A DOUBLE-BLIND, PLACEBO-CONTROLLED INVESTIGATION OF INTER-INDIVIDUAL VARIABILITY IN THE PHARMACOLOGIC RESPONSE TO NON-STEROIDAL ANTI-INFLAMMATORY DRUGS

Regulatory Sponsor:	Garret A. FitzGerald, MD Institute for Translational Medicine and Therapeutics
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Study Product:	Celecoxib (Celebrex) Naproxen (Naprosyn)
Protocol Number:	820715
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List of Abbreviations

CABG: Coronary artery bypass graft
COX: Cyclooxygenase
CTRC: Clinical and Translational Research Center
FDA: Food and Drug Administration
GFR: Glomerular filtration rate
LCL: Lymphoblastoid cell line
NSAID: Non-steroidal anti-inflammatory drug
PBMC: Peripheral blood mononuclear cells
PG: Prostaglandin
PGD-M: Prostaglandin D_2 metabolite
PGE-M: Prostaglandin E_2 metabolite
PGI-M: Prostaglandin I_2 metabolite
PUFA: Polyunsaturated fatty acid
TxA_2: Thromboxane A_2
Tx-M: Thromboxane A_2 metabolite

Study Summary

Title	Investigation of inter-individual variability in the pharmacologic response to non-steroidal anti-inflammatory drugs
Short Title	Variability in response to non-steroidal anti-inflammatory drugs
Protocol Number	IRB # 820715
Phase	Phase 1
Methodology	Randomized, double-blind, placebo-controlled trial
Study Duration	3 years
Study Center(s)	Single-center
Objectives	The objectives of the study are: 1. To characterize the variability in the pharmacologic response to NSAIDs in healthy adults 2. To investigate clinical, genetic, and environmental factors that contribute to this variability 3. To explore the dynamics and variability of the molecular NSAID response network using “omics” data 4. To identify candidate genetic modifiers in the function of the COX pathway at baseline and in response to NSAIDs 5. To establish a well-phenotyped cohort of subjects, with biobanked samples, that can be re-contacted about participation in future studies of NSAID response
Number of Subjects	288
Diagnosis and Main Inclusion Criteria	Adult men and women greater than 18 years of age who are in good health based on medical history, physical examination, vital signs, and laboratory tests. Volunteers with adequately controlled hypertension and hyperlipidemia (total cholesterol of ≤270 mg/dL) may participate in the study.
Study Product, Dose, Route, Regimen	Celecoxib (Celebrex), 100 or 200 mg orally twice daily Naproxen (Naprosyn), 250 or 500 mg orally twice daily
Duration of administration	7 days
Reference therapy	Placebo
Statistical Methodology	The effect of clinical, genetic, and environmental variables on response to NSAID treatment will be evaluated. Exploratory transcriptomic, proteomic, metabolomic, and microbiome analyses will be performed.

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1 Introduction

This document is a protocol for a human research study. This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

This protocol is part of the collaborative effort by the Personalized NSAID Therapy Consortium (www.pentaconhq.org), a group of ~35 investigators in 18 institutions, funded by the National Heart Lung and Blood Institute to explore approaches to improve the prediction of risk and benefit of NSAIDs for individuals.

1.1 Background

Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used for the treatment of inflammatory pain. Pain is a highly subjective experience, and selecting an analgesic regimen that provides optimal pain relief for a specific patient can be challenging. Moreover, patients often express a preference for a particular NSAID, raising the possibility that the efficacy in relieving pain is variable among individuals. However this has never been studied systematically. The clinical decision-making process has been further complicated by the recognition that NSAIDs cause serious thrombotic adverse events in some patients.[1] Elucidating the factors that influence an individual patient's risk of cardiovascular complications and the likelihood of analgesic efficacy will enable clinicians to prescribe NSAIDs rationally in order to maximize their therapeutic benefit while minimizing the risk of adverse cardiovascular events.

NSAIDs are a chemically diverse class of therapeutic agents that exert their analgesic and anti-inflammatory effects via inhibition of cyclooxygenase (COX)-1 and/or COX-2, enzymes that catalyze the first committed step in prostaglandin (PG) synthesis. PGs produce a diverse array of biologic effects via activation of prostanoid receptors, and play important roles in a variety of pathologic and homeostatic processes.[2] COX-2 is readily induced in response to pro-inflammatory stimuli and has been considered the primary source of inflammatory PGs. In contrast, the production of PGs with homeostatic functions, such as gastric epithelium cytoprotection, has been ascribed to COX-1, which is constitutively expressed in most tissues.[2] Consequently, COX-2-selective NSAIDs, including rofecoxib, valdecoxib, and celecoxib, were developed in order to retain the anti-inflammatory and analgesic effects of inhibition of COX-2-derived PG formation, while avoiding the gastrointestinal toxicity of traditional NSAIDs (i.e. aspirin, ibuprofen, naproxen, etc) that inhibit both isoforms. Although fewer gastrointestinal complications were observed in clinical trials, treatment with COX-2-selective NSAIDs increased the risk of serious cardiovascular adverse events, including myocardial infarction, stroke, and heart failure.[1,3]

The risk of thrombotic events associated with the use of NSAIDs, particularly those selective for COX-2, is mediated via suppression of COX-2-derived prostacyclin formation in endothelial and vascular smooth muscle cells.[4,5] Prostacyclin possesses potent anti-thrombotic and vasodilatory effects, and thus acts as a general inhibitor of platelet activation *in vivo.*[2] Traditional NSAIDs also inhibit COX-2 in the vasculature, but the associated risk of thrombosis is mitigated to some extent by inhibition of formation of thromboxane A_2 (TxA_2), a COX-1-derived PG released by activated platelets that promotes platelet activation and aggregation.[1,3] Thus, the risk of thrombosis for a particular NSAID is dependent upon its relative selectivity for COX-2 over COX-1.[3,6] In addition to their effects on vascular PG production, all NSAIDs inhibit renal PG formation, resulting in sodium retention and hypertension, which may further augment cardiovascular risk.[1,3,7]

Currently, it is recommended that NSAIDs be avoided or used only for a limited duration in patients classified as high cardiovascular risk.[8] These recommendations are supported by studies demonstrating that even short-term NSAID use increased the incidence of cardiovascular events in patients undergoing coronary artery bypass grafting[9,10] and following a myocardial infarction.[11,12] However, long-term treatment with COX-2-selective NSAIDs also increased the incidence of cardiovascular events in patients considered to be at low baseline risk,[13,14] consistent with risk transformation due to atherogenesis and indicating traditional cardiovascular risk factors alone are not sufficient to guide therapeutic decisions. Thus, additional studies are necessary to define comprehensively the factors that modify the cardiovascular risk of NSAID use and facilitate the progressive personalization of NSAID therapy.

1.2 Investigational Agent

Celecoxib and naproxen are FDA approved medications for pain and inflammatory diseases. The study drugs will be all administered at FDA approved therapeutic doses.

Celecoxib is a non-steroidal anti-inflammatory drug that selectively inhibits COX-2. It is approved for the treatment of osteoarthritis (100 mg twice daily), rheumatoid arthritis (up to 200 mg twice daily), juvenile rheumatoid arthritis (based on body weight up to 200 mg per day in two doses), ankylosing spondylitis (up to 400 mg per day in one or divided in two doses), acute pain (400 mg initially, followed by an additional 200 mg dose if needed on the first day), gout (800 mg initially followed by 400 mg every 12 hours for 7 days), and primary dysmenorrhea (200 mg twice daily). Celecoxib is contraindicated in patients with known hypersensitivity to celecoxib, aspirin, or other NSAIDs, in patients who have demonstrated allergic-type reactions to sulfonamides, and in patients who have experienced asthma, urticaria, or allergic-type reactions after taking aspirin or other NSAIDs. Severe anaphylactoid reactions to NSAIDs, some of them fatal, have been reported in such patients. Celecoxib is also contraindicated for the treatment of perioperative pain in patients who have recently undergone coronary artery bypass graft (CABG) surgery. Like other NSAIDs, celecoxib carries the risk of gastrointestinal ulceration, bleeding, and perforation. All of the above risks are considered low with a short period (7 days) of administration in subjects who will be thoroughly interviewed as to whether they have allergies to NSAIDs or NSAID-containing medications.

Naproxen is a non-steroidal anti-inflammatory drug that inhibits both COX-1 and COX-2. It is approved for the treatment of osteoarthritis (250-500 mg twice daily), rheumatoid arthritis (250-500 mg twice daily), juvenile rheumatoid arthritis (10 mg/kg/day in two divided doses), ankylosing spondylitis (250-500 mg twice daily), gout (750 mg initially followed by 250 mg every 8 hours), and acute pain, including bursitis, tendinitis, and primary dysmenorrhea (500 mg initially followed by 250 mg every 6-8 hours or 500 mg every 12 hours as needed). Naproxen is contraindicated in patients with known hypersensitivity to naproxen, aspirin, or other NSAIDs and in patients who have experienced asthma, urticaria, or allergic-type reaction following aspirin or other NSAID administration. Severe anaphylactoid reactions to NSAIDs, some of them fatal, have been reported in such patients. Naproxen is also contraindicated for the treatment of perioperative pain in patients who have recently undergone coronary artery bypass graft (CABG) surgery. Like other NSAIDs, naproxen carries the risk of gastrointestinal ulceration, bleeding, and perforation. All of the above risks are considered low with a short period (7 days) of administration in subjects who will be thoroughly interviewed as to whether they have allergies to NSAIDs or NSAID-containing medications.

1.3 Clinical Data to Date

We have previously demonstrated high inter-individual variability in the pharmacologic response to a single dose of the COX-2 selective NSAIDs celecoxib and rofecoxib,[15] but the inter-individual variability in response to a traditional and COX-2 selective NSAID at steady state has not been evaluated. The outcome of drug treatment in an individual patient reflects a complex interaction of multiple factors, including demographic and clinical variables, genetic variation, gene and protein expression levels, drug exposure/pharmacokinetics, and environmental exposures. To date, a comprehensive investigation of how these factors influence the pharmacologic response to NSAIDs has not been performed. Preliminary studies in our laboratory suggest that the expression of COX-1 and COX-2 in lymphoblastoid cell lines (LCLs) varies among individuals by greater than 50-fold and greater than 300-fold, respectively. However, it is unknown whether this inter-individual variability in gene expression influences the pharmacologic response to NSAIDs. Moreover, how variation in the genome, transcriptome, proteome, microbiome and metabolome influences the pharmacologic response to traditional and COX-2 selective NSAIDs has not been investigated to date. Such studies may provide insight into the biologic networks that influence the outcome of NSAID therapy and provide the requisite preliminary data to design prospective studies to identify biomarkers to personalize NSAID therapy.

1.4 Dose Rationale and Risk/Benefits

Celecoxib will be administered at a dose of 100 mg (low dose arm) or 200 mg (high dose arm) orally twice daily for 7 days. Naproxen will be administered at a dose of 250 mg (low dose arm) or 500 mg (high dose

arm) orally twice daily for 7 days. These dose regimens are clinically used in the treatment of rheumatoid arthritis, and are being used in the Prospective Randomized Evaluation Of Celecoxib Integrated Safety Vs Ibuprofen Or Naproxen (PRECISION) trial, [16] which is a prospective trial comparing the cardiovascular safety of celecoxib, naproxen, and ibuprofen. The half-lives of celecoxib and naproxen are 11 hours and 12-15 hours, respectively. Thus, the treatment duration of 7 days was chosen to ensure that drug plasma concentrations have reached steady state.

NSAIDs are generally considered safe drugs for short term treatments, and some NSAIDs are available as over-the-counter (OTC) painkillers in low doses. For example, naproxen is available OTC as a 200 mg dose. Higher does, including the doses used here (250 mg and 500 mg) require a prescription. Celecoxib is a prescription medicine at all doses. All NSAIDs may cause allergic reactions including asthma, urticaria, or allergic-type skin reactions and severe anaphylactic reactions. All NSAIDs can lead to the onset of new hypertension or worsening of preexisting hypertension. All NSAIDs may cause renal adverse events, including fluid retention and edema, and renal toxicity. All NSAIDs may cause gastrointestinal adverse events including heartburn, diarrhea, abdominal distress, nausea and vomiting, indigestion, constipation, abdominal cramps or pain, fullness of GI tract (bloating and flatulence), ulceration and bleeding. Chronic use of NSAIDs may increase the risk of myocardial infarction, stroke and thrombosis, although this has only been shown for celecoxib in randomized trials. Central nervous system adverse effects include dizziness, headache, nervousness, and tinnitus.

Here the treatment duration will be short (7 days) when compared to treatment durations required to relieve symptoms of arthritis or spondylitis. Thus, the risks from study drugs are deemed low, particularly as these are volunteers without prior history of gastrointestinal or renal disease, and without previous serious cardiovascular events such as myocardial infarction, stroke, revascularization procedures and thrombotic events. Severe adverse events of the study drugs such as bleeds, gastrointestinal bleeds, hypertension, renal failure, myocardial infarction and stroke, drug allergies and death are rare.

2 Study Objectives

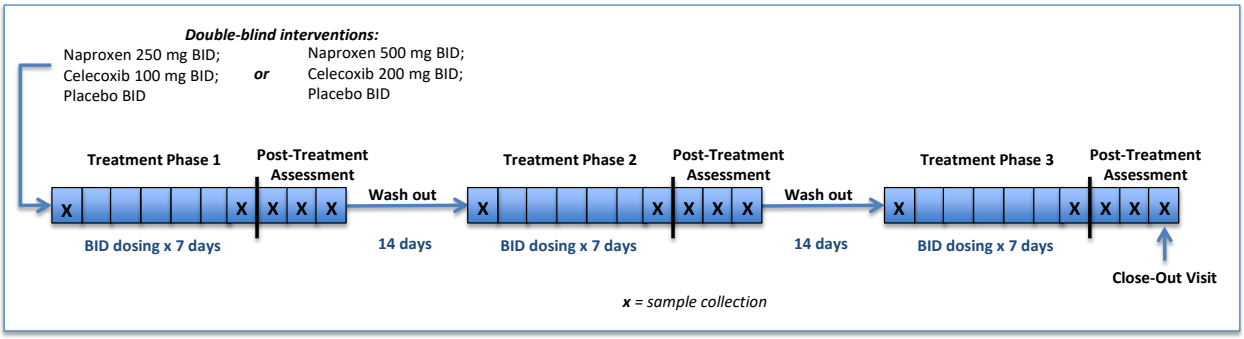
There are several objectives for this study:

1. To determine the range of variation of *ex vivo* and *in vivo* markers of COX function at baseline and at steady state concentrations of naproxen and celecoxib in comparison to placebo
2. To explore factors that may account for the observed variability in COX function at baseline and following NSAID treatment, including demographic factors, pharmacokinetics, variability in COX pathway gene expression, and the gut microbiome
3. To explore the dynamics and variability of the molecular NSAID response network using “omics” data
4. To identify candidate genetic modifiers in the function of the COX pathway at baseline and in response to NSAIDs
5. To establish a well-phenotyped cohort of subjects, with biobanked samples, that can be re-contacted about participation in future studies of NSAID response.

