

**PROTOCOL TITLE:** Investigating the stability, variability and mechanism of incorporation of lipid mediators into eccrine sweat

**1) Protocol Title** Investigating the stability, variability and mechanism of incorporation of lipid mediators into eccrine sweat

**2) Author(s) of Protocol** John W. Newman, Ph.D. -USDA Western Human Nutrition Research Center and Department of Nutrition, University of California Davis

**UC Davis Researcher**

**Researcher from other institution**

**Private Sponsor**

**Cooperative Group**

**Other:** \_\_\_\_\_

**3) IRB Review History**

N/A

**4) Objectives**

Validate the utility of sweat as a non-invasive sample for the pharmacological investigation of skin biology

Specific Aim 1: Develop and Report Validated Protocols for the Collection and Quantification of Lipid Mediator Profiles in Sweat by evaluating:

- 1) Comparison of pharmacological and physiological methods of sweat stimulation (i.e. comparing the lipid mediator profile of sweat collected following stimulation by pilocarpine nitrate to sweat collected following stimulation by light exercise)
- 2) Site-specificity of sweat lipid mediator composition (i.e. comparing the lipid mediator profile of sweat collected from the volar forearm to sweat collected from the anterior distal thigh and/or lower back)
- 3) Inter- and intra-individual variability of the sweat mediator lipidome

Specific Aim 2: Evaluating the impact of systemic cyclooxygenase inhibition on the plasma and sweat mediator lipidome

**5) Background**

Blood and urine represent common human biofluids that have been extensively studied in the context of pharmacokinetic and metabolomic analyses. However, the collection of these biofluids is either physically or culturally invasive and therefore subject compliance is sometimes difficult to achieve. The use of non-invasive matrices such as hair, oral fluid, sweat and tears has improved subject compliance in pharmacokinetic studies, and recently there has been interest in conducting metabolomic analyses using sweat for the same reason. While sweat has been previously used to diagnose cystic fibrosis in neonates and infants and to detect illicit drugs in forensic settings, it has rarely been used in metabolomic analyses due to a lack of uniform sweat collection protocols that provide a reproducible and measureable volume of sweat. With advances in technology, the Macroduct® sweat collection system was developed and validated by Wescor, Inc. for the reproducible collection of neonatal sweat for the diagnosis of cystic fibrosis. More recently, the Macroduct® system was used in a proof-of-principle study

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to conduct an untargeted metabolomic analysis of human sweat, and has since been used to develop a screening tool for lung cancer based on the sweat metabolite profile.

As can be seen from the cited studies above, sweat has predominantly been used to study pulmonary diseases. However, given its proximity to the skin, there is also the potential for sweat to be an informative non-invasive matrix in cutaneous research. Current cutaneous research methods are dependent on tissue biopsies, which are again invasive methods, and therefore not suitable for repeated temporal sampling. To the best of our knowledge, only a single study exists that examines the composition of sweat in the context of cutaneous disease, and this study demonstrated that subjects with atopic dermatitis, psoriasis or hyperhidrosis exhibited increased prostaglandin E2 (PGE2) levels relative to healthy control.

PGE2 is a member of a class of compounds known as bioactive lipid mediators, which regulate inflammatory and immune responses. These mediators, which include an array of oxygenated lipids (“oxylipins”), endocannabinoids and ceramides, are generally produced locally via a variety of biosynthetic pathways in response to extracellular stimuli and function similarly to local hormones or autacoids. Additionally, ceramides play an important structural role in the epidermal barrier. Lipid mediators have been studied previously in cutaneous research, but have depended on a limited number of analytical targets and/or focused on a single class of analytes, preventing the identification of mediator pathway cross-talk. Our laboratory has developed methods to quantify over 125 bioactive lipid targets, most not previously studied in the context of cutaneous biochemistry. Understanding the actions and interactions of these lipid mediators in the skin could improve treatment modalities, thus improving skin care and reducing the burden of skin conditions on subjects with cutaneous diseases.

Recently, we conducted a study which demonstrated differences in the sweat lipid mediator profiles of subjects with and without atopic dermatitis using the Macroduct® sweat collection system on the volar bilateral forearm of human subjects (IRB #605131). However, little is known about the source of lipid mediators in sweat, the effects of different sweat stimulation techniques on lipid mediator composition as well as the site-specificity of the sweat mediator lipidome. Collecting such baseline data is critical for the evaluation of routine mediator lipidomic analyses of sweat as a non-invasive tool to support studies investigating cutaneous inflammatory responses, dietary manipulation, and skin diseases lacking biomarkers and therapeutic targets.

