared thermography camera will be used to measure skin temperature in the supraclavicular (i.e., collar bone) region. This provides a non-invasive estimate of brown adipose tissue activation (6).

- PAR-Q+: Participants will complete this questionnaire to ensure they meet the inclusion and exclusion criteria.

- 3-Day Dietary Log: Myfitnesspal.com (Under Armour) is a dietary log that will be used to quantify the type and amount of food and fluid that participants consume 3 days prior to each study visit.

- Body Composition: Participants will have their body composition measured using DEXA.

- Whole Blood Collection: 15 mL of whole blood will be obtained from an antecubital vein at pre-mirabegron/placebo and at 15 minutes post protocol using standard aseptic techniques defined by the World Health Organization.

- Fecal Sample Collection: Participants will be given fecal collection kits (DNA Genotek) and will be given verbal instructions from the collection kit directions on how to obtain samples as well as the pamphlet that contains these directions the after the study visits of Specific Aim 1. Participants will be instructed to obtain samples from their first bowel movement after each study visit.

Timeline of Procedures

Specific Aim 1

- Informed Consent/Screening Visit: Once participants arrive at the laboratory, an investigator will obtain written informed consent after all procedures and study risks are fully explained and all questions answered. After obtaining written informed consent, participants will complete a Health History and Demographics Questionnaire, an International Physical Activity Questionnaire (IPAQ), Physical Activity Readiness Questionnaire (PAR-Q+). An experienced investigator will administer these questionnaires. These individuals can fully explain any technical terms and answer any inquiries related to the questionnaire. A resting heart rate and arm blood pressure measurement will be done after the questionnaires are complete. Participants will then have their body composition measured using DEXA. If DEXA cannot be performed during this visit, an additional visit can be scheduled. Participants will also visit with Dr. Watters to review medical history and resting blood pressure and heart rate measurements to review any contraindications for taking mirabegron. The visit with Dr. Watters can be in person or through a scheduled Zoom call to reduce participant scheduling burden. In the case of a Zoom call, Dr. Watters will be provided the medical history and resting blood pressure and heart rate measurements prior to the call.

- Study Visits: Mirabegron has a half-life of ~50 hours so all study visits will be separated by at least a 10-day washout period. Our goal will be to have 10-14 days in between study visits. Participants will report to the temperature and humidity-controlled laboratory in the morning following a 12-hour fast, and 24-hour abstention from exercise, caffeine, and alcohol. Participants will bring their dietary logs for review to ensure a consistent diet prior to each study visit. Participants will consume a light standard breakfast (237 ml Ensure Original; 220 kcal) upon arrival and be instrumented for skin temperature (12-sites: calf, shin, front of the thigh, back of the thigh, chest, upper back, forehead, lower back, abdomen, forearm, hand, and foot; iButtons) core temperature (rectal thermistor), indices of shivering (surface mechanomyography using triaxial accelerometers at 3 anatomic sites (chest, upper back, and thigh) and the bedside shivering assessment scale), brachial artery blood pressure (brachial artery auscultation), and heart rate (3-lead ECG). Thermal perceptions will be assessed using Likert scales for thermal discomfort and thermal sensation. Participants will be shirtless (sports bra for women) and will wear shorts throughout the study. After 20 minutes of quiet resting in the supine position, 5 minutes of baseline measurements will be taken, thermal perceptions and an infrared thermography image of the supraclavicular fossa will be obtained as an indicator of brown adipose tissue activation (6), and a whole blood sample will be obtained. Whole blood samples will be allowed to clot at room temperature (~15-30 minutes). The clot will be removed via centrifuging the samples at 1,500 g for 10 minutes. The serum samples will then be frozen (-80°C) until they are shipped on dry ice to Rutgers University for analysis. Participants will then ingest mirabegron (100 mg, 150 mg, or 200 mg) or placebo. Thirty minutes after mirabegron or placebo has been ingested, participants will enter the whole-body indirect calorimeter; the internal temperature of the calorimeter will be set to 20°C (68°F). This will elicit a mild cold stress over the 6 hours of observation in the whole-body indirect calorimeter. The whole-body indirect calorimeter provides an accurate and continuous measure of thermogenesis (in the form of energy expenditure) that can be used over