3 Study Design

3.1 General Design

The study is a double-blind, placebo-controlled trial investigating the pharmacologic response to celecoxib and naproxen in healthy adults. Subjects will be assigned to one of two treatment arms: a low dose arm and a high dose arm. Each treatment phase will be 7 days followed by a 3 day post-treatment assessment and a 2 week wash-out period. The order of the three treatment phases (celecoxib, naproxen, and placebo) will be randomized. The study schema is shown in the figure below:



Qualified subjects will have approximately 9 weeks of participation in this study (1 week/treatment phase x 3 treatment phases + 3 days/post-treatment assessment x 3 post-treatment assessments + 2 weeks/washout phase x 2 washout phases).

3.2 Primary Study Endpoints

The primary study endpoints are COX-1 and COX-2 activity as assessed *ex vivo* using whole blood assays and *in vivo* by measuring urinary excretion of prostaglandin metabolites.

3.3 Secondary Study Endpoints

Secondary outcome variables include:

- Celecoxib and naproxen pharmacokinetic profiles and drug exposure at steady state.
- 24 hr ambulatory blood pressure at steady state.
- Renal function as assessed by glomerular filtration rate (GFR), sodium & potassium excretion, aldosterone, and creatinine in 12-hour urine as well as renin, creatinine and urea nitrogen in plasma
- mRNA expression of genes in the COX pathway in lymphoblastoid cell lines derived from peripheral blood mononuclear cells (PBMCs).
- Analysis of the gut microbiome at steady state drug concentrations.
- Urinary and plasma metabolomics at trough and peak drug concentrations at steady state.
- Whole blood transcriptomics and plasma proteomics at trough and peak drug concentrations at steady state.
- DNA sequencing to identify genetic polymorphisms that modify the response to NSAIDs
- Nutritional assessment: 3-day food records, 24-hour dietary recalls and concentrations/ratios of major n-3/n-6 PUFAs in red blood cell membranes

3.4 Primary Safety Endpoints

Safety endpoints include:

- Renal function as assessed by glomerular filtration rate (GFR), sodium & potassium excretion, and creatinine in 12-hour urine as well as creatinine and urea nitrogen in plasma
- 24 hr ambulatory blood pressure at steady state

4 Subject Selection and Withdrawal

4.1 Inclusion Criteria

1. Men and women greater than 18 years of age
2. Subjects must be in good health based on medical history, physical examination, vital signs, and laboratory tests. In order to ensure sufficient enrollment of subjects in the higher age groups, volunteers with the following conditions may participate in the study:
    a. Adequately controlled hypertension, with diastolic blood pressure ≤100 mmHg at screening.
    b. Total cholesterol of ≤270 mg/dL
3. Body mass index (BMI) between 18 and 30 kg/m^2.

4. Has not used tobacco products, including smoking cessation nicotine-containing products (e.g., nicotine patch, nicotine gum), for at least the 3 months prior to screening.
5. Female subjects of child bearing potential must be using a medically acceptable method of contraception (oral contraception, Depo-Provera injection, IUD, condom with spermicide, diaphragm, cervical cap, progestin implant, abstinence, tubal ligation, oopherectomy, TAH) throughout the entire study period. All female subjects must consent to a serum and urine pregnancy test at screening and close-out and a urine pregnancy test just prior to the start of each treatment phase of the study, which must be negative at all time points.
6. All subjects must consent to a urine drug test at screening. Results must be negative. A positive result will be reported to the subject.
7. Does not consume more than 1 alcoholic beverage per day on average.
8. Able and willing to refrain from alcohol use within 48 hours prior to the first dose of study drug and during the study period until the final study visit.
9. Able to understand and comply with study procedures.
10. Able and willing to provide written informed consent prior to any study procedures being performed.

4.2 Exclusion Criteria

1. Female subjects who are pregnant or nursing a child.
2. Subjects who have received an investigational drug or used an experimental medical device within 30 days prior to screening, or who gave a blood donation of ≥ one pint within 8 weeks prior to screening.
3. Subjects with any coagulation, bleeding or blood disorders.
4. Subjects who are sensitive or allergic to celecoxib (Celebrex) or naproxen (Naprosyn) or their components.
5. Subjects who are sensitive or allergic to aspirin or other NSAIDs.
6. Subjects with documented history of any gastrointestinal disorders, including bleeding ulcers.
7. History of significant cardiovascular disease (including stroke or TIA), renal, hepatic, respiratory (except infections which longer > 6 months prior to screening), immune, endocrine, hematopoietic disorder or neurological disorders.
8. History of cancer within the last 5 years (except for cutaneous basal cell or squamous cell cancer resolved by excision, or carcinoma in situ of the cervix adequately treated).
9. Has taken any prescription medication other than hormone replacement therapy (including males taking testosterone as a hormone replacement to treat a documented low testosterone level), thyroid replacement hormones, anti-hyperlipidemic agents, or anti-hypertensive medications. Individuals taking other/additional chronic stable medications can be considered on a case-by-case basis for inclusion in the study if agreed upon by judgment of the investigators.
10. Has taken the following NSAID or antisecretory agents within 2 weeks prior to study drug administration:
    a. Nonsteroidal anti-inflammatory drugs (NSAIDs) including acetaminophen or other medications for pain, including aspirin or aspirin-containing products
    b. Proton pump inhibitors, including Prilosec®, Prevacid®, Aciphex®, Protonix®, or Nexium® (antacid medications, including OTC products, are not permitted within 24 hours of dosing)
    c. H_2 blockers, including Tagamet®, Zantac®, Axid®, or Pepcid®
11. Has ever taken the following anti-platelet or anti-coagulant agents:
    a. Any anti-platelet agent, including Aggrenox®, Brilinta®, Plavix®, Ticlid®, Pletal®, ReoPro®, Integrilin®, Aggrastat®, Persantine®, or Effient®
    b. Any anti-coagulant including Arixtra®, Coumadin®, acenocoumarol, Lovenox®, phenprocoumon, phenindione, heparin, Exanta®, Pradaxa®, argatroban, lepirudin, hirudin, bivalirudin, or Xarelto®
12. Used dietary or herbal supplements containing salicylates, Vitamin E, fish oil, or any other herbal supplements, within 14 days of study drug administration.

13. Subjects with any abnormal laboratory value or physical finding that according to the investigator may interfere with interpretation of the study results, be indicative of an underlying disease state, or compromise the safety of a potential subject.
14. Subjects who have had a history of drug or alcohol abuse within the last 6 months.
15. Subjects who are unwilling to provide a blood sample for genetic analyses and creation of a lymphoblastoid cell line.

4.3 Subject Recruitment and Screening

Healthy volunteers will be recruited from the database of healthy volunteers at the Institute for Translational Medicine and Therapeutics (ITMAT), through flyer, and by word of mouth.

Prior to enrollment, all volunteers will be asked to complete an online screening questionnaire to determine their eligibility. The screening questionnaire was prepared using Redcap, an on-line research electronic data capturing software. Volunteers will be sent an invitation email through RedCap with instructions to complete the questionnaire and a link. They will also have the option to complete the questionnaire over the phone by contacting the clinical study coordinator. The screening questionnaire can be accessed at https://redcap.med.upenn.edu/surveys/?s=FWCDYTXTE3 .

Volunteers who meet inclusion criteria based on the screening questionnaire will undergo a screening visit, during which a complete medical history, physical examination, hematology and blood chemistries, urinalysis, urine drug test and urine pregnancy test (female subjects only) will be obtained.

4.4 Early Withdrawal of Subjects

4.4.1 When and How to Withdraw Subjects

All subjects are free to withdraw from participation in this study at any time, for any reason, and without prejudice.

The Investigator may terminate a subject from the study at any time for intolerable or unacceptable AEs, intercurrent illness, noncompliance with protocol requirements, administrative reasons, or in the Investigator's opinion to protect the subject's best interest.

If a subject is withdrawn before completing the study, the reason for withdrawal will be entered on the appropriate case report form (CRF). Whenever possible and reasonable, the evaluations which were to be conducted at the completion of the study should be performed at the time of premature discontinuation.

4.4.2 Data Collection and Follow-up for Withdrawn Subjects

Whenever possible and reasonable, the evaluations which were to be conducted at the completion of the study should be performed at the time of premature discontinuation. To encourage subjects to participate in the end-of-study clinic visit, up to 10 contacts will be initiated via e-mail, text message or phone by study coordinators. If this fails to motivate the subject, up to 2 phone calls will be initiated by the PI, Co-PI, or qualified sub-investigator (MD) in order to schedule the exit visit.

5 Study Drug

5.1 Description

Celecoxib and naproxen are FDA approved medications for pain and inflammatory diseases. The study drugs will be all administered at FDA approved therapeutic doses.

Celecoxib is a prescription drug that will be administered as commercially available 100 mg (low dose group) and 200 mg (high dose group) capsules. Naproxen is a prescription drug that will be administered as commercially available 250 mg (low dose group) and 500 mg (high dose group) tablets.

5.2 Treatment Regimen

During each treatment phase, subjects will receive celecoxib, naproxen, or placebo for 7 days. Subjects will be assigned to either a high dose arm (celecoxib 200 mg BID, naproxen 500 mg BID, or placebo) or a low dose arm (celecoxib 100 mg BID, naproxen 250 mg BID, or placebo). Subjects will be instructed to take the study medications twice a day (at approximately 8 AM and 8 PM) on an empty stomach with a full glass of water.

5.3 Method for Assigning Subjects to Treatment Groups

Subjects will be assigned alternately to the low dose or high dose treatment arm in blocks of 36 subjects (i.e. subjects 1-36 will be assigned to the low dose arm; subjects 37-72 will be assigned to the high dose arm, etc).

The order of administration of the three study drugs will be randomized. The independent study statistician will prepare randomization lists according to this schedule which will be sent to the investigational pharmacist (UPENN Investigational Drug Service). When notified that a subject has completed the screening period and is ready to begin treatment, the investigational pharmacist will select the next randomization label in sequence and dispense the assigned study treatment for that subject.

5.4 Preparation and Administration of Study Drug

Study drug will be maintained and dispensed by the Investigational Drug Service (IDS) of the University of Pennsylvania (3600 Spruce St, Ground Maloney, Philadelphia, PA 19104, contact person: Dr. Kenneth Rockwell Jr., Phone: 215-349-8817 / Fax 215-349-5132). Commercially sourced celecoxib and naproxen will be blinded by IDS to match the placebo by over-encapsulation. As noted in section 5.7 (Packaging), the IDS will prepare blister packs of blinded study drug. The study drugs will be administered orally.

5.5 Subject Compliance Monitoring

Subjects will be instructed to take the study medications twice a day (at approximately 8 AM and 8 PM) on an empty stomach with a full glass of water. During each treatment phase, subjects will receive a reminder to take each study medication dose by email and/or text message twice a day. Reminder emails will be sent through Redcap, an on-line research electronic data capturing software, and will include a unique link to a survey to confirm taking the dose. Text message reminders will be sent through the HIPAA-compliant Clienttell system in use at the University of Pennsylvania Health System. The daily administration of drugs will be monitored by requesting the patient to confirm taking each pill at the time of dosing through Redcap or by the subject replying to the text message. Those forgetting to take the pill will be reminded by phone. Meanwhile, pill count and plasma drug concentration will also be used to verify adherence.

5.6 Prior and Concomitant Therapy

A full medication history will be taken at the screening visit. Subjects will be permitted to take oral contraceptives, hormone replacement therapy, thyroid replacement hormones, anti-hyperlipidemic agents, or anti-hypertensive agents during the study. Individuals taking other/additional chronic stable medications can be considered on a case-by-case basis for inclusion in the study if agreed upon by judgment of the investigators.

Subjects will not be permitted to take NSAIDs, antisecretory agents, or dietary or herbal supplements for 2 weeks prior to study drug administration and throughout the study. Any subject who has ever taken an anti-platelet or anti-coagulant medication will not be enrolled in the study (see Exclusion Criteria).

5.7 Packaging

Study drug will be supplied in bulk shipments and blinded by over-encapsulation by the IDS. The IDS will package blinded study medication on-site, as blister packs containing twelve capsules per blister pack. Each blister pack represents the drug supply for at-home drug treatment for a single treatment phase. The last dose, which will be administered on Day 7 in the CTRC, will be packaged separately. Subject

study drug supply will be packaged in kits of 3 blister packs plus the last study medication dose, one for each treatment phase. Each kit will represent the entire drug supply for one study subject.

5.8 Blinding of Study Drug

Blinding of the study drugs will be done by over-encapsulation at the Investigational Drug Service. All subjects and research staff will be blinded as to the sequence of study medications until the subject has completed the study. Codes linking randomization number for each subject to actual treatment will be secured in a sealed, opaque envelope and maintained in a locked drawer in the IDS. Research subjects will be given the emergency contact number for the study during the consenting process (see Attachment 2)

See section 8.4 (Unblinding Procedures) for a description of the process for unblinding a study subject.

5.9 Receiving, Storage, Dispensing and Return

5.9.1 Receipt of Drug Supplies

Upon receipt of the of the study treatment supplies, an inventory will be performed and a drug receipt log filled out and signed by the person accepting the shipment. The IDS designated staff will count and verify that the shipment contains all the items noted in the shipment inventory. Any damaged or unusable study drug in a given shipment will be documented in the study files. The investigator will notify the supplier of any damaged or unusable study treatments that were supplied to the investigator's site.

5.9.2 Storage

Stock study drug and drug packaged in patient kits within the IDS, and study drug that has been dispensed to the Investigator, will be stored in a locked cabinets with climate control maintaining the temperatures within a range of 20°-25° C (68°-77° F). Access to study drug within the IDS will be controlled by the IDS staff, and access to study drug dispensed to the Investigator will be controlled by the Investigator and the Investigator's study staff.

5.9.3 Dispensing of Study Drug

All study drugs will be dispensed as subject-specific kits by the IDS to the investigator or their designated study staff. The investigator or designated study staff will then dispense the appropriate amount of study drug from the study kit to the research subject assigned to that kit. For safety, the first dose will be administered while at the CTRC including a post-dose observational period to screen for acute adverse effects.

5.9.4 Return or Destruction of Study Drug

At the completion of the study, there will be a final reconciliation of drug shipped, drug consumed, and drug remaining. This reconciliation will be logged on the drug reconciliation form, signed and dated. Any discrepancies noted will be investigated, resolved, and documented prior to return or destruction of unused study drug. Drug destroyed on site will be documented in the study files.