## **6) Inclusion and Exclusion Criteria**

### Inclusion criteria

- 20-40 y
- Male
- Weight > 110 pounds

### Exclusion criteria

- Diagnosed active chronic diseases for which the individual is currently taking daily medication, including but not limited to:
  - Diabetes mellitus

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- Cardiovascular disease
- Cancer
- Gastrointestinal disorders
- Kidney disease
- Liver disease
- Bleeding disorders
- Asthma
- Autoimmune disorders
- Hypertension
- Osteoporosis
- Recent minor surgery (within 4 wk) or major surgery (within 16 wk)
- Recent antibiotic therapy (within 4 wk)
- Recent hospitalization (within 4 wk)
- Use of over-the-counter or prescription medications at the time of the study that directly affect endpoints of interest (e.g. hyperlipidemia, glycemic control, steroids, statins, anti-inflammatory agents, and weight loss aids)
- Adults who are not able to consent
- Under current medical supervision
- Ibuprofen intolerance or allergy
- Those with a bleeding disorder
- Current enrollee in a clinical research study.
- Individuals with blood clotting or platelet defect disorders
- Individuals with orthopedic limitations or cardiovascular risk that preclude participation in the physiological stimulation of sweat by light exercise portion of the study
- Individuals who are trained athletes or that regularly perform physical activity defined as “vigorous” by the Centers for Disease Control and Prevention

## **7) Number of Subjects**

The target sample size is 10 participants that complete the study visits. In addition, we aim to enroll additional participants not to exceed 20% of the calculated sample size to account for participants contributing incomplete or invalid data for various reasons including study withdrawal, compromised sample conditions, etc. Thus a total of 12 people will be enrolled. However due to subject withdrawal and non-compliance, we plan to now enroll 5 additional subjects, bringing the total number of enrolled subjects to 17.

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## **8) Recruitment Methods**

Participants will be recruited from Davis, Woodland, and the greater Sacramento area using the following methods:

- WHNRC study subject referrals
- Personal contact

WHNRC Human Studies office maintains a list of people who have participated in previous studies, and who wish to be contacted about new studies. In addition, Dr. Sivamani has conducted previous studies from which there is a list of interested persons. The contact will be in the form of an email providing a study description. It will be up to the individual to respond if they wish to participate.

## **9) Compensation to the Subjects**

Participants will be compensated a reasonable hourly wage for their time contributions to the study. Participants will be compensated \$10 for Visit 1, \$10 for Visit 2, \$10 for Visit 3, and \$45 for Visit 4. Total possible compensation will be \$75 per participant and will be pro-rated based on time involved. The compensation will be provided in the form of a check written to the volunteer.

## **10) Study Timelines**

The study will be conducted over a one-year period. Each subject's participation will consist of four visits. The first three visits will last approximately 1 hour each, the fourth visit will last approximately 4 hours. The duration of a single subject's participation in the study is not expected to exceed six months.

Additionally, subjects expressing an interest in the study will be screened in-person or by telephone and eligibility for enrollment in the study will be determined by verbal completion of the eligibility screening questionnaire with a trained study staff member. If a subject is determined to be eligible for participation, they will be given further information about the study procedures and consent protocol and emailed a copy of the consent document. Time will be allocated at the first study visit to complete the consent document and answer any questions related to the study.

## **11) Study Endpoints**

- Primary endpoints:
  - Changes in sweat oxidized lipid, endocannabinoid and ceramide concentrations following exercise-induced sweating relative to sweat collection following pilocarpine iontophoresis
  - Changes in sweat oxidized lipid, endocannabinoid and ceramide concentrations due to sweat collection from the anterior distal thigh and lower back compared to sweat collected from the volar forearm
  - Changes in sweat oxidized lipid, endocannabinoid and ceramide concentrations following consumption of ibuprofen relative to baseline

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- Changes in plasma oxidized lipid, endocannabinoid and ceramide concentrations following consumption of ibuprofen relative to baseline
- Secondary endpoints:
  - Changes in plasma and sweat ibuprofen concentrations following oral administration
  - Relative abundance of sweat metabolites derived from primary metabolism covering carbohydrates and sugar phosphates, amino acids, hydroxyl acids, free fatty acids, purines, pyrimidines, aromatics, exposome-derived chemicals
  - Relative abundance of sweat metabolites derived from complex lipids covering ceramides, sphingomyelins, cholesteryl esters, oxysterols, lyso- and phospholipids, mono-, di- and triacylglycerols, galactosyl- and glucuronylipids

## 12) Procedures Involved

### Prior to enrollment in study

All volunteers will be subjected to either an in-person interview at the WHNRC or to a telephone interview during which their eligibility and willingness to participate in the study will be determined. Twelve subjects eligible and willing to participate will be enrolled in the study and scheduled to begin participation at least seven days after enrollment.