long periods of time in a stable environment. Our whole-body calorimeter is designed to perform human studies that are up to 48 hours in duration and contains a private washroom. While in the whole-body indirect calorimeter, participants will be instructed to be sedentary and will be allowed to watch television or read. In order to stay consistent between study visits, participants will be instructed to spend the same amount of time watching television or reading for each study visit. Every 30 minutes, we will record skin temperatures, core temperature, blood pressure, and heart rate. Thermogenesis will be determined by having participants lie supine for 30 minutes and resting energy expenditure will be calculated using the abbreviated Weir formula every hour (5). Thermal perceptions and an infrared thermography image will also be obtained at these time points. At approximately the midpoint of the 6-hour measurement period, participants will have the opportunity to use the restroom and an additional urine sample will be collected at this time. At the end of the 6-hour measurement period, participants will exit the whole-body indirect calorimeter and final measurements of skin temperature, core temperature, heart rate, and blood pressure will be obtained as well as a final infrared thermography image and a whole blood sample. After the study, participants will be given a mylar blanket to re-warm themselves prior to leaving the laboratory. Participants will be given a fecal collection kit that they will take with them. Instructions will be provided (both verbal and written) on the proper handling of their sample. If participants complete the informed consent/screening visit and all study visits, we expect that they will spend a total of ~33 hours in the study.

Specific Aim 2

- Informed Consent/Screening Visit: Once participants arrive at the laboratory, an investigator will obtain written informed consent after all procedures and study risks are fully explained and all questions answered. After obtaining written informed consent, participants will complete a Health History and Demographics Questionnaire, an International Physical Activity Questionnaire (IPAQ), Physical Activity Readiness Questionnaire (PAR-Q+). An experienced investigator will administer these questionnaires. These individuals can fully explain any technical terms and answer any inquiries related to the questionnaire. A resting heart rate and arm blood pressure measurement will be done after the questionnaires are complete. Participants will then have their body composition measured using DEXA. If DEXA cannot be performed during this visit, an additional visit can be scheduled. Participants will also visit with Dr. Watters to review medical history and resting blood pressure and heart rate measurements to review any contraindications for taking mirabegron. The visit with Dr. Watters can be in person or through a scheduled Zoom call to reduce participant scheduling burden. In the case of a Zoom call, Dr. Watters will be provided the medical history and resting blood pressure and heart rate measurements prior to the call.

- Study Visits: Mirabegron has a half-life of ~50 hours so all study visits will be separated by at least a 10-day washout period. Our goal will be to have 10-14 days in between study visits. Participants will report to the temperature and humidity-controlled laboratory in the morning following a 12-hour fast, and 24-hour abstention from exercise, caffeine, and alcohol. Participants will bring their dietary logs for review to ensure a consistent diet prior to each study visit. On arrival, participants will provide a urine sample to ensure they are hydrated via urine specific gravity. Participants will consume a light standard breakfast (237 ml Ensure Original; 220 kcal) and enter the

empty water immersion tank. Then they will be instrumented for the measurement of heart rate (3-lead ECG), blood pressure (brachial artery auscultation), core temperature (rectal thermistor), and indices of shivering (surface mechanomyography using triaxial accelerometers at 3 anatomic sites (chest, upper back, and thigh), oxygen consumption via ventilation and expired gases, and the bedside shivering assessment scale). Thermal perceptions will be assessed using Likert scales for thermal discomfort and thermal sensation. Following 10 minutes of resting baseline measurements, infrared thermography will then be used to measure skin temperature of the supraclavicular fossa as an estimate of brown adipose tissue activation and thermal perceptions will be assessed. The participants will then ingest either mirabegron (100 mg) or a placebo tablet. The dose of mirabegron will be determined from the results of Specific Aim 1. The dose of mirabegron the elicits the greatest increase in thermogenesis in Specific Aim 1 will be used in Specific Aim 2. The measurement of supraclavicular fossa skin temperature (infrared thermography) and thermal perceptions will be assessed every 10 minutes prior to the progressive cold-water challenge. After 30 minutes of seated rest, pre-immersion measurements of heart rate, blood pressure, core temperature, shivering, and thermal perceptions will be conducted and an infrared thermography image will be taken. Then, the water immersion tank will be filled up to the participant's neck with 35°C (95°F) water. Heart rate, blood pressure, core temperature, and shivering indices will be continuously measured. Infrared thermography and thermal perceptions will be obtained every 5 minutes throughout the progressive cold-water challenge. The water will be cooled at a rate of 12°C per hour until the water temperature reaches 10°C (50°F), the participant can no longer tolerate the cold water, or core temperature reaches 35.5°C (95.9°F). This is 0.5°C higher than the clinical definition of hypothermia. Also, the risks associated with hypothermia are core body temperature-dependent and not dependent on time (7). Upon completion of the progressive cold water challenge, the water in the tank will be rapidly emptied. Participants will gently towel dry and will be able to don a circulating warm water perfused suit and/or use a mylar blanket to reestablish core temperature. If participants complete the informed consent/screening visit and all study visits, we expect that they will spend a total of ~11 hours in the study.