6 Study Procedures

See Attachment 1 for the Study Procedure Flowchart.

6.1 Laboratory screens for compliance

6.1.1 Cotinine

Urinary cotinine, the nicotine metabolite indicating recent tobacco or nicotine use, will be qualitatively assessed per One-Step Rapid Nicotine (COT) Test, Craig Medical Distribution, Inc., 1185 Park Center Drive, Vista, CA (or similar). This immunoassay detects cotinine at a cut-off sensitivity level of 200 ng/ml.

6.1.2 Protocol Non-Conform Drugs

Urine will be used to assess intake of protocol non-conform drugs by means of the Rapid Detect 10 Panel Test Dip Card, Rapid Detect, Inc., 804 South Broadway, Poteau, OK (or similar). The drug screen includes for example Amphetamines (AMP), cocaine (COC), Tetrahydrocannabinol (THC), methamphetamine (mAMP), opiates (OPI), Phencyclidine (PCP), benzodiazepines (BZO), tricyclic antidepressants (TCA), barbiturates (BAR), and methadone (MTD).

6.1.3 Pregnancy test

Serum beta-HCG concentrations and a standard pregnancy urine dip test will be used to rule out pregnancy.

6.1.4 NSAID plasma concentrations

Prior to beginning each treatment phase, the concentrations of the four most commonly used NSAIDs in subjects' plasma will be quantified by LC-MS/MS. This will be done to confirm that subjects have not used any NSAIDs prior to beginning the treatment phase. During each treatment phase, study drug plasma concentrations will be quantified by LC-MS/MS to verify compliance and evaluate the pharmacokinetic profile of the study drugs. These assays are routinely performed in the Center for Experimental Therapeutics mass spectrometry laboratories.

6.1.5 COX-1 acetylation

COX-1 acetylation will be quantified to confirm that the subjects have not used any aspirin or aspirin-containing products.

Peripheral blood anticoagulated with citrate solution will be centrifuged immediately (180 g,10 min, 21^{o}C) using a swing bucket centrifuge. Platelet rich plasma (PRP) will be removed, diluted with an equal volume PIPES buffer (pH 6.8), and 1 μM prostaglandin (PG) E_1 added and washed with PIPES buffer once. The platelets will be lyzed with RIPA buffer supplemented with protease inhibitor cocktail (Roche, Mannheim, Germany) at room temperature for 30 min. The soluble proteins in the supernatant will be collected by centrifugation at 4^{o}C for 10 min. Proteins will be separated on NuPAGE® precast gels (Invitrogen) and stained with colloidal blue staining kit (Invitrogen). The molecular weight region corresponding to purified COX-1 (Cayman Chemical, Ann Arbor, MI) will be excised and cut into small pieces, which were then de-stained in 40% acetonitrile solution and dried. The gel pellet will be rehydrated in 20 μl of 12 ng/μl trypsin in 0.01% ProteaseMAX™ Surfactant (Promega, Madision, WI): 50mM NH4HCO3 for 10 min, which is covered with 30 μl of 0.01% ProteaseMAX™ Surfactant: 50mM NH4HCO3 and 10 μl heavy internal standards (250 fmol). Peptide standards IGAPFSLK, IGAPFS[+ace]LK and LVLTVR, and their heavy isotopic labeled peptides IGAPFSL[^{13}C6;^{15}N]K, IGA[^{13}C3;^{15}N]PFS[+ace]LK and LVLTV[^{13}C5;^{15}N]R were synthesized by Thermo Scientific (Bremen, Germany) for isotope dilution mass spectrometry. The protein digestion will be performed at 37°C for 2 hours.

Peptide liquid chromatography-mass spectrometry will be performed on an Eksigent NanoLC-2D system (Eksigent Technologies, Dublin, CA) coupled to a Thermo LTQ-FT mass spectrometer. Solvents used will be 0.5% ACN/0.1% formic acid (mobile phase A) and 98% ACN/0.1% formic acid (mobile phase B). Peptides will be loaded on an IntegraFrit™ trap column (10 mm×75 μm, New Objective, Woburn, MA), then the LC gradient run at 1 μL/min to separate peptides on a Magic C18 column (50 mm×0.2 mm, Michrom BioResources). A linear gradient of mobile phase B will be developed to elute peptides from 5% to 30% B at 1 min to 20 min. Nano electrospray ionization will be performed in the positive ion mode. The mass spectrometer was operated in MRM mode including 10 transitions in a single LC−MRM/MS analysis. The transitions for non-acetylated peptides, acetylated peptides and control peptides will be monitored. Typical instrument settings included a capillary temperature of 200 °C using nitrogen as an auxiliary gas. Helium is used as the collision gas. The activation energy (Act. Q) is 0.35 (arbitrary units). The MRM peak areas will be extracted and analyzed manually.

6.2 Questionnaires

6.2.1 Food questionnaires

6.2.1.1 3-day food record

The 3-day food record will be collected and analyzed by using Nutrition Data System for Research (NDSR).

6.2.1.2 24-hour dietary recall

The 24-hour dietary recalls will be collected using Nutrition Data System for Research (NDSR), a computer-based software application developed at the University of Minnesota Nutrition Coordinating Center (NCC) that facilitates the collection of recalls in a standardized fashion.[17] Dietary intake data gathered by interview is governed by a multiple-pass interview approach.[18] Five distinct passes provide multiple opportunities for the participant to recall food intake. The first pass involves obtaining from the participant a listing of all foods and beverages consumed in the previous 24 hours. This listing is reviewed with the participant for completeness and correctness (second pass). The interviewer then collects detailed information about each reported food and beverage, including the amount consumed and method of preparation (third pass). In the optional fourth pass, the interviewer then probes for commonly forgotten foods. Finally, the detailed information is reviewed for completeness and correctness (fifth pass).

Dietary supplement use will be assessed in conjunction with collection of 24-hour dietary recalls using the Dietary Supplement Assessment Module included in NDSR.[19] Use of all types of dietary supplements and non-prescription antacids are queried in the module.

The NCC Food and Nutrient Database serves as the source of food composition information in NDSR. [20] This database includes over 18,000 foods including 7,000 brand name products. Ingredient choices and preparation methods provide more than 160,000 food variants. Values for 163 nutrient, nutrient ratios and other food components are generated from the database. The USDA Nutrient Data Laboratory is the primary source of nutrient values and nutrient composition. These values are supplemented by food manufacturers' information and data available in the scientific literature.[21] Standardized, published imputation procedures are applied to minimize missing values.[22]

6.2.2 International Physical Activity Questionnaire

The physical activity will be assessed using the “International Physical Activity Questionnaire” (October 2002) in the “long last 7 days self-administered format” for “use with young and middle-aged adults (15-69 years)” (Source: www.ipaq.ki.se). This questionnaire comprises five activity domains asked independently and has been frequently validated internationally to obtain comparable data on health–related physical activity. The scoring protocol for the IPAQ long form (updated Nov 2005) is available at www.ipaq.ki.se/IPAQ.asp?mnu_sel=EEF&pg_sel=IIA. The questionnaire is provided in the Attachments.

6.3 Measurements of COX activity

The activity of COX-1 and -2 will be assessed by the use of two *ex vivo* assays: inhibition of platelet TxA_2 formation, which is reflective of COX-1 activity;[23] and inhibition of PGE_2 formation by monocytes in which COX-2 is induced by endotoxin (LPS) and constitutive COX-1 is blocked by aspirin.[24] The urinary prostaglandin metabolite, 2,3 dinor-6 keto $PGF_{1\alpha}$ (PGI-M) is measured by GC/MS, the thromboxane metabolite, 11-dehydro thromboxane B_2 (Tx-M) and 7α-hydroxy-5, 11-diketotetranorprostane-1, 16-dioic acid (PGE-M), are quantified as its methoxyamine derivative by LC/MS/MS. These assays are routinely performed in the Center for Experimental Therapeutics mass spectrometry laboratories with inter- and intra-assay variability of 4-8%.

6.4 Genetic Analyses

Genetic analysis will be performed using High Throughput Sequencing (HTS) technology. These analyses will be performed at the High Throughput Sequencing Facility of the Penn Genomics Frontiers Institute (PGFI) using the Illumina HiSeq 2000 platform. Both whole exome and whole genome sequencing will be performed. This will allow high quality variant calling of approximately 3 million SNPs and insertions and deletions (Indels). It will also allow identifying Copy Number Variations (CNV) and other structural rearrangements. Analysis will be performed on de-identified samples and data sets.

6.5 In-vitro studies on Lymphoblastoid Cell Lines

Blood will be drawn for isolation of lymphocytes and other mononuclear cells (PBMCs) from whole blood using a standard Ficoll Paque density gradient centrifugation method. Mononuclear cells will be washed twice with saline solution for the generation of Epstein-Barr virus immortalized lymphoblastoid cell lines (LCLs) following standard methodology.[25]

Baseline and cytokine induced COX-1 and -2 expression in freshly purified PBMCs and in LCLs will be detected by RT-PCR and Western blot and peptide mass spectrometry. Cyclooxygenase function will be quantitated by measuring COX products in the absence and the presence of COX inhibitors using mass spectrometry. Molecular mechanisms underlying variable COX expression and function will be elucidated using promoter studies, gene expression studies, proteomics studies and DNA sequencing. The B-cell immortalization method and the above mentioned in-vitro methodologies are routinely performed in the Institute for Translational Medicine and Therapeutics. Gene expression studies will involve both micro array technology and RNA sequencing technology. Data analysis will be based on standard software packages in Bioconductor (www.bioconductor.org) using R (cran.r-project.org). RNA sequence analysis will be performed using in-house software.

Storage of cell lines and separation of personal information from stored biological material and data will be handled analogous to the handling of stored DNA samples in compliance with lawful privacy rules.

6.6 Transcriptomics

Gene expression profiling will be performed on whole blood and/or PBMCs. Gene expression studies will involve both micro array technology and RNA sequencing technology. Data analysis will be based on standard software packages in Bioconductor (www.bioconductor.org) using R (cran.r-project.org). RNA sequence analysis will be performed using in-house software.

6.7 Proteomics

Plasma proteomics will be performed using the SomaLogic proteomics assay.[26]

6.8 Metabolomics on Plasma and Urine Samples

Plasma and urine samples will be de-identified by transferring it into a new tube, which is labeled with a code (similar to the DNA tube labeling code) instead of the subject study number. This code will be generated using a random number generator. The study coordinator will maintain a table which contains the association between sample code and study subject information, which will not be given to the personnel who conduct and interpret the metabolomics analyses. Only non-PHI demographic information such as age, sex, race, ethnicity, BMI will be used for the analysis of the metabolomics results. Only aggregate statistics, which do not allow the identification of individual subjects, will be published. The metabolomics analysis will involve NMR spectroscopy and liquid chromatography mass spectrometry measurements. These analyses will be conducted by our collaborator, Dr. Julian Griffin at Cambridge University, UK. These measurements are entirely exploratory. Aspects such as intraindividual and interindividual variability will be assessed and can inform the design of future prospective studies into the metabolomics effects of NSAIDs.

6.9 Ambulatory Blood Pressure Monitoring (ABPM)

Ambulatory blood pressure monitors (ABPM) manufactured by Spacelab Healthcare are readily available to the investigators to assess ABP in this study protocol. The 90207 ABP monitors are compact and

lightweight to optimize subject comfort and a choice of 5 cuff sizes further aids comfort while also maximizing accuracy (retrieved from the website of the manufacturer at http://www.spacelabshealthcare.com/en/, accessed August 28, 2012).

The manufacturer indicates that the 92506 ABP Report Management System software, also readily available to the investigators, supports compliance with FDA and HIPAA requirements for integrity, availability, security, and confidentiality of protected health information (retrieved from the website of the manufacturer at http://www.spacelabshealthcare.com/en/, accessed August 28, 2012).

ABPM will be guided by the AHA Scientific Statement, Council on High Blood Pressure[27]. Recommendations relevant to the present study are:

- Select ABP cuff size according to Table 1,
- Select the non-dominant upper arm for ABPM measurements,
- Instruct the patient to hold the arm still by their side while the device is taking a reading.

Table 1 Cuff Sizes for ABPM

Cuff Size*	Arm circumference [cm]	Arm circumference [inches]
Small Adult	17-26	7-10
Adult	24-32	9-13
Large Adult	32-42	13-17
Extra Large Adult	38-50	15-20

* The bladder of the cuff should encircle at least 80% of the arm circumference. [27]

The monitors for the 48-hr ambulatory blood pressure measurements will be preset to assess BP every 15 minutes in the daytime (0600h to 2200h) and every 30 minutes during the nighttime (2200h-0600h). As ABPM will be assessed over an extended period of time, 48 hours, the daytime intervals may be set to assess BP every 20 minutes to decrease the burden on the subjects and increase compliance. Preset ranges for acceptable BP measurements are 60–250 mmHg systolic, 30-200 mmHg diastolic, and 40-230 mmHg for mean arterial values. ABP readings should cover ≥ 80% of the expected readings with interruptions of less than 1 hour. Awake and asleep times will be determined by patient diaries. This protocol is well established. [28,29]

6.10 Stool Samples for Microbiome Analyses

Stool samples will be collected by subjects either at home or in a private bathroom within the CTRC. Subjects will be supplied with a collection kit and verbal and written instructions for stool collection at the time of consent. Stool samples will be kept frozen in a -80°C freezer until analysis. Stool form will be assessed by means of the Bristol Stool Chart, a 7-point scale.[30]

6.11 Microbiome Analyses

DNA from stool samples, mouth and fecal swabs stored at -80ºC will be extracted from each sample; PCR amplified using 16S primers, and subjected to 454/Roche pyrosequencing. At least 1000 sequence reads will be used to characterize each community. The 16S sequence reads are then aligned using the NAST and GreenGenes servers and inserted into a well characterized phylogenetic trees of 16S sequences, allowing phylogenetic placement of each sequence read. As a first step in analyzing the global effects of each treament, we will compare microbial communities using UniFrac, which quantifies the similarities among microbial communities based on phylogenetic distances. To compare two communities, sequences from both communities are placed on a common phylogenetic tree generated using ARB. The fraction of the branch length on the tree unique to each community is then measured. This provides a objective measure of community similarity based on the amount of shared

evolutionary history. To compare multiple communities, distances between all pairs of communities are computed to generate a distance matrix and Principal Coordinate Analysis is used to plot communities in a scatter plot along orthogonal axes of maximal variance. Such scatter plots can be generated taking into account the abundance as well as the presence of each taxa (weighted UniFrac), or using only presence/absence information (unweighted UniFrac). The two methods thus address different questions--weighted analysis allows differences in proportional representation of community members to be assessed, while unweighted analysis discloses changes in community composition. Measures of alpha diversity such as the Chao1 and Shannon Indices will also be used to characterize communities.