### Study Design and Visits

Subjects will participate in four study visits, separated by at least one week. On each of the study days, participants will arrive in the morning after an overnight 12 hour fast (ad lib water consumption will be allowed and encouraged). Collection procedures will be performed at approximately the same time of the day to avoid circadian effects. Subjects will also be asked to not apply any cream or medication to their body 24 hours before each study visit and refrain from use of non-steroidal anti-inflammatory drugs such as ibuprofen for at least 48 hours prior to each study visit.

- *Study Visit 1:* After obtaining written informed consent, sweat will be collected using the Macroduct® sweat collector from the volar forearm following stimulation of sweating by pilocarpine iontophoresis as part of the Webster Sweat Induction System. Following sweat collection, subjects will be given a 15-minute break after which they will undergo up to 15-minutes of light exercise on a stationary bicycle to stimulate sweat by physiological methods (see below). Following the light exercise, sweat will be collected from the opposite volar forearm using the Macroduct® sweat collector. The study visit is complete after the second sweat collection.
- *Study Visit 2:* Sweat will be collected using the Macroduct® sweat collector from the volar forearm and anterior distal thigh following stimulation of sweat by pilocarpine iontophoresis as part of the Webster Sweat Induction System. Collections will occur simultaneously at both sites. The study visit is complete after both sweat collections.

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- *Study Visit 3:* Sweat will be collected using the Macroduct® sweat collector from both volar forearms and the lower back following stimulation of sweat by pilocarpine iontophoresis as part of the Webster Sweat Induction System. Collections will begin at approximately 10 minute intervals at all three sites. The study visit is complete after all three sweat collections.
- *Study Visit 4:* A baseline sweat sample will be collected using the Macroduct® sweat collector from the volar forearm following stimulation of sweating by pilocarpine iontophoresis as part of the Webster Sweat Induction System. A blood sample (~ 6 mL) will be collected in EDTA-treated Vacutainer® tubes following venipuncture of the opposite antecubital vein. Following sweat and blood collection, subjects will consume 400 mg of ibuprofen orally. Blood and sweat collections will occur at 30 minutes, 2 hours and 4 hours post-ibuprofen consumption. The study visit is complete after the blood and sweat collections at 4 hours post-ibuprofen consumption. Total blood drawn per participant over the course of the study will be ~ 24 mL. Prior to Study Visit 4, participants will undergo a video interview with the study physician (and co-PI of the study) Dr Raja Sivamani, during which time Dr Sivamani will obtain a medical history from the subjects and review the risks and side effects of ibuprofen with the subjects.

Participants will remain fasted during study visits (ad lib water consumption will be allowed and encouraged). At the end of the study visit, subjects will be allowed access to the WHNRC dining room where snacks such as juice, granola bars, oatmeal, dried fruit, coffee, tea and hot cocoa will be made available for their consumption.

All blood and sweat samples collected will be measured for metabolites derived from normal biological processes which will include inflammatory mediators. Additionally, blood and sweat collected during visit 4 will also be used to measure ibuprofen levels at each collection time.

Description of the “light exercise”

Sweat stimulation by light exercise, such as riding a stationary bicycle with resistance, has been used in several recent studies to collect sweat for ionic analysis. In the proposed study, subjects will ride a cycle ergometer (Monark ergometer) with resistance for 10-15 minutes in a temperature and humidity monitored room. Resistance (in kg\*m/min) will be set so as to elevate heart rate to that evinced during moderate (60-80% VO<sub>2</sub>max) steady state aerobic exercise and pedal cadence will be set using a metronome at 70 rpm. Heart rate will be continuously monitored by sensors during the exercise. Additionally, subjects will be fitted with air collection masks connected to a metabolic cart in order to continuously monitor oxygen consumption, respiratory quotient and substrate use in order to ensure achievement of steady state aerobic exercise. All procedures are non-invasive and should cause minimal subject discomfort.