Specific Aim 3

The serum and fecal samples that were obtained during the study visits for Specific Aim 1 will be used for the Specific Aim 3. Serum samples will be frozen at -80°C until they are shipped on dry ice to Rutgers University for analyses. Participants will be given the option to directly send their fecal samples to Rutgers University (shipping will be paid for by the study) or they can bring their samples in to the lab and a staff member will ship the samples to Rutgers University. All samples will be de-identified.

8.0 Study Calendar

Specific Aims 1 and 3

	Screening	Study Visits 1-4			
	Day 1	Day 2	Day 3	Day 4	Day 5
STUDY PROCEDURES					
Height	X				
Weight	X	X	X	X	X

Urine pregnancy		X	X	X	X
Heart rate (electrocardiogram)		X	X	X	X
Arm Blood Pressure	X	X	X	X	X
Core temperature		X	X	X	X
Skin temperature		X	X	X	X
Perceptual scales		X	X	X	X
Bedside shivering scale		X	X	X	X
Surface mechanomyography		X	X	X	X
Resting energy expenditure		X	X	X	X
International Physical Activity Questionnaire (IPAQ)	X				
Health History Questionnaire	X				
Health History Update Questionnaire		X	X	X	X
Infrared Thermography Images		X	X	X	X
PAR-Q+	X				
3-Day Dietary Log		X	X	X	X
Body Composition	X				
Whole Blood		X	X	X	X
Fecal Samples		X	X	X	X

Specific Aim 2

	Screening	Study Visits 1-2	
	Day 1	Day 2	Day 3
STUDY PROCEDURES			
Height	X		
Weight	X	X	X
Urine pregnancy		X	X
Urine Specific Gravity		X	X
Heart rate		X	X
Arm Blood Pressure	X	X	X
Arterial oxygen saturation		X	X
Partial pressure of end tidal CO_2		X	X
Ventilation		X	X
Core temperature		X	X
Skin temperature		X	X
Perceptual scales		X	X
Bedside shivering scale		X	X
Surface mechanomyography		X	X
International Physical Activity Questionnaire (IPAQ)	X		
Health History Questionnaire	X		
Health History Update Questionnaire		X	X
Infrared Thermography Images		X	X
PAR-Q+	X		
3-Day Dietary Log		X	X
Body Composition	X		

Specific Aim 3

See table for Specific Aims 1 and 3 above.

9.0 Reportable Events

Safety will be constantly monitored during all data collection activities. This will occur on a participant-by-participant basis. If a suspected adverse event occurs in accordance with IU HRPP Policy on Reportable Events, the principal investigator will immediately report to the IRB and cooperate with the IRB in any necessary investigation. All safety data will also be reviewed in weekly lab meetings, particularly as it relates to changes to the risk-benefit ratio.

If any of the following adverse events, which are specific to the procedures conducted in this study, were to occur, they will be immediately reported to the IRB using standard operating procedures.

- Core temperature drops below 35.0°C
- Infection at venous blood draw site
- Loss of confidentiality
- Injury requiring hospitalization and/or activation of EMS.
- Death

Other events deemed as events occurring outside the criteria above will be reported to the IU IRB reported within 5 business days using standard operating procedures. If any event occurs that would potentially impair the health of the participant, the experiment will be halted, and the IRB will be notified.

Any incident, experience, or outcome that meets all the following criteria will also be reported:

(1) unexpected (in terms of nature, severity, or frequency) given
   a. the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and
   b. the characteristics of the participant population being studied;

(2) related or possibly related to participation in the research (in this guidance document, possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and

(3) suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Participants will be informed of possible risks before consenting to participate through the informed consent document. Details of foreseeable risks and discomforts for the procedures, and steps taken to lessen the probability and/or magnitude of these risks are noted below.

- Acute Mirabegron ingestion: Mirabegron ingestion in the population that will be studied is deemed safe (3) and will be prescribed under the direction of our study physician, Dr. Andrew Watters. The doses that will be used in this study have mild cardiovascular (heart rate and blood pressure) effects that are associated with the dose of mirabegron (e.g., the greater the dose of mirabegron, the greater the cardiovascular response (2, 3). Acute oral administration of 200 mg of mirabegron has been shown to increase heart rate by ~8 beats per minute and systolic blood pressure by ~10 mmHg with no changes in diastolic blood pressure (2). These increases in heart rate and blood pressure are far below the heart rate and blood pressure

responses associated with moderate to heavy exercise intensities that are achieved in healthy 18-40 year old men and women.