6.12 Study Visits

6.12.1 Screening Visit

All subjects will be asked to come to the Clinical and Translational Research Center (CTRC) at the Hospital of the University of Pennsylvania for the screening visit. During this visit, a complete medical history, physical examination, and the following laboratory tests will be obtained:

- Hematology: white blood cell count, red blood cell count, hemoglobin, hematocrit, MCV, MCH, MCHC, RCDW, and platelet count.
- Blood Chemistries: sodium, potassium, chloride, urea nitrogen, creatinine, fasting glucose, albumin, alkaline phosphatase, fasting cholesterol, HDL cholesterol and LDL cholesterol, triglycerides, ALT, AST, LDH, total bilirubin, GGT, uric acid, and phosphate.
- Urinalysis
- Serum and urine pregnancy test for women of childbearing potential
- Urine drug test for all subjects

Subjects will be asked to discontinue all aspirin and aspirin-containing products and herbal and dietary supplements, including vitamins and anti-oxidants, two weeks prior to beginning the treatment phase. The use of other NSAIDs and acetaminophen will be discontinued for two weeks prior to the study. Also, subjects will be asked to refrain from alcohol, caffeine, and high fat foods for 24 hours prior to all study visits and to refrain from alcohol use throughout the study.

Subjects will be asked to complete a physical activity questionnaire. They will also be asked to complete a 3-day food record to record everything they eat for 3 consecutive days.

6.12.2 Visit 1 (Baseline Visit)

Subjects will arrive in a fasting state at the CTRC at approximately 7:30 AM and will be admitted as outpatients. A urine pregnancy test will be performed in women of childbearing potential. All assessments are experimental and will be carried out for research purposes.

Blood will be drawn at approximately 7:50 AM for measurement of:

- COX-1 and COX-2 activity
- NSAID plasma concentration
- COX-1 acetylation
- PBMC isolation for transformation to LCLs
- DNA isolation

Spot urine will be collected for measurement of:

- Tx-M
- PGI-M
- PGE-M
- PGD-M
- Metabolomic analysis
- Urinary electrolytes, aldosterone, creatinine clearance, and glomerular filtration rate (GFR).

The study medication will be administered at 8 AM. Subjects will be given the study medication for the treatment phase. They will be instructed to take the medication two times a day on an empty stomach at approximately 8 AM and 8 PM with a full glass of water.

6.12.3 Visit 2 (Whole-Day Visit)

Subjects will arrive in a fasting state at the CTRC at approximately 7:30 AM on Treatment Day 7 and will be admitted as outpatients. All assessments are experimental and will be carried out for research purposes.

Blood pressure will be measured for 24 hours using an ambulatory blood pressure monitor. Activity will be measured using an actigraph digital activity recorder. A stool sample will be collected for assessment of the gut microbiome, and subjects will complete a questionnaire regarding food consumption over the previous 24 hours.

Blood will be drawn at approximately 7:50 AM for measurement of:

- COX-1 and COX-2 activity
- Transcriptomic analysis
- Proteomic analysis
- Metabolomic analysis
- NSAID plasma concentration
- Renin activity, creatinine, and urea nitrogen
- Concentrations/ratios of major n-3/n-6 PUFAs in red blood cell membranes

Spot urine will be collected for measurement of:

- Tx-M
- PGI-M
- PGE-M
- PGD-M
- Metabolomic analysis
- Urinary electrolytes, aldosterone, creatinine clearance, and glomerular filtration rate (GFR).

The study medication will be administered at 8 AM.

Blood will be drawn 0.5, 1, 2, 4, 8, and 12 hours after administration of the study medication for measurement of:

- COX-1 and COX-2 activity
- NSAID plasma concentration

Spot urine will be collected 1, 2, 4, 8, and 12 hours after administration of the study medication for measurement of:

- Tx-M
- PGI-M
- PGE-M
- PGD-M

At 4 hours after administration of the study medication (the presumed peak of drug concentrations), blood will also be drawn for measurement of:

- Transcriptomic analysis
- Proteomic analysis
- Metabolomic analysis

Urine will be collected over 12 hours for measurement of:

- Urinary electrolytes, aldosterone, creatinine clearance, and GFR.

Subjects will be discharged after the 12 hour time point (approximately 8 PM).

6.12.4 Visit 3 (Post-Treatment Visit 1)

Subjects will return to the CTRC at approximately 7:30 AM on the day after completing the treatment phase (Post-Treatment Assessment Day 1). At approximately 8 AM a spot urine sample will be collected for measurement of:

- Tx-M
- PGI-M
- PGE-M
- PGD-M
- Metabolomic analysis
- Urinary electrolytes, aldosterone, creatinine clearance, and glomerular filtration rate (GFR).

Blood samples will be drawn for measurement of:

- COX-1 and COX-2 activity
- NSAID plasma concentration

The ambulatory blood pressure monitor and actigraph will be removed, and subjects will be discharged.

6.12.5 Visit 4 (Post-Treatment Visit 2)

Subjects will return to the CTRC at approximately 7:30 AM two days after completing the treatment phase (Post-Treatment Assessment Day 2). At approximately 8 AM a spot urine sample will be collected for measurement of:

- Tx-M
- PGI-M
- PGE-M
- PGD-M
- Metabolomic analysis
- Urinary electrolytes, aldosterone, creatinine clearance, and glomerular filtration rate (GFR).

Blood samples will be drawn for measurement of:

- COX-1 and COX-2 activity
- NSAID plasma concentration

Subjects will be discharged.

6.12.6 Visit 5 (Post-Treatment Visit 3)

Subjects will return to the CTRC at approximately 7:30 AM three days after completing the treatment phase (Post-Treatment Assessment Day 3). At approximately 8 AM a spot urine sample will be collected for measurement of:

- Tx-M
- PGI-M
- PGE-M
- PGD-M
- Metabolomic analysis
- Urinary electrolytes, aldosterone, creatinine clearance, and glomerular filtration rate (GFR).

Blood samples will be drawn for measurement of:

- COX-1 and COX-2 activity
- NSAID plasma concentration

Subjects will be given the study medication for the next treatment phase and will be discharged. After a two week wash-out period, subjects will begin the next treatment phase and return to the CTRC for a baseline visit on Treatment Day 1. A close-out visit will occur after all three treatment phases and post-treatment assessments have been completed.

6.12.7 Close-out Visit

After completing all three treatment phases, subjects will attend a close-out visit to conclude their participation in the study. This visit will occur concurrently with the final Post-Treatment Visit (Post-Treatment Visit 3 of Treatment Phase 3). During this visit, a physical examination and the following laboratory tests will be obtained:

- Hematology: white blood cell count, red blood cell count, hemoglobin, hematocrit, MCV, MCH, MCHC, RCDW, and platelet count.
- Blood Chemistries: sodium, potassium, chloride, urea nitrogen, creatinine, fasting glucose, albumin, alkaline phosphatase, fasting cholesterol, HDL cholesterol and LDL cholesterol, triglycerides, ALT, AST, LDH, total bilirubin, GGT, uric acid, and phosphate.
- Urinalysis
- Serum and urine pregnancy test for women of childbearing potential

After this visit, the subject's participation in the study will end.

7 Statistical Plan

7.1 Sample Size Determination

144 subjects per treatment arm (low dose and high dose; total N=288) will be studied to compare the variability in COX-1 and COX-2 activity as the primary outcome in measurements on 12 individuals per group. These groups are 3 DRUGs (placebo, celecoxib, naproxen) via randomization, 2 DOSEs (high and low) via randomization, 2 SEXes (female : male = 1:1), 3 AGE groups (18y to 40y, 41y to 60y, 61y to 85y = 1:1:1), and 2 RACEs (African American, Caucasian = 1:1). Importantly, individuals from ethnicities or races other than African American and Caucasian will be invited to participate in the study; however, there will not be specific sample size targets in the other groups. We expect that less than 10% of the population will be Hispanic or Asian or belong to another race. Thus, we will explore the outcomes in these groups, but will not have the statistical power for formal primary analyses.

7.2 Statistical Methods

The primary hypothesis of this study is that one or more clinical, genetic, and environmental factor affects variability in COX-1 and COX-2 activity following placebo and NSAID treatment. Calculations may involve change from baseline or a summary of longitudinal measures such as the area under the curve, but for simplicity of illustration we describe only a single post-treatment value. For 80% power and a two-sided significance level of 0.05, with n=12 per group we can detect a ratio of standard deviations of 2.42 (calculated based on the F-distribution for the ratio of variances). For example, analysis of the data from our prior study[15] provides an estimate of inter-subject standard deviation of 41.2; thus we could in this metric have adequate power to identify a standard deviation of 99.7. A 95% confidence interval on an estimated standard deviation of 41.2 with n=12 would range from approximately 29.2 to 70.0. For the paired comparisons between treatments within a dose group, the detectable ratio in standard deviations (and width of a confidence interval) will be smaller.

Exploratory transcriptomic, proteomic, and metabolomics analyses will be conducted by PENTACON collaborators using a variety of modelling techniques, such as Bayesian networks and ODE modelling. Microbiome analyses will utilize weighted and unweighted UniFrac, as well as measures of alpha diversity such as the Chao1 and Shannon Indices, to characterize communities.

7.3 Subject Population(s) for Analysis

The statistical analyses will include the all-randomized population.

8 Safety and Adverse Events

8.1 Definitions

Unanticipated Problems Involving Risk to Subjects or Others

Any incident, experience, or outcome that meets all of the following criteria:

- Unexpected in nature, severity, or frequency (i.e. not described in study-related documents such as the IRB-approved protocol or consent form, the investigators brochure, etc)
- Related or possibly related to participation in the research (i.e. 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)
- Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic, or social harm).

Adverse Event
An ***adverse event*** (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

Serious Adverse Event
Adverse events are classified as serious or non-serious. A ***serious adverse event*** is any AE that is:

- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

All adverse events that do not meet any of the criteria for serious should be regarded as ***non-serious adverse events***.

Adverse Event Reporting Period
The study period during which adverse events must be reported is normally defined as the period from the initiation of any study procedures to the end of the study treatment follow-up. For this study, the study treatment follow-up is defined as 30 days following the last administration of study treatment.

Preexisting Condition
A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

General Physical Examination Findings
At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an adverse event.

Post-study Adverse Event

All unresolved adverse events should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator should instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study. The investigator should notify the study sponsor of any death or adverse event occurring at any time after a subject has discontinued or terminated study participation that may reasonably be related to this study. The sponsor should also be notified if the investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that has participated in this study.

Abnormal Laboratory Values
A clinical laboratory abnormality should be documented as an adverse event if any one of the following conditions is met:

- The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality
- The abnormality suggests a disease and/or organ toxicity
- The abnormality is of a degree that requires active management; e.g. change of dose, discontinuation of the drug, more frequent follow-up assessments, further diagnostic investigation, etc.

Hospitalization, Prolonged Hospitalization or Surgery
Any adverse event that results in hospitalization or prolonged hospitalization should be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for and adverse event.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should ***not*** be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

Incidental Pregnancies
Pregnancy tests will be done by serum and urine concentrations of beta-HCG for the screening and close-out visit and by urine concentrations of beta-HCG for the remaining visits prior to each blinded drug administration. This frequent testing for pregnancies and the use of medically accepted methods of birth control will prevent incidental pregnancies in most, if not all, enrolled women.

Any pregnancy that inadvertently occurs in a study subject will be reported to the IRB as an unanticipated problem. The follow-up will consist of:

- Counseling the participant to contact and inform the primary care provider and an obstetrician or maternal-fetal specialist.
- A report summarizing the relevant study procedures which includes an unblinded log of drug/placebo, dosage, dates (& times if available) of study drug administration, results of laboratory blood and urine screens to the treating obstetrician or maternal-fetal specialist as well as the primary care physician (if available).
- The request to notify the study site about any adverse events which occurred for the mother and/or child during pregnancy and childbirth. To assist in this request, the study site will contact the treating obstetrician or maternal-fetal specialist twice by phone or in print after the calculated date of delivery.
- Final reporting of the outcome to the IRB.

8.2 Recording of Adverse Events

At each contact with the subject, the investigator must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded immediately in the source document, and also in the appropriate adverse event module of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results should recorded in the source document, though should be grouped under one diagnosis.

All adverse events occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period must be followed up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation should be recorded and reported immediately.

8.3 Reporting of Serious Adverse Events and Unanticipated Problems

Investigators and the protocol sponsor must conform to the adverse event reporting timelines, formats and requirements of the various entities to which they are responsible, but at a minimum those events that must be reported are those that are:

- related to study participation,
- unexpected, and
- serious or involve risks to subjects or others
  (see definitions, section 8.1).

If the report is supplied as a narrative, the minimum necessary information to be provided at the time of the initial report includes:

- Study identifier
- Study Center
- Subject number
- A description of the event
- Date of onset
- Current status
- Whether study treatment was discontinued
- The reason why the event is classified as serious
- Investigator assessment of the association between the event and study treatment

8.3.1 Investigator reporting: notifying the Penn IRB

This section describes the requirements for safety reporting by investigators who are Penn faculty, affiliated with a Penn research site, or otherwise responsible for safety reporting to the Penn IRB. The University of Pennsylvania IRB (Penn IRB) requires expedited reporting of those events related to study participation that are unforeseen and indicate that participants or others are at increased risk of harm. The Penn IRB will not acknowledge safety reports or bulk adverse event submissions that do not meet the criteria outlined below. The Penn IRB requires researchers to submit reports of the following problems within 10 working days from the time the investigator becomes aware of the event:

- Any adverse event (regardless of whether the event is serious or non-serious, on-site or off-site) that occurs any time during or after the research study, which in the opinion of the principal investigator is:

  Unexpected (An event is “unexpected” when its specificity and severity are not accurately reflected in the protocol-related documents, such as the IRB-approved research protocol, any applicable investigator brochure, and the current IRB-approved informed consent document and other relevant sources of information, such as product labeling and package inserts.)

  AND

  Related to the research procedures (An event is “related to the research procedures” if in the opinion of the principal investigator or sponsor, the event was more likely than not to be caused by the research procedures.)

Reporting Process

Unanticipated problems posing risks to subjects or others as noted above will be reported to the Penn IRB using the form: “Unanticipated Problems Posing Risks to Subjects or Others Including Reportable Adverse Events” or as a written report of the event (including a description of the event with information regarding its fulfillment of the above criteria, follow-up/resolution and need for revision to consent form and/or other study documentation).

Copies of each report and documentation of IRB notification and receipt will be kept in the Clinical Investigator’s study file.