Sweat collection following stimulation of sweating by pilocarpine iontophoresis using the Webster Sweat Induction System

The Macroduct® sweat collection system and the Webster Sweat Inducer are FDA-approved medical devices routinely used for sweat collection in neonates and infants in order to diagnose cystic fibrosis. The protocols for these devices are well established and freely available at <http://wescor.com/translations/Translations/M2551-7A-EN.pdf>. In brief, the collection site will be cleansed with a 70% solution of isopropanol followed by distilled water, and sweating will be

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stimulated using the Webster Sweat Inducer. This procedure consists of attaching positive and negative electrodes, each containing a manufacturer-supplied pilocarpine gel disk, to the collection site and applying a 1.5 mA current across the electrodes for five minutes. The area under the positive electrode will be marked using a felt-tip marker and electrodes will then be removed and the collection site will be cleansed with distilled water. The Macroduct<sup>®</sup> sweat collector will then be attached to marked site and secured using a tourniquet or bandage to maximize cutaneous contact. The collection device will remain in place for up to 30 minutes after which it will be removed and the collected sweat will be exuded into a collection vial and stored until analysis.

### **13) Data and Specimen Banking**

All banked specimens will be stored in -20 °C or -80 °C freezers. Participants will be given the option to provide consent for future, undetermined use of their specimens by initialing the designated section on a signed consent document and a verbal agreement of ‘yes’ during the discussion of consent for participation at the enrollment visit. Any archived blood or sweat samples of participants having provided the described consent may be used at a later date for purposes not specified by this study. All identifiable subject information will be removed except for assigned subject ID codes. If the subject does not agree to future use of specimens, all remaining specimens will be destroyed.

### **14) Data Management and Confidentiality**

Identifiable information linked with the participant identification code will be stored separately from any data collected during the study and will only be made accessible to the study Principal Investigators and the Coordinator. Data will be collected on hard copy forms and computer-based questionnaires using only the participant identification code. Completed forms will be kept in locked files at the WHNRC in rooms that are also locked. Electronic files will be kept in folders on the WHNRC fileserver, which is password-protected and will be limited to access for key study personnel only. The WHNRC fileserver is located behind a hardware firewall and is backed up off-site weekly, at a minimum. In the case that data needs to be stored on a laptop computer, the laptop will be encrypted. Data entered manually will be double entered and compared to identify data entry errors.

### **15) Provisions to Monitor the Data to Ensure the Safety of Subjects**

This study is an observational study and involves an intervention with an FDA-approved over-the-counter non-steroidal anti-inflammatory drug. The Co-Investigator for the study, Dr. Raja Sivamani, will provide oversight for this part of the research and review the study participants’ medical history prior to prescribing the ibuprofen to each subject. Nevertheless, it does present several challenges and various methods of data collection that participants will be subject to. In order to ensure the safety of participants during administration of challenges or specimen collection, all aspects of the study will be carried out by trained study personnel. The other collections involve non-invasive minimal risk activities. Participants will spend the study visit day in private rooms in the Metabolic Research Unit of the WHNRC containing hospital beds for rest and privacy during data and specimen collection. A unit monitor will remain in the unit (but outside of the participant room) as a contact person for participants should any reactions,

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concerns, questions, or emergencies arise throughout the visit. Participants will never be left alone in the Metabolic Research Unit.

The exercise-induced sweat stimulation (exercise bicycle) will be administered by a qualified physiologist and staff who, prior to the test, will assess the subject's risk level using the risk factors established by the American College of Sports Medicine: a) family history of premature heart disease, cigarette smoking, hypertension, dyslipidemia, impaired fasting glucose, obesity, and sedentary lifestyle. Those subjects falling in the low and moderate risk categories will be further evaluated by the physiologist who will verbally administer the Physical Activity Readiness Questionnaire prior to the test. If the subject responds 'yes' to any of the questions, they will not perform the test. For those cleared for testing, heart rate, pulse, and blood pressure will be measured prior to administration of the challenge. During the test, participants will be asked to rate their level of perceived exertion, and if level reaches a level of 'extremely hard' or if the participant loses balance, becomes dizzy, or other adverse reaction occurs during the test, it will be stopped immediately.

Venipuncture blood draws will be conducted by licensed, trained and experienced phlebotomists. If excessive bruising or discomfort occurs, no further venipunctures will be conducted.