- Head Out Water Immersion: Water temperature during Specific Aim 2 will be perceived as being cold and rectal temperature will decrease and participants might shiver. We will constantly monitor rectal temperature during the study visits for Specific Aim 2. Any risks are reversible with removal from the cold water and warming with warm water perfused suits and/or mylar blankets, which are located in the laboratory. The protocol will be immediately terminated if core temperature drops to 35.5°C or if the participant does not want to continue. This safety cutoff is 0.5°C higher than the clinical definition of hypothermia and we don't expect core temperature to drop to 35.5°C. For instance, 60 minutes of 15°C water immersion decreased core temperature less than 1°C (8). Another study safely had participants sit in 10°C (50°F) water up to the mid-sternum for 90 minutes and core temperature only decreased to 36.2°C (9). In a case study, 88 minutes of full body immersion in crushed ice lowered core temperature from 37.6°C to 37.0°C (10). Also, the risks associated with hypothermia are core body temperature-dependent and do not appear to be dependent on time (7).

- Whole blood sample: Participants will likely experience discomfort during venous blood draws and in rare circumstances may feel lightheaded or faint. This risk will be minimized by the venipuncture always occurring in the semi-recumbent or supine position. There is a rare risk of infection following venous blood draws. This risk will be minimized by blood draws only being conducted by trained laboratory personnel.

- Rectal temperature: There is an extremely low risk of rectal perforation. This risk will be minimized by using lubricating jelly, while rectal temperature will not be measured in participants with any known chronic (e.g., cancer, surgery, etc) or acute conditions (e.g., diarrhea, constipation, etc.) (or history of disorders) of the rectum. The temperature probe will be self-inserted during instrumentation processes and removed prior to the participant leaving the laboratory. Any risks will be reversed with removal.

- Loss of confidentiality: There is a potential risk for loss of confidentiality. Every effort will be made to keep all subject information confidential. This risk will be alleviated by only using de-identified information.

- Other risks: There may be other risks that we cannot predict.

10.0 Data Safety Monitoring

Dr. Zachary Schlader, who is independent from the study, will be responsible for data and safety monitoring. Dr. Schlader is an experienced environmental physiologist who specializes in thermoregulatory physiology. He is experienced to recognize adverse physiological responses to cold exposure." Data quality, participant recruitment, accrual, retention, outcome and adverse event data will be monitored on a bi-weekly basis.

Data will be monitored by participant number. Thus, analysis of data will be performed without any identifying features to individuals. Statistical software will be used to analyze data for significance. Results will be reported through traditional scientific outlets such as peer-reviewed manuscript and presentations at national and international meetings. Communication with the IRB regarding safety will be reported directly by the principal investigator.

11.0 Study Withdrawal/Discontinuation

Participants can voluntarily withdraw from the study at any time by contacting any of the researchers on the study via any available means (personal contact, email, phone, etc.). If a researcher on the project wishes to withdraw a participant from the study, they will contact the participant directly (personal contact, phone, email, etc.). Possible indications for withdrawal by a researcher include a change in their health history that excludes them from participation and an inability to keep appointments or follow or understand the rules of the protocol. Data collected up to the time of withdraw (either voluntary or by the study team) may be used for analysis.

12.0 Statistical Considerations

Specific Aim 1
Power Analysis: Using data from Loh et al. (2) we expect to observe an effect size (*f*) of 0.90 for the comparison of resting energy expenditure in the placebo condition versus 200 mg of mirabegron. Given this effect size, 10 participants will be needed to achieve a power of 0.99 with an alpha level set at 0.05. To account for possible attrition, we plan on recruiting up to 30 participants.

Statistical Approach: We will compare the primary (i.e., thermogenesis) and secondary (i.e., core temperature, skin temperature, heart rate, blood pressure, indices of shivering, thermal perceptions, and infrared thermography images) dependent variables using mixed effects ANOVA (i.e., mirabegron dose x time) followed by Tukey's post hoc procedure if a significant interaction or main effect is found from the ANOVA analyses. We will also calculate the thermogenesis area under the curve for each dose of mirabegron. These values will be compared using repeated measures ANOVA followed by Tukey's post hoc procedure if a significant main effect is found from the ANOVA analysis. We will also perform correlation analyses to determine the relation between body composition and our primary and secondary dependent variables. The alpha level will be set at $P < 0.05$ for all analyses.

Specific Aim 2
Power Analysis: Similar to Specific Aim 1 and the data from Loh et al. (2), we expect to observe an effect size (*f*) of 0.90 for the comparison of rectal temperature in the placebo condition versus the mirabegron condition. Given this effect size, 10 participants will be needed to achieve a power of 0.99 with an alpha level set at 0.05. To account for possible attrition, we plan on recruiting up to 30 participants.