Other Reportable events:
For clinical drug trials, the following events are also reportable to the Penn IRB:

- Any adverse experience that, even without detailed analysis, represents a serious unexpected adverse event that is rare in the absence of drug exposure (such as agranulocytosis, hepatic necrosis, Stevens-Johnson syndrome).
- Any adverse event that would cause the sponsor to modify the investigators brochure, protocol or informed consent form, or would prompt other action by the IRB to assure protection of human subjects.
- Information that indicates a change to the risks or potential benefits of the research, in terms of severity or frequency. For example:
  - An interim analysis indicates that participants have a lower rate of response to treatment than initially expected.
  - Safety monitoring indicates that a particular side effect is more severe, or more frequent than initially expected.
  - A paper is published from another study that shows that an arm of your research study is of no therapeutic value.
- Change in FDA safety labeling or withdrawal from marketing of a drug, device, or biologic used in a research protocol.
- Breach of confidentiality
- Change to the protocol taken without prior IRB review to eliminate apparent immediate hazard to a research participant.
- Incarceration of a participant when the research was not previously approved under Subpart C and the investigator believes it is in the best interest of the subject to remain on the study.
- Complaint of a participant when the complaint indicates unexpected risks or the complaint cannot be resolved by the research team.
- Protocol violation (meaning an accidental or unintentional deviation from the IRB approved protocol) that in the opinion of the investigator placed one or more participants at increased risk, or affects the rights or welfare of subjects.

8.3.2 Investigator reporting: Notifying a non-Penn IRB

Investigators who are not Penn faculty or affiliated with a Penn research site are responsible for safety reporting to their local IRB. Investigators are responsible for complying with their local IRB’s reporting requirements, though must submit the required reports to their IRB no later than 10 working days. Copies of each report and documentation of IRB notification and receipt will be kept in the investigator’s study file.

8.3.3 Sponsor reporting: Notifying the FDA

N/A

8.3.4 Sponsor reporting: Notifying participating investigators

N/A

8.4 Unblinding Procedures

While the safety of the subject always comes first, it is still important to seriously consider if unblinding the study therapy is necessary to ensure a subject's safety. Authorization for unblinding must be given by the principal investigator or Co-PI.

To break the code, contact the **24 hour emergency number** at the **Investigational Drug Service** at the University of Pennsylvania School of Medicine, Institute for Translational Medicine and Therapeutics:

1-800-670-3151

The reporting of the unblinding procedure will follow the same timeline requirements for investigator reporting of SAEs (i.e. notification of sponsor within 24 hours by phone or fax, followed by a written narrative of the event within 48 hours.). The unblinding of study medication will be documented in the subject's source document.

8.5 Stopping Rules

A subject may withdraw from the trial at any time with or without giving reasons. A subject may withdraw her/his subject consent at any time. The participation of a subject may, at any moment, be terminated by the investigator, if he/she considers that it will be in the subject's best interest. Subjects may be withdrawn at the discretion of the Investigator for reasons of medical prudence.

8.6 Medical Monitoring

It is the responsibility of the Principal Investigator to oversee the safety of the study at his/her site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan (see section 9 Auditing, Monitoring and Inspecting). Medical monitoring will include a regular assessment of the number and type of serious adverse events.

8.6.1 Data Monitoring Committee

For this investigator-initiated study, the investigators will provide continued regulatory oversight of study activities in order to ensure that regulatory requirements and GCP guidelines are being followed. Therefore, an external monitor will not be appointed. A brief check-list is being kept to facilitate the documentation of the investigators oversight of this clinical trial. All investigators have access to the study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc).

A subject may withdraw from the trial at any time with or without giving reasons. A subject may withdraw her/his subject consent at any time. The participation of a subject may, at any moment, be terminated by the investigator, if he/she considers that it will be in the subject's best interest. Subjects may be withdrawn at the discretion of the Investigator for reasons of medical prudence.

9 Data Handling and Record Keeping

9.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain

permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

This study produces a considerable amount of health-related data which includes genomic-related data. Potential risks are centered on psychological and social risks for the research participant and, possibly, their family.

Risks associated with genetic testing are for example:

- Risks related to broad sharing of phenotype and genomic data (e.g. genotype or DNA sequence).
- Risks of the data sharing model for the study (e.g. the possibility that the coded data may be released to members of the public, insurers, employers, and law enforcement agencies).
- Risks of receiving information that is unwanted by the participant.
- Risks of computer security breaches or other unanticipated distributions arising from maintaining data in an electronic format.
- Risks to relatives or identifiable populations or groups.
- The uncertainty of findings related to genetic risk for a given disease or trait.
- Privacy risks, both those known and those unforeseen at this time.

In order to minimize risks associated with health-related data including genetic testing, the following measures to protect data and subject confidentiality as well as subject privacy will be put in place:

- Collection of personal information will be limited to that which is essential to the research.
- Participants will be assigned a unique study-related identification (ID) number. This ID number along with the subject initials will be used for collecting and maintaining data sets. This ID number will be further used when data is being submitted for the statistical analyses; personal identifiable information, such as the participants names or initials will be removed; however, data such as age (but not date of birth), gender, ethnicity, will remain part of the data sets.
- Data will be stored in a secure location. All data captured in paper form will be kept in a locked file cabinet where there is limited non-study personnel access. Investigators, project managers, coordinators or properly trained office personnel will maintain keys. Optional, paper CRFs can be scanned and stored electronically in a secured data base for archival purposes.
- Personnel access to data that can personally identify research subjects will be restricted to key study personnel. Upon project initiation, all study personnel will sign confidentiality agreements. Study personnel will be trained on the proper handling of confidential data.
- The ultimate disposition or destruction of the code linking the data to personal identifiers will be executed seven (7) years after completion of research, i.e. counting from the day study data will have been published. As this coincides with the time period the study essential documents will be retained, execution of this plan is facilitated.
- In the case the investigators need to protect their research data, for example for the genetic information obtained from the subjects, legal protection can be obtained in form of a Certificate of Confidentiality (CoC).
- Unauthorized disclosure of personal information will be reported to the IRB.
- Electronic study data will be coded and the linking set will be stored in a separate password protected location from the coded data. This will be considered de-identified data (i.e. any identifying information such as name or SS# will be replaced with a code and only a few authorized people will have access to this code to link samples and data back to personal identifiers).

Federal legislation (The Genetic Information Nondiscrimination Act, or GINA) was passed that will provide baseline protection against discrimination in employment and health insurances decisions across the nation.

9.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in

source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

9.3 Case Report Forms

The study case report form (CRF) is the primary data collection instrument for the study. Whenever possible, Redcap, an on-line research electronic data capturing software, will be used as the CRF to collect study data. All data requested on the CRF must be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, write "N/D". If the item is not applicable to the individual case, write "N/A". If a paper CRF is used, all entries should be printed legibly in black ink. If any entry error has been made, to correct such an error, draw a single straight line through the incorrect entry and enter the correct data above it. All such changes must be initialed and dated. DO NOT ERASE OR WHITE OUT ERRORS. For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date it.

9.4 Records Retention

The ultimate disposition or destruction of the code linking the data to personal identifiers will be executed seven (7) years after completion of research, i.e. counting from the day study data will have been published. As this coincides with the time period the study essential documents will be retained, execution of this plan is facilitated.

10 Study Monitoring, Auditing, and Inspecting

10.1 Study Monitoring Plan

This study will be monitored according to the monitoring plan (see attachments). The investigator will allocate adequate time for such monitoring activities. The Investigator will also ensure that the monitor or other compliance or quality assurance reviewer is given access to all the above noted study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit.

10.2 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the EC/IRB, the sponsor, government regulatory bodies, and University compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices.

11 Ethical Considerations

This study is to be conducted accordance with applicable US government regulations and international standards of Good Clinical Practice, and applicable institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted independent Ethics Committee (EC) or Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the EC/IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the sponsor before

commencement of this study. The investigator should provide a list of EC/IRB members and their affiliate to the sponsor.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. See Attachment 2 for a copy of the Subject Informed Consent Form. This consent form will be submitted with the protocol for review and approval by the EC/IRB for the study. The formal consent of a subject, using the EC/IRB-approved consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent.

12 Study Finances

12.1 Funding Source

This study is financed through a grant from the US National Institute of Health, Heart, Lung, and Blood Institute (Personalization of Therapeutic Efficacy and Risk, HL117798).

12.2 Conflict of Interest

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by a properly constituted Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study sponsor prior to participation in this study. All University of Pennsylvania investigators will follow the applicable University conflict of interest policies.

12.3 Subject Stipends or Payments

Subjects will be compensated a total of $525; i.e. $30/week of drug intervention x 3; plus $100/12-hr visit to the CTRC including collection of biospecimens x 3; plus $15/post-treatment assessment visit x 3 visits x 3.

13 Publication Plan

The Principal Investigator and Co- Principal Investigators hold jointly the primary responsibility for publication of the results of this study. Neither the complete nor any part of the results of the study carried out under this protocol, nor any of the information provided by investigators for the purposes of performing the study, will be published or passed on to any third party without the consent of the study sponsor-investigator. Any investigator involved with this study is obligated to provide the sponsor with complete test results and all data derived from the study.

14 Trial Registration

This study will be registered in ClinicalTrials.gov.

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16 Attachments

Attachment 1: Study Procedures Flowchart
Attachment 2: Sample Consent Form
Attachment 3: Subject Handout: Summary of All Study Visits
Attachment 4: Subject Handout: Confirmation of Study Drug Dosing Using Redcap

Attachment 5: Medical Monitoring Plan
Attachment 6: Physical Activity Questionnaire
Attachment 7: Three-Day Food Record
Attachment 8: Study Advertisement Flyer
Attachment 9: Text of Electronic Advertisement

16.1 Study Procedures Flowchart

Event	Screening Visit	Treatment Phase 1			Post-Treatment Assessment 1				Treatment Phase 2			Post-Treatment Assessment 2				Treatment Phase 3			Post-Treatment Assessment 3		
Study Day		1	2-6	7	8	9	10		25	26-30	31	32	33	34		49	50-54	55	56	57	58[4]
Hours in CTRC	2	1	0	13	1	1	1		1	0	13	1	1	1		1	0	13	1	1	1
Informed Consent	X																				
Medical History	X																				
Physical Examination	X																				X
Screening Labs	X																				X
Urine Drug Test	X																				
Urine Pregnancy Test	X	X							X							X					X
Serum Pregnancy Test	X																				X
Urinalysis	X																				X
Questionnaires (3-day food record and physical activity questionnaire)	X																				
Study Drug Treatment		X	X	X					X	X	X					X	X	X			
DNA		X							X							X					
PBMCs		X							X							X					
Questionnaire (24-hour food recall)				X							X							X			
Stool Sample				X							X							X			
Plasma Drug Levels		X		X[1]	X	X	X		X		X[1]	X	X	X		X		X[1]	X	X	X
COX-1 acetylation		X							X							X					
COX-1 and COX-2 Activity		X		X[1]	X	X	X		X		X[1]	X	X	X		X		X[1]	X	X	X
Urinary Prostaglandins and Other Metabolites		X		X[1]	X	X	X		X		X[1]	X	X	X		X		X[1]	X	X	X
Transcriptomics				X[2]							X[2]							X[2]			
Proteomics				X[2]							X[2]							X[2]			
Metabolomics				X[2]							X[2]							X[2]			
24 hr Blood Pressure Monitoring				X[3]							X[3]							X[3]			

Washout – 2 weeks (between Treatment Phase 1 and Post-Treatment Assessment 1)
Washout – 2 weeks (between Post-Treatment Assessment 2 and Treatment Phase 3)

[1] Blood for plasma drug levels and COX-1 and COX-2 activity will be drawn before and 0.5, 1, 2, 4, 8, and 12 hours after taking the morning dose of study drug. Spot urine will be collected before and 1, 2, 4, 8, and 12 hours after taking the morning dose of study drug. 12 hr urine will be collected for assessment of creatinine clearance.

[2] Blood for transcriptomics, proteomics, and metabolomics will be drawn before and 4 hours after taking the morning dose of study medication.

[3] Subjects will wear an ambulatory blood pressure and activity monitor for 24 hrs. The monitor will be removed when they return to the CTRC for the first Post-Treatment Assessment.

[4] The Close-Out Visit will occur concurrently with the final visit of Post-Treatment Assessment 3.

16.2 Sample Consent Form

UNIVERSITY OF PENNSYLVANIA RESEARCH SUBJECT INFORMED CONSENT AND HIPAA AUTHORIZATION FORM

Protocol Title: A double-blind, placebo-controlled investigation of inter-individual variability in the pharmacologic response to non-steroidal anti-inflammatory drugs

Principal Investigator: Garret A. FitzGerald, MD
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10-122 SCTR
Philadelphia, PA 19104
215-898-1184

Co-Investigator: Tilo Grosser, MD
215-573-7600

Emergency Contact: **Department of Medicine Resident on call:**
215-662-6059

Why am I being asked to volunteer?

You are being invited to participate in a research study to investigate what causes differences in response to non-steroidal anti-inflammatory drugs (NSAIDs). Your participation is voluntary which means you can choose whether or not you want to participate. If you choose not to participate, there will be no loss of benefits to which you are otherwise entitled. Before you can make your decision, you will need to know what the study is about, the possible risks and benefits of being in this study, and what you will have to do in this study. The research team is going to talk to you about the research study, and they will give you this consent form to read. You may also decide

to discuss it with your family, friends, or family doctor. You may find some of the medical language difficult to understand. Please ask the study doctor and/or the research team about this form. If you decide to participate, you will be asked to sign this form.

What is the purpose of this research study?

The purpose of this research study is to measure the difference in response to non-steroidal anti-inflammatory drugs (NSAIDs) among people. We will also study what factors, like age, sex, or genetic background, cause these differences.

NSAIDs are painkillers, like Motrin, Advil or Aleve, that are commonly used to treat headaches and joint pain. They relieve pain by inhibiting one or both of the enzymes (COX-1 and COX-2) that produce metabolites called prostaglandins.

This study will compare two NSAIDs – celecoxib and naproxen – to placebo (a pill that does not have any medication). We will measure the amounts of prostaglandins in your blood and urine to study how you respond to each of these NSAIDs.

We will collect your DNA. Human DNA is organized in pieces called genes that provide instructions to make our bodies work. We will use your DNA to study how your genetic background affects your response to celecoxib and naproxen.

We will also create a cell line from your blood sample. These cells will be similar or identical to you genetically. Cell lines will be de-identified with a separate ID number and will not contain any information linked to you. The cell line will be used to measure the amounts of genes that are expressed by your cells. They will also be used in future studies to study how your cells respond to different treatments in a test tube. Your cell line will not be shared with other researchers. Your blood and cell line will be used for research purposes only and will not be sold. The blood samples and cell line will be stored for no longer than 10 years after which time they will be destroyed.

In the future, the information we learn in this study may help doctors choose which NSAID to prescribe to a specific patient so that he or she will have a good response and a low chance of side effects.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

How long will I be in the study? How many other people will be in the study?