Sweat-stimulation by pilocarpine iontophoresis may cause minor discomfort to subjects in the form of slight stinging or prickling at the site of administration. Subjects will be asked periodically during iontophoresis if the discomfort is unbearable. If the subject responds 'yes', the iontophoresis procedure will be stopped immediately.

If at any time a participant refuses to continue participation or refuses to participate in a particular aspect of the study, they will be excluded from all or part of the remaining procedures according to their wishes.

## **16) Withdrawal of Subjects**

During the consent process, participants will be informed that they can withdraw from the study at any time without any risk of repercussion or penalty. Compensation for participant time in the study will be pro-rated to reflect the withdrawal time. Specimens previously collected during the study will be used as specified.

## **17) Risks to Subjects**

There is a small risk of loss of balance, dizziness, or shortness of breath that could result from the 30 min light exercise to stimulate sweat production (exercise bicycle). The use of an exercise bicycle is expected to result in increased heart rate and sweating but may present additional risks to individuals in very poor cardiovascular health or with undiagnosed cardiovascular risk factors such as family history of heart disease, hypertension, high cholesterol, type 2 diabetes, harmful use of alcohol, tobacco use, stress, physical inactivity, obesity and unhealthy diet. All of these risk factors will be evaluated prior to having participants engage in the test. Potential cardiovascular-related serious adverse events include stroke and myocardial infarction, which could result in death.

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There is a small risk of bruising as a result of blood sample collection. A licensed, trained phlebotomist will collect blood samples to minimize bruising.

The measurement of metabolic rate uses an air collection mask that fits snugly over the nose and mouth. This may create a feeling of claustrophobia in some individuals. Participants may request that the mask be readjusted or removed at any time.

Sweat collection procedures pose minimal risks to the subjects as they are all noninvasive. The Webster Sweat Inducer uses electricity for iontophoresis based induction of sweating. Physical risks of the sweat collection may include:

- The use of the device will require pressing on the skin. If a subject bruises easily, they may develop a bruise at the location of the measurements although this would be unusual.
- Iontophoretic induction of sweating using the Webster Sweat Inducer may cause contact dermatitis that may be adequately managed with topical steroids. It is also possible, although unlikely, that the iontophoretic procedure may cause burns to the subject. The device manufacturer has estimated that the risk of burns to be 1 in 50,000 iontophoretic procedures. It should be noted that both the Macroduct device and Webster Sweat Inducer have been cleared for sweat collection by the FDA and is frequently used on infants and neonates with rare adverse effects reported.
- Iontophoresis may also cause minor discomfort to subjects in the form of slight stinging or prickling at the site of administration. However, the procedure has been approved by the FDA and any discomfort should cease immediately after iontophoresis is completed (~ 5 min).

As the amount of ibuprofen provided (400 mg) is within the recommended over-the-counter dosage for this product, we do not anticipate any risks associated with administration of this drug. However, ibuprofen intake has been known to produce an upset stomach including nausea, bloating and gas; diarrhea; constipation; headaches; dizziness; nervousness; itching skin or a rash; blurred vision or ringing ears in certain individuals. Prior to prescription of the ibuprofen, Dr Raja Sivamani will review the participants' medical history and inform them of the risks and potential side effects associated with ibuprofen intake.

We will do our best to make sure that the personal information in the subjects' study record will be kept private. However, no study can guarantee 100% protection of private information despite all of our measures to protect each subject's privacy. Subjects will be made aware of this during the consent process.

## **18) Potential Benefits to Subjects**

There are no direct benefits to the participants. Results from this study will benefit the scientific and medical communities and provide information that may be used to develop non-invasive sampling methods to support future cutaneous research studies.

## **19) Vulnerable Populations**

UC-Davis Students, Employees and USDA Employees

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**20) Multi-Site Research**

N/A

**21) Community-Based Participatory Research**

N/A

**22) Sharing of Results with Subjects**

No results will be shared with participants.

**23) Setting**

All in-person contact visits will occur at the USDA Western Human Nutrition Research Center on the UC Davis campus (430 West Health Sciences Dr., Davis, CA 95616).

The facilities at the Western Human Nutrition Research Center were designed specifically to support human trials and include a clinical wing with private bedrooms for all-day study visits, a physiology laboratory with instrumentation for anthropometric measurements, body composition testing, and a reception area for screening potential participants. In addition, the second floor is laboratory space for processing, analyzing and storing biological specimens.