Statistical Approach: The primary (i.e., core temperature deflection point) and secondary (i.e., onset of shivering inflection point) dependent variables from the mirabegron and placebo trials will be compared using paired t-tests to determine if mirabegron improves cold-water immersion tolerance. Mixed effects ANOVA will be used to compare the remaining dependent variables between conditions (i.e., mirabegron vs. placebo) and across time. If a significant

interaction or main effect results from the ANOVA analyses, we will use the Holm-Sidak post hoc test to determine where differences exist. The alpha level will be set at $P < 0.05$ for all analyses.

Specific Aim 3
Power Analysis: This aim is being conducted as an exploratory aim in conjunction with Specific Aim 1. As such, to be consistent, we expect to observe an effect size (f) of 0.90 for the comparison of the gut microbiota in the placebo condition versus 200 mg of mirabegron. Given this effect size, 10 participants will be needed to achieve a power of 0.99 with an alpha level set at 0.05. To account for possible attrition, we plan on recruiting up to 30 participants.

Statistical Approach:
Principal Coordinate Analysis will be performed with group samples and any outliers will be identified. T-tests, and/or ANOVA will be performed to identify significant differences. Statistical analysis to detect variation between samples will be carried out using student t-test or Mann–Whitney test depending on data distribution. Multivariate statistics will be incorporated in a web-based tool Metastats, which can reveal, with high confidence, discriminatory functions between the replicated metagenome dataset of the gut microbiota of humans. In addition, the ShotgunFunctionalizeR and/or Primer E packages will be used for this analysis.

13.0 Statistical Data Management

Primary data will be collected via a data acquisition system (PowerLab) and stored electronically on a password protected computer. The storage location will be backed up manually every week. Quality assurance steps will be confirmed by plotting the data prior to formal analyses. Outliers will be identified and checked for authenticity in the database and other original data documents. Extraction and cleaning of data that will be used for analysis will be carried out after each study visit.

14.0 Privacy/Confidentiality Issues

Informed consent will be obtained, and the testing procedures will be completed in a closed, private laboratory setting at the School of Public Health (SPH), with only the participant and investigators present.

All testing will take place in a closed, private laboratory setting in the School of Public Health. All participants will be assigned an identification number, which will then be used when recording and analyzing data. A code list containing the participants names and identification numbers will be stored separately in a locked filing cabinet and will only be available to the investigators. The reports generated as a result of this investigation will not identify individual subjects. Data will be collected and stored on password protected computers located in the Human Performance Laboratories, which are always kept locked. Any paper data will be stored in a locked filing cabinet inside a locked office. Primary data are backed up by manual download to an encrypted flash drive once per week, which is then stored in a separate locked office in a locked filing cabinet accessible only to the investigators.

While testing is in progress, a sign will be outside the laboratory door to prevent people not associated with the study from entering.

15.0 Follow-up and Record Retention

We anticipate that all participants will be enrolled within 24 months. Any identifiable data (e.g., signed consent forms) will be maintained as required by law. De-identified data will be stored indefinitely. Only investigators will have full access to data. All electronic data will be kept on password protected computers, while paper data will remain in locked file cabinets.

16.0 References

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17.0 Appendix

17.1 Payment schedule

Specific Aim 1: Participants will be compensated in the form of a gift card for $495 for completing the informed consent/screening visit and the four study visits. Compensation will occur on or after the final visit to the laboratory. If participants do not complete the study, they will be compensated for their time spent in the laboratory at a rate of $15/hour paid by gift card.

Specific Aim 2: Participants will be compensated in the form of a gift card for $165 for completing the informed consent/screening visit and both study visits. Compensation will occur on or after the final visit to the laboratory. If participants do not complete the study, they will be compensated for their time spent in the laboratory at a rate of $15/hour paid by gift card.

Specific Aim 3: Participants will be compensated in the form of a gift card for $100 for completing the informed consent/screening visit and brining their samples to the laboratory. Compensation will occur on or after the final visit to the laboratory. If participants do not complete the study, they will be compensated at a rate of $25/sample paid by gift card.

17.2 Attachments

The following Questionnaires have been included as attachments:

- International Physical Activity Questionnaire (IPAQ)
- Physical Activity Readiness Questionnaire Plus (PAR-Q+)
- Healthy History and Demographics Questionnaire
- Health History Update Questionnaire

The following Recruitment tools have also been included as attachments:

- Recruitment flyer
- Email script
- Verbal script
- Phone script