You will be in the study for approximately 9 weeks.

We will enroll 288 people in the study. The study will last approximately 3 years.

What am I being asked to do?

This study will compare how you respond to celecoxib, naproxen, and placebo. There are 3 treatment phases during the study. During each treatment phase, you will take either celecoxib, naproxen, or placebo two times a day for 7 days. You will take each of these study medications in a random order. The study will be double-blinded. This means that neither you nor the study team will know the order that you take the study medications during the study.

After each treatment phase, there is a post-treatment assessment phase that is 3 days long. The treatment phases are separated by washout periods that are 14 days long.

On the first day of each treatment phase, you will attend a baseline study visit at the Clinical and Translational Research Center (CTRC) at the Hospital of the University of Pennsylvania. This visit will last approximately 1 hour.

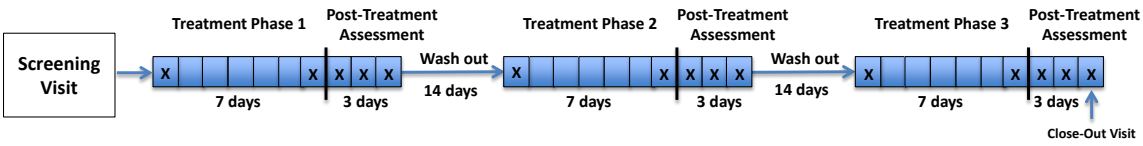
On the last day of each treatment phase (Day 7), you will attend a whole-day study visit at the CTRC. During this visit, blood will be drawn from a vein in your arm, and urine and stool will be collected. This visit will last approximately 13 hours.

The post-treatment assessment phase will happen for the 3 days after each treatment phase. You will attend a post-treatment study visit at the CTRC on all 3 days. During these visits, blood will be drawn from a vein in your arm, and urine will be collected. These visits will last approximately 1 hour each.

You will then wait 2 weeks before taking the next study medication.

The study visits will be repeated for the next treatment phase. When you have completed all 3 treatment phases, you will attend a close-out visit and your participation in the study will end.

This information is shown in the diagram below:



Before each visit, you will need to fast the night before by not eating or drinking anything but water from 9PM. It is encouraged to drink at least 2, 8 ounces of water the morning before you arrive. Here is a description of what will happen at each CTRC visit:

Screening Visit: Before you begin the study, you will attend a screening visit. A physical exam will be performed. Blood will be drawn from a vein in your arm for routine screening labs, including a complete blood count and blood chemistries. You will be asked to provide a urine sample for urinalysis and a urine drug test. If you are a woman of child-bearing age, serum and urine pregnancy tests will be performed.

You will be asked to complete a questionnaire about your level of physical activity. You will also be asked to record everything you eat for three days before you begin taking the study medication. You will then be allowed to go home.

If you are able to be in the study, a member of the study team will contact you to schedule your study visits. You will be asked to stop taking any vitamins or dietary supplements for 2 weeks before you begin the study. You will also be asked to stop taking aspirin and other NSAIDs for 2 weeks before you begin the study. You will be asked to avoid alcohol use throughout the study and to avoid alcohol, caffeine, and high-fat foods for 24 hours before each study visit.

Before each study visit, you should not eat any food starting from 9:00 PM the evening before. You may drink plain water throughout the night and in the morning before each study visit.

Visit 1 (Baseline Visit): You will arrive at the CTRC at approximately 7:30 AM. Several blood samples will be drawn from a vein in your arm. These will be used to measure COX-1 and COX-2 activity and to confirm that you have not taken any aspirin or other NSAIDs. Blood will also be drawn to collect DNA and white blood cells. The white blood cells will be used to make a cell line for future studies.

You will be asked to provide a urine sample. This will be used to measure levels of prostaglandins. If you are a woman of child-bearing age, a urine pregnancy test will be performed.

At approximately 8:00 AM, you will take your first dose of study medication. You will be given enough study medication for the next 6 days. You will then be allowed to go home.

You will take the study medication 2 times a day, at approximately 8:00 AM and 8:00 PM. You should take the medication on an empty stomach with a full glass of water. You will receive a reminder by email and/or text message when it is time to take the study medication. You will confirm when you take each dose through an online program

called Redcap or by replying to the text message. There are instructions on how to use Redcap in a separate handout. If you forget to take a dose, you will receive a phone call to remind you.

Visit 2 (Whole-Day Visit): You will return to the CTRC on Day 7 at approximately 7:30 AM. When you arrive, you will put on a blood pressure and activity monitor that will record your blood pressure and activity throughout the day. You will fill out a questionnaire about the foods you have eaten during the past day.

At approximately 7:50 AM, several blood samples will be drawn from a vein in your arm. These will be used to measure COX-1 and COX-2 activity, the amount of study medication in your blood, and the levels of genes, proteins, and metabolites.

You will be asked to provide a urine sample. This will be used to measure levels of prostaglandins and other metabolites.

At approximately 8:00 AM, you will take your morning dose of the study medication. Blood samples will be drawn 30 minutes, 1, 2, 4, 8, and 12 hours after you take the medication. These will be used to measure COX-1 and COX-2 activity and the amount of study drug in your blood. Extra blood samples will be drawn 4 hours after you take the study medication. These will be used to measure the levels of genes, proteins, and metabolites.

You will be asked to provide a urine sample 1, 2, 4, 8, and 12 hours after taking the study medication to measure levels of prostaglandins. Your urine will be collected throughout the day to measure how well your kidneys are working.

You will be asked to provide a stool sample at some point during the day.

You will be allowed to go home after the last blood draw and urine collection, at approximately 8:00 PM. You will continue to wear the blood pressure and activity monitor until you return to the CTRC the next day.

Visit 3 (Post-Treatment Visit 1): You will return to the CTRC at 7:30 AM the next morning. The blood pressure and activity monitor will be removed. At approximately 8:00 AM, blood will be drawn from a vein in your arm. This will be used to measure COX-1 and COX-2 activity and the amount of study medication in your blood. You will be asked to provide a urine sample to measure the levels of prostaglandins. You will then be allowed to go home.

Visit 4 (Post-Treatment Visit 2): You will return to the CTRC at 7:30 AM the next morning. At approximately 8:00 AM, blood will be drawn from a vein in your arm. This will be used to measure COX-1 and COX-2 activity and the amount of study medication

in your blood. You will be asked to provide a urine sample to measure the levels of prostaglandins. You will then be allowed to go home.

Visit 5 (Post-Treatment Visit 3): You will return to the CTRC at 7:30 AM the next morning. At approximately 8:00 AM, blood will be drawn from a vein in your arm. This will be used to measure COX-1 and COX-2 activity and the amount of study medication in your blood. You will be given the study medication for the next treatment phase, which you will begin taking in 2 weeks. You will then be allowed to go home.

These visits will be repeated for the next 2 treatment phases. When you have completed all 3 treatment phases, you will attend a close-out visit to conclude your participation in the study.

Close-Out Visit: The close-out visit will happen on the same day as the last Post-Treatment Visit. A physical exam will be performed. Blood will be drawn from a vein in your arm for routine screening labs, including a complete blood count and blood chemistries. You will be asked to provide a urine sample for urinalysis. If you are a woman of child-bearing age, serum and urine pregnancy tests will be performed. After this visit, your participation in the study will end.

This information is shown as a flow chart in the Handout.

What are the possible risks or discomforts?

Celecoxib and naproxen are NSAIDs that have been approved by the Food and Drug Administration (FDA) for pain and inflammatory diseases. FDA is the agency that oversees food and drug safety.

NSAIDs are generally considered safe drugs for short term treatments. Some NSAIDs are available as over-the-counter painkillers in low doses. All NSAIDs may cause allergic reactions, like asthma, itching, or allergic-type skin reactions and severe anaphylactic reactions. All NSAIDs can raise your blood pressure and can affect your kidneys. All NSAIDs can cause gastrointestinal side effects, such as heartburn, diarrhea, abdominal distress, nausea and vomiting, indigestion, constipation, abdominal cramps or pain, gas and bloating, ulcers and bleeding. Using NSAIDs for a long period of time can increase the risk of a heart attack or stroke. We believe that the chance of serious side effects from taking celecoxib or naproxen for a short period of time during this study (7 days) is very small.

The most common risks related to drawing blood from your arm are brief pain and/or bruising.

Reproductive risks: Because of the effects of celecoxib and naproxen, there could be serious harm to unborn children or children who are breast-feeding. These effects could also harm the mother. It is also possible that harmful side effects that are not yet known could happen to both the mother and unborn or breast-feeding child. If you are currently pregnant, it is important that you inform the investigator because you will not be able participate in the study. If you are able to become pregnant, you will be given a serum and urine pregnancy test before entry into the study. You are asked to use a medically accepted method of birth control (such as oral contraceptives, intra-uterine device (IUD), or condom with spermicide) while you participate in the study.

You should not become pregnant while you are taking this drug. If you do become pregnant, you must tell the investigators and consult an obstetrician or maternal-fetal specialist. The investigators will provide information about the study medications that you were taking and your lab results to the obstetrician or maternal-fetal specialist. The investigators will also contact the obstetrician or maternal-fetal specialist to find out about any adverse events that occurred during the pregnancy or childbirth. This information will be reported to the Institutional Review Board (IRB) at the University of Pennsylvania. The IRB is the committee that oversees clinical research.

Genetic Testing: You are being asked to provide blood (amount equivalent to 2 teaspoons) for the analysis of your genes, or DNA. This will involve analyzing the sequence or codes of your genes. The resulting information about you will be used for research on how your genes affect your response to celecoxib and naproxen.

You are also being asked to provide blood to create a cell line. These cells will be similar or identical to you genetically. The cell line will be used to measure the amounts of genes that are expressed by your cells. They will also be used in future studies to study how your cells respond to different treatments in a test tube. The results of research on your cell line will be shared with other researchers in online databases. Your name or other identifiers will not be included in these databases. Your cell line will not be shared with other researchers.

Your decision to be in this study and provide blood for DNA and to create a cell line is entirely up to you. If you do not want to provide blood for DNA or to create a cell line, you cannot be in the study. If you change your mind, you may withdraw or take away your permission to use your blood or cell line at any time. You may request that your DNA or cell line be destroyed and not used for future research. You do this by sending written notice to the investigator for the study. If you withdraw your permission, the study team may be able to use any research results that have already been collected.

Information gained from research on your DNA or cell line may be used for the development of diagnostic procedures or new treatments. Your blood, DNA, or cell line will not be sold to any person, institution, or company for financial gain or commercial profit. Neither you nor your heirs will gain financially from discoveries made using the information and/or specimens that you provide.

There is a risk that someone could get access to the data we have stored about you. In some cases, it could be used to make it harder for you to get or keep a job or insurance. There are laws against the misuse of genetic information, but they may not give full protection. We believe that the chance these things will happen is very small, but we cannot make guarantees.

A Federal law, called the Genetic Information Nondiscrimination Act (GINA), generally makes it illegal for health insurance companies, group health plans, and most employers to discriminate against you based on your genetic information. This law generally will protect you in the following ways:

• Health insurance companies and group health plans may not request your genetic information that we get from this research.

• Health insurance companies and group health plans may not use your genetic information when making decisions regarding your eligibility or premiums.

• Employers with 15 or more employees may not use your genetic information that we get from this research when making a decision to hire, promote, or fire you or when setting the terms of your employment.

Be aware that this Federal law does not protect you against genetic discrimination by companies that sell life insurance, disability insurance, or long-term care insurance.

You will not be informed of the results of the research performed on your genetic blood sample or cell line. Reports about research done with your blood or cell line will not be given to you or your doctor. These reports will not be put in your health records; however, the results of this research may be used in reports and research publications. The investigators do not wish to identify you in connection with this research, and will use procedures designed to prevent the results of this research from being linked to you. For example, a separate study ID and the date the specimen is drawn will be the only information on the label on the tubes that contain your DNA or cell line. If your blood or other health information is used in these reports, you will not be identified by name, address, or phone number.

The results of research on your DNA and cell line will be shared with other researchers outside of Penn through online scientific databases. Your name or other identifiers will not be included in these databases. There is a risk that someone could trace the information in a scientific database back to you. Even without your name or other

identifiers, your genetic information is unique to you. We believe that the chance that someone will identify you is very small, but the risk may grow in the future as people come up with new ways of tracing information.

What if new information becomes available about the study?

During the course of this study, we may find more information that could be important to you. This includes information that, once learned, might cause you to change your mind about being in the study. We will notify you as soon as possible if such information becomes available.

What are the possible benefits of the study?

You are not expected to get any benefit from being in this research study. The information that is gained from the study will be used to better understand why people respond to NSAIDs differently. In the future, this knowledge may help doctors choose which NSAID to prescribe to a specific patient.

What other choices do I have if I do not participate?

Taking part in this study is your choice. You can choose to take part or not take part. Choosing not to take part will not affect the choices you have regarding your medical care. If you choose to take part, you can change your mind at any time.

Will I be paid for being in this study?

You will receive a total of $525 for completing the study. This is broken down as $30 for each week of study drug treatment plus $100 for each whole-day visit to the CTRC plus $15 for each post-treatment visit.

Please note: In order to be compensated for your participation in this study, you must provide your Social Security Number. Additionally, please note that the University of Pennsylvania is required to report to the IRS any cumulative payments for participation in research studies that exceed a total of $600 in a calendar year.

Will I have to pay for anything?

There are no costs to you or your insurance. All the tests done during the study are for research purposes only.

What happens if I am injured from being in the study?

We will offer you the care needed to treat injuries directly resulting from taking part in this research. We may bill your insurance company or other third parties, if appropriate, for the costs of the care you get for the injury, but you may also be responsible for some of them.

There are no plans for the University of Pennsylvania to pay you or give you other compensation for the injury. You do not give up your legal rights by signing this form.

If you think you have been injured as a result of taking part in this research study, tell the person in charge of the research study as soon as possible. The researcher's name and phone number are listed in the consent form.

When is the Study over? Can I leave the Study before it ends?

This study is expected to end after all participants have completed all visits, and all information has been collected. This study may also be stopped at any time by your physician, the study Sponsor, or the Food and Drug Administration (FDA) without your consent because:

- The Primary Investigator feels it is necessary for your health or safety. Such an action would not require your consent, but you will be informed if such a decision is made and the reason for this decision.
- You have not followed study instructions.
- The Sponsor, the study Principal Investigator, or the Food and Drug Administration (FDA) has decided to stop the study.

If you decide to participate, you are free to leave the study at any time. Leaving the study will not interfere with your future care.

What information about me may be collected, used or shared with others?

The following protected health information will be collected:

- Name, address, telephone number, email address, and social security number
- Age, race, gender, and date of birth
- Medical record number
- Account number
- Personal and family history
- Results of tests and procedures
- Current and past medications or therapies

Why is my information being used?