**24) Resources Available**

Dr. John W. Newman is a senior scientist at the USDA Western Human Nutrition Research Center and holds an adjunct appointment in the Department of Nutrition at UC Davis. Dr. Newman will serve as PI for this project and is considered a leading expert in the field of metabolomics/lipomic analyses.

Dr Raja K. Sivamani is an assistant professor of clinical dermatology at the UC Davis School of Medicine and a board certified dermatologist. His expertise centers on general dermatology including medical, surgical, and cosmetic services. Dr Sivamani will serve as co-PI for this project and oversee use of the Macroduct sweat collection and Webster sweat induction devices as well as oversee administration of ibuprofen to study volunteers.

The human studies coordinator at the WHNRC, Dr. Ellen Bonnel, will be assisting the investigators with all phases of this study. Dr. Bonnel has held this position since 1999. In addition, to provide additional support for this study the WHNRC has a full complement of personnel with hands-on experience with phlebotomy expertise, and laboratory stress training. All necessary research equipment for this study is available at the WHNRC.

Justin Waller, M.S., is the physiologist and physiology support laboratory supervisor at the WHNRC since February 2016. Mr. Waller has previous clinical and exercise physiology research experience with a variety of populations, including endurance athletes, post-bariatric surgery patients and breast cancer patients. His areas of expertise are in cardiovascular, endocrine and metabolic responses to aerobic exercise and he has previous experience validating commercial heart rate sensors and wearables.

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**25) Prior Approvals**

N/A

**26) Provisions to Protect the Privacy Interests of Subjects**

Each subject will be assigned a unique numerical identification code which will be linked to all data files. Data is managed through the Western Human Nutrition Research Center's Laboratory Information Management System, using the identification codes only. Only the investigators, the sponsors, and the WHNRC analytical laboratory director will have access to this system. Personal identifiers such as name, date of birth, social security number, driver's license number, address, phone number, email address etc. will not be linked to the data files. While the study is active, this personal information will be stored in a locked file cabinet in the Western Human Nutrition Research Center's Human Studies Office, which is a secure office, along with signed consent forms. Only those individuals who are listed as study personnel responsible for consenting subjects will have access to these files. All records containing personal information will be destroyed upon completion of the study.

**27) Compensation for Research-Related Injury**

If subjects are injured as a result of participating in this study, USDA and the University of California will provide necessary medical treatment. The USDA and the University of California do not normally provide any other form of compensation for injury.

**28) Economic Burden to Subjects**

Transportation to the USDA WHNRC in Davis and related costs will be the responsibility of participants. Complimentary on-site parking will be provided. Compensation for participation sufficient to outweigh transportation costs.

**29) Consent Process**

Eligibility for participation in the study will be determined upon participant completion of a telephone interview with study personnel. Eligible volunteers will be invited to visit the USDA WHNRC for an in-person discussion of the consent document with authorized study personnel. Volunteers will be allowed time for personal review of the document and given the opportunity for a question and answer session, as needed. If the volunteer wishes to proceed with participation in the study, the consent form must be signed and dated by both the volunteer and the authorized study personnel.

**30) Process to Document Consent in Writing**

SOP: Written Documentation of Consent (HRP-091) procedures will be followed. The consent form must be signed and dated by both the volunteer participant and authorized study personnel. A copy of signed consent forms will be provided to the subject upon request and the originals will be kept in a locked cabinet at the WHNRC human studies office.

**31) Drugs and Devices**

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Subjects will be administered 400 mg ibuprofen orally at study visit 4. Ibuprofen is an FDA-approved over-the-counter non-steroidal anti-inflammatory drug. Although the drug is not being used for an anti-inflammatory effect, it is being utilized in FDA approved dosing. Ibuprofen will be sourced from the UC Davis Health System's Investigational Drug Pharmacy following prescription to the study subjects by Dr Sivamani and will be stored in a locked medication container at room temperature at the WHNRC prior to administration to the study subjects. The ibuprofen will be packaged by the Investigational Drug Pharmacy into individual doses labelled with subject ID numbers, and will be transferred to the subjects by the study coordinator, Mr. Karan Agrawal under instructions from Dr Sivamani.

Sweat collections will be conducted using the Macroduct® sweat collector, an FDA-approved medical device routinely used for sweat collection in neonates and infants. The device will be used according to manufacturer instructions and for its designated purpose.

Sweat stimulation will be achieved by pilocarpine iontophoresis using the Webster Sweat Inducer, FDA-approved medical device routinely used for sweat collection in neonates and infants. The device will be used according to manufacturer instructions and for its designated purpose.