Your information is used by the research team to contact you during the study. Your information and results of tests and procedures are used to:

- do the research
- oversee the research
- to see if the research was done right.

Who may use and share information about me?

The following individuals may use or share your personal health information for this research study:

The investigator for the study and the study team

Authorized members of the workforce of the UPHS and the School of Medicine and the University of Pennsylvania support offices who may need access to your information in the performance of their duties (for example, for research oversight and monitoring).

Who, outside of the School of Medicine, might receive my information?

Coded data may be shared with external partners at other academic or commercial research laboratories. These external partners may include the National Institutes of Health or other multi-institutional consortia. For external research, all direct identifiers will be removed. However, some indirect identifiers, such as dates of service, may be released.

Once your personal health information is disclosed to others outside the School of Medicine, it may no longer be covered by federal privacy protection regulations.

The Principal Investigator or study staff will inform you if there are any additions to the list above during your active participation in the trial. Any additions will be subject to University of Pennsylvania procedures developed to protect your privacy.

How long may the School of Medicine use or disclose my personal health information?

Your authorization for use of your personal health information for this specific study does not expire.

Your information may be held in a research database. However, the School of Medicine may not re-use or re-disclose information collected in this study for a purpose other than this study unless:

You have given written authorization

The University of Pennsylvania's Institutional Review Board grants permission

As permitted by law

Can I change my mind about giving permission for use of my information?

Yes. You may withdraw or take away your permission to use and disclose your health information at any time. You do this by sending written notice to the investigator for the study. If you withdraw your permission, the study team may be able to use the information that had already been collected before receiving your written request. If you withdraw your permission, you will not be able to stay in this study.

What if I decide not to give permission to use and give out my health information?

Then you will not be able to be in this research study.

You will be given a copy of this Research Subject Consent Form and HIPAA Authorization describing your confidentiality and privacy rights for this study.

By signing this document you are permitting the School of Medicine to use and disclose personal health information collected about you for research purposes as described above.

Who can see or use my information? How will my personal information be protected?

We will do our best to make sure that the personal information obtained during the course of this research study will be kept private. However, we cannot guarantee total privacy. Your personal information may be given out if required by law.

We will remove your name and other identifiers from your samples and information, and replace them with a code number. Only the study team and approved Penn researchers will have access to the code. All people with access to the code must sign an agreement to keep your identity a secret. None of your personal identifiers will be shared outside Penn.

If information from this study is published or presented at scientific meetings, your name and other personal information will not be used. The Institutional Review Board (IRB) at the University of Pennsylvania may review your research records. The IRB is the committee that oversees clinical research.

Electronic Medical Records and Research Results

What is an Electronic Medical Record?

An Electronic Medical Record (EMR) is an electronic version of the record of your care within a health system. An EMR is simply a computerized version of a paper medical record.

If you are receiving care or have received care within the University of Pennsylvania Health System (UPHS) (outpatient or inpatient) and are participating in a University of Pennsylvania research study, results of research-related procedures (i.e. laboratory tests, imaging studies and clinical procedures) may be placed in your existing EMR maintained by UPHS.

If you have never received care within UPHS and are participating in a University of Pennsylvania research study that uses UPHS services, an EMR will be created for you for the purpose of maintaining any results of procedures performed as part of this research study. The creation of this EMR is required for your participation in this study. In order to create your EMR, the study team will need to obtain basic information about you that would be similar to the information you would provide the first time you visit a hospital or medical facility (i.e. your name, the name of your primary doctor, the type of insurance you have). Results of research procedures performed as part of your participation in the study (i.e. laboratory tests, imaging studies and clinical procedures) may be placed in this EMR.

Once placed in your EMR, these results are accessible to appropriate UPHS workforce members that are not part of the research team. Information within your EMR may also be shared with others who are determined by UPHS to be appropriate to have access to your EMR (e.g. health insurance company, disability provider, etc).

Who can I call with questions, complaints or if I'm concerned about my rights as a research subject?

If you have questions or concerns about this study, you should contact the Principal Investigator listed on page one of this form. If you have questions or concerns about your rights as a research subject, you should contact the Office of Regulatory Affairs at the University of Pennsylvania by calling (215) 898-2614.

When you sign this form, you are agreeing to take part in this research study. This means that you have read the consent form, your questions have been answered, and you have decided to volunteer. Your signature also means that you are permitting the University of Pennsylvania to use your personal health information collected about you for research purposes within our institution. You are also allowing the University of Pennsylvania to disclose that personal health information to outside organizations or people involved with the operations of this study.

Someone from the study team may contact me with offers to take part in other studies. You may still take part in the study if you select “NO”.

YES_____ NO_____

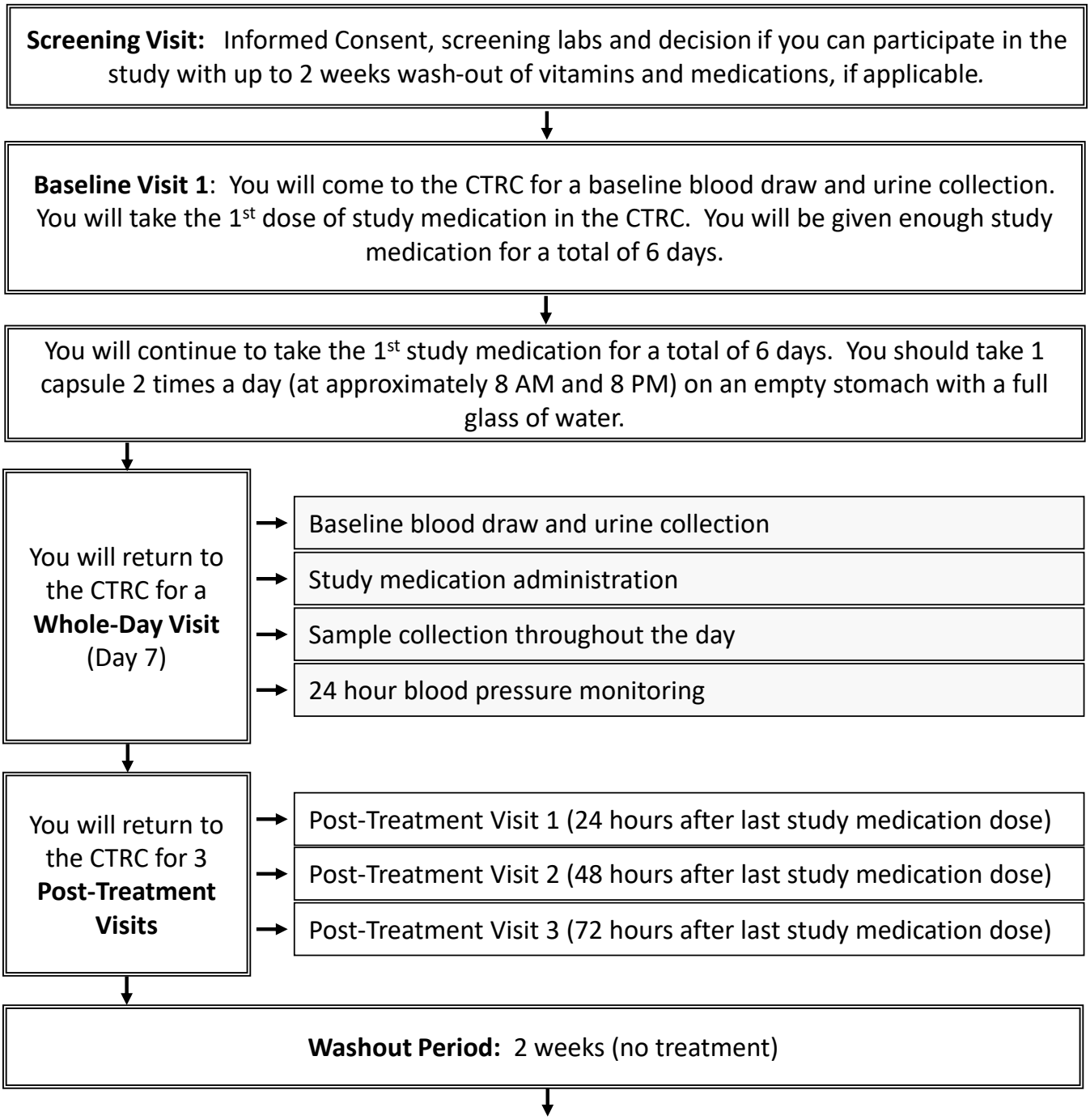
(initials) (initials)

A copy of this consent form will be given to you.

Name of Subject (Please Print)	Signature of Subject	Date

Name of Person Obtaining Consent (Please Print)	Signature	Date

16.3 Subject Handout: Summary of All Study Visits



Continued on Page 2

Continued from Page 1

Baseline Visit 2: You will come to the CTRC for a baseline blood draw and urine collection. You will take the 1st dose of study medication in the CTRC. You will be given enough study medication for a total of 6 days.

You will continue to take the 2nd study medication for a total of 6 days. You should take 1 capsule 2 times a day (at approximately 8 AM and 8 PM) on an empty stomach with a full glass of water.

You will return to the CTRC for a **Whole-Day Visit** (Day 7)

- Baseline blood draw and urine collection
- Study medication administration
- Sample collection throughout the day
- 24 hour blood pressure monitoring

You will return to the CTRC for 3 **Post-Treatment Visits**

- Post-Treatment Visit 1 (24 hours after last study medication dose)
- Post-Treatment Visit 2 (48 hours after last study medication dose)
- Post-Treatment Visit 3 (72 hours after last study medication dose)

Washout Period: 2 weeks (no treatment)

Continued on Page 3

Continued from Page 2

Baseline Visit 3: You will come to the CTRC for a baseline blood draw and urine collection. You will take the 1st dose of study medication in the CTRC. You will be given enough study medication for a total of 6 days.

You will continue to take the 3rd study medication for a total of 6 days. You should take 1 capsule 2 times a day (at approximately 8 AM and 8 PM) on an empty stomach with a full glass of water.

You will return to the CTRC for a **Whole-Day Visit** (Day 7)

- Baseline blood draw and urine collection
- Study medication administration
- Sample collection throughout the day
- 24 hour blood pressure monitoring

You will return to the CTRC for 3 **Post-Treatment Visits**

- Post-Treatment Visit 1 (24 hours after last study medication dose)
- Post-Treatment Visit 2 (48 hours after last study medication dose)
- Post-Treatment Visit 3 (72 hours after last study medication dose) and **Close-out Visit**

Please note that the length of the three "Whole Day Visits" amounts to approximately 13 hours each starting at 7:30 AM. The "Baseline Visits" and "Post-Treatment Visits" only take about 1 hour each to be completed.
Please also note that each "Baseline Visit," "Whole Day Visit," and "Post-Treatment Visit" requires you to fast from 9 PM the evening before; plain water can be consumed throughout the night and morning before each the visit.

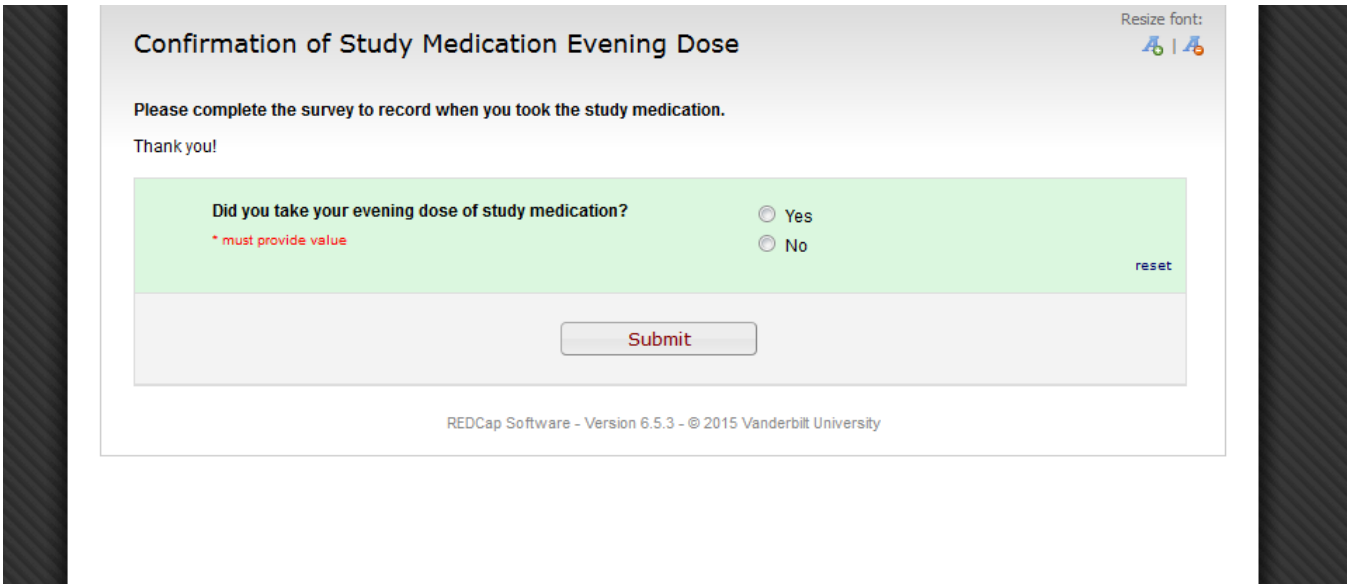
16.4 Subject Handout: Confirmation of Study Drug Dosing Using Redcap

You will receive a reminder email when it is time to take your study medication. This email will include a link to confirm when you took the study medication using Redcap. This link is unique to you and should not be forwarded to others.

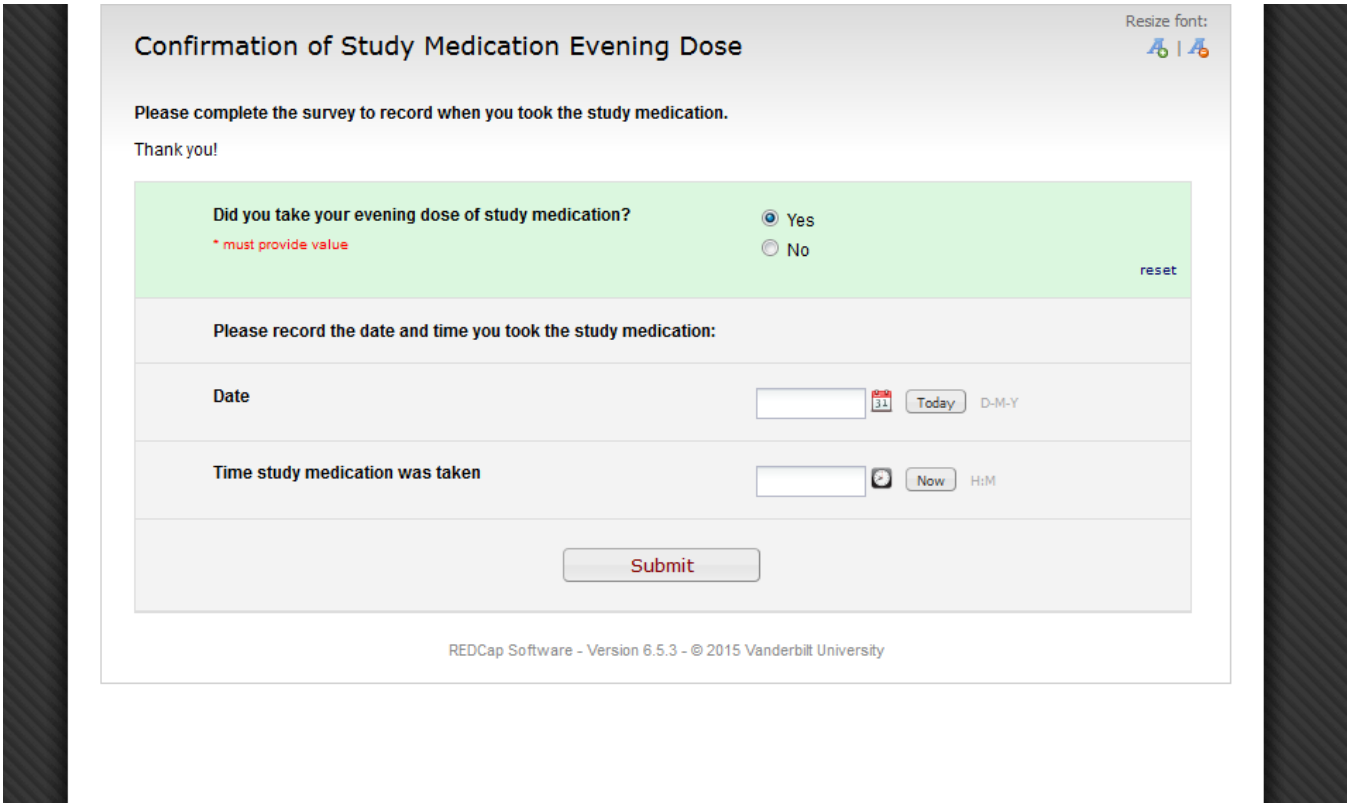
To use RedCap, you will need to be able to access the internet. If you are unable to access the internet, you can call the study coordinator at 215-662-4652 to confirm that you have taken the study medication.

To use Redcap:

1. Go to the link provided in the email reminder.
2. You will see a survey like the one below:



3. If you skipped your dose, select “No” and click “Submit”. If you took your dose, select “Yes”, and additional questions will appear so that you can record the date and time you took the dose:



4. Enter the information and click “Submit”.

16.5 Medical Monitoring

Medical Monitoring Plan

Medical monitoring visits are conducted on a regular basis each time n=10±2 subjects have been closed out of this study part. Additional visits may be scheduled as required.

Purpose

The medical monitor will be reviewing subjects' lab work, SAE/AE reporting and subjects' overall study course to monitor for missed AEs/SAEs**.**

Per clinical trial protocol the following safety parameters will be included:

- Laboratory screen at the inclusion visit (panels: CBC PLT, CMP, GFR, GGT, LDH, Uric, UA Dip)
- Renal function as assessed by glomerular filtration rate (GFR), sodium & potassium excretion, and creatinine in 12-hour urine as well as creatinine and urea nitrogen in plasma during treatment phase
- 24 hr ambulatory blood pressure during treatment phase
- Laboratory screen at the exit visit (panels: CBC PLT)

The medical monitor will use the template below to review subjects' folders. Furthermore, the medical monitor will provide a brief synopsis of major findings in writing.

Subject Binder: ID _____ _____ _____ **(Initials** _____ _____ _____ **)**

Medical Monitoring

Lab Work

Please specify which labs are being reviewed
Screening ☐ Close-out visit ☐ Both ☐

1. Are all out-of-range values accounted for and reviewed by the investigator?
   Yes ☐ No ☐
   If "No", Please Explain:
   _____
   _____
   _____
   _____
   _____
   _____

2. Are the ratings "Not Clinically Significant (NCS)" and "Clinically Significant (CS)", when indicated, appropriate for the out-of-range values?
   Yes ☐ No ☐
   If "No", Please Explain:
   _____
   _____
   _____
   _____
   _____
   _____

Adverse Events (AE)

1. Are all Adverse Events accounted for and reviewed by the investigator?
   Yes ☐ No ☐ N/A ☐ (No AE occurred)
   If "No", Please Explain:
   _____
   _____
   _____
   _____
   _____
   _____
   _____

Subject Binder: ID _____ _____ _____ (Initials _____ _____ _____)

2. Do all Adverse Events have a corresponding completed adverse event form?
   Yes ☐ No ☐ N/A ☐ (No AE occurred)
   If "No", Please Explain:
   _____
   _____
   _____
   _____

3. Has the overall study course for the subject for Adverse Events been reviewed?
   Yes ☐ No ☐ N/A ☐ (No AE occurred)
   If "No", Please Explain:
   _____
   _____
   _____
   _____

4. Have Adverse Events been identified, investigated, reported, and all necessary follow-ups performed?
   Yes ☐ No ☐ N/A ☐ (No AE occurred)
   If "No", Please Explain:
   _____
   _____
   _____
   _____

Serious Adverse Events (SAE)

Please describe the serious adverse event and the action taken following event:

_____
_____
_____
_____N/A
☐ (No SAE occurred)

1. Are all Serious Adverse Events accounted for and reviewed by the investigator?
   Yes ☐ No ☐ N/A ☐ (No SAE occurred)
   If "No", Please Explain:
   _____
   _____
   _____
   _____
   _____
   _____

Subject Binder: ID _____ _____ _____ (Initials _____ _____ _____)

2. Do all Serious Adverse Events have a corresponding completed Serious Adverse Events Form?
   Yes ☐ No ☐ N/A ☐ (No SAE occurred)
   If "No", Please Explain:
   _____
   _____

3. Has the IRB been notified within 5 calendar days of the SAE?
   Yes ☐ No ☐ N/A ☐ (No SAE occurred)
   If "No", Please Explain:
   _____
   _____
   _____

4. Has the FDA been notified of SAE within 7 days if unexpected and life-threatening, or fatal, and within 15 days if unexpected and not life-threatening?
   Yes ☐ No ☐ N/A ☐ (No SAE occurred)
   If "No", Please Explain:
   _____
   _____
   _____

5. Has the overall study course for the subject for Serious Adverse Events been reviewed?
   Yes ☐ No ☐ N/A ☐ (No SAE occurred)
   If "No", Please Explain:
   _____
   _____
   _____

6. Have Serious Adverse Events been identified, investigated, reported, and all necessary follow-ups performed?
   Yes ☐ No ☐ N/A ☐ (No SAE occurred)
   If "No", Please Explain:
   _____
   _____
   _____

Medical Monitor's Name (print): _____

Medical Monitor's Signature: _____

Date: _____

16.6 Physical Activity Questionnaire

Physical Activity Questionnaire

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE
(October 2002)

LONG LAST 7 DAYS SELF-ADMINISTERED FORMAT

FOR USE WITH YOUNG AND MIDDLE-AGED ADULTS (15-69 years)

The International Physical Activity Questionnaires (IPAQ) comprises a set of 4 questionnaires. Long (5 activity domains asked independently) and short (4 generic items) versions for use by either telephone or self-administered methods are available. The purpose of the questionnaires is to provide common instruments that can be used to obtain internationally comparable data on health–related physical activity.

Background on IPAQ
The development of an international measure for physical activity commenced in Geneva in 1998 and was followed by extensive reliability and validity testing undertaken across 12 countries (14 sites) during 2000. The final results suggest that these measures have acceptable measurement properties for use in many settings and in different languages, and are suitable for national population-based prevalence studies of participation in physical activity.

Using IPAQ
Use of the IPAQ instruments for monitoring and research purposes is encouraged. It is recommended that no changes be made to the order or wording of the questions as this will affect the psychometric properties of the instruments.

Translation from English and Cultural Adaptation
Translation from English is encouraged to facilitate worldwide use of IPAQ. Information on the availability of IPAQ in different languages can be obtained at www.ipaq.ki.se. If a new translation is undertaken we highly recommend using the prescribed back translation methods available on the IPAQ website. If possible please consider making your translated version of IPAQ available to others by contributing it to the IPAQ website. Further details on translation and cultural adaptation can be downloaded from the website.

Further Developments of IPAQ
International collaboration on IPAQ is on-going and an ***International Physical Activity Prevalence Study*** is in progress. For further information see the IPAQ website.

More Information
More detailed information on the IPAQ process and the research methods used in the development of IPAQ instruments is available at www.ipaq.ki.se and Booth, M.L. (2000). *Assessment of Physical Activity: An International Perspective.* Research Quarterly for Exercise and Sport, 71 (2): s114-20. Other scientific publications and presentations on the use of IPAQ are summarized on the website.

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** and **moderate** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal.

PART 1: JOB-RELATED PHYSICAL ACTIVITY

The first section is about your work. This includes paid jobs, farming, volunteer work, course work, and any other unpaid work that you did outside your home. Do not include unpaid work you might do around your home, like housework, yard work, general maintenance, and caring for your family. These are asked in Part 3.

1. Do you currently have a job or do any unpaid work outside your home?

   ☐ Yes

   ☐ No → ***Skip to PART 2: TRANSPORTATION***

The next questions are about all the physical activity you did in the **last 7 days** as part of your paid or unpaid work. This does not include traveling to and from work.

2. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, heavy construction, or climbing up stairs **as part of your work**? Think about only those physical activities that you did for at least 10 minutes at a time.

   _____ **days per week**

   ☐ No vigorous job-related physical activity → ***Skip to question 4***

3. How much time did you usually spend on one of those days doing **vigorous** physical activities as part of your work?

   _____ **hours per day**
   _____ **minutes per day**

4. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads **as part of your work**? Please do not include walking.

_____ **days per week**

☐ No moderate job-related physical activity → ***Skip to question 6***

5. How much time did you usually spend on one of those days doing **moderate** physical activities as part of your work?

_____ **hours per day**
_____ **minutes per day**

6. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **as part of your work**? Please do not count any walking you did to travel to or from work.

_____ **days per week**

☐ No job-related walking → ***Skip to PART 2: TRANSPORTATION***

7. How much time did you usually spend on one of those days **walking** as part of your work?

_____ **hours per day**
_____ **minutes per day**

PART 2: TRANSPORTATION PHYSICAL ACTIVITY

These questions are about how you traveled from place to place, including to places like work, stores, movies, and so on.

8. During the **last 7 days**, on how many days did you **travel in a motor vehicle** like a train, bus, car, or tram?

_____ **days per week**

☐ No traveling in a motor vehicle → ***Skip to question 10***

9. How much time did you usually spend on one of those days **traveling** in a train, bus, car, tram, or other kind of motor vehicle?

_____ **hours per day**
_____ **minutes per day**

Now think only about the **bicycling** and **walking** you might have done to travel to and from work, to do errands, or to go from place to place.

10. During the **last 7 days**, on how many days did you **bicycle** for at least 10 minutes at a time to go **from place to place**?

_____ **days per week**

☐ No bicycling from place to place → ***Skip to question 12***

11. How much time did you usually spend on one of those days to **bicycle** from place to place?

_____ **hours per day**
_____ **minutes per day**

12. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time to go **from place to place**?

_____ **days per week**

☐ No walking from place to place → ***Skip to PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY***

13. How much time did you usually spend on one of those days **walking** from place to place?

_____ **hours per day**
_____ **minutes per day**

PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY

This section is about some of the physical activities you might have done in the **last 7 days** in and around your home, like housework, gardening, yard work, general maintenance work, and caring for your family.

14. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, chopping wood, shoveling snow, or digging **in the garden or yard**?

_____ **days per week**

☐ No vigorous activity in garden or yard → ***Skip to question 16***

15. How much time did you usually spend on one of those days doing **vigorous** physical activities in the garden or yard?

_____ **hours per day**
_____ **minutes per day**

16. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, sweeping, washing windows, and raking **in the garden or yard**?

_____ **days per week**

☐ No moderate activity in garden or yard → ***Skip to question 18***

17. How much time did you usually spend on one of those days doing **moderate** physical activities in the garden or yard?

_____ **hours per day**
_____ **minutes per day**

18. Once again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, washing windows, scrubbing floors and sweeping **inside your home**?

_____ **days per week**

☐ No moderate activity inside home → ***Skip to PART 4: RECREATION, SPORT AND LEISURE-TIME PHYSICAL ACTIVITY***

19. How much time did you usually spend on one of those days doing **moderate** physical activities inside your home?

_____ **hours per day**
_____ **minutes per day**

PART 4: RECREATION, SPORT, AND LEISURE-TIME PHYSICAL ACTIVITY

This section is about all the physical activities that you did in the **last 7 days** solely for recreation, sport, exercise or leisure. Please do not include any activities you have already mentioned.

20. Not counting any walking you have already mentioned, during the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **in your leisure time**?

_____ **days per week**

☐ No walking in leisure time → ***Skip to question 22***

21. How much time did you usually spend on one of those days **walking** in your leisure time?

_____ **hours per day**
_____ **minutes per day**

22. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like aerobics, running, fast bicycling, or fast swimming **in your leisure time**?

_____ **days per week**

☐ No vigorous activity in leisure time → ***Skip to question 24***

23. How much time did you usually spend on one of those days doing **vigorous** physical activities in your leisure time?

_____ **hours per day**
_____ **minutes per day**

24. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like bicycling at a regular pace, swimming at a regular pace, and doubles tennis **in your leisure time**?

_____ **days per week**

☐ No moderate activity in leisure time → ***Skip to PART 5: TIME SPENT SITTING***

25. How much time did you usually spend on one of those days doing **moderate** physical activities in your leisure time?
_____ **hours per day**
_____ **minutes per day**

PART 5: TIME SPENT SITTING

The last questions are about the time you spend sitting while at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading or sitting or lying down to watch television. Do not include any time spent sitting in a motor vehicle that you have already told me about.

26. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekday**?

_____ **hours per day**
_____ **minutes per day**

27. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekend day**?

_____ **hours per day**
_____ **minutes per day**

This is the end of the questionnaire, thank you for participating.

The source of this questionnaire is: www.ipaq.ki.se

16.7 Three-day food record

3-Day Diet Record Instructions

You will need to write down ALL food and beverages consumed on the assigned day(s). Please take the diet record with you all day during the day(s) you are recording intake and record items at the same time you consume any food or beverage. There are four columns that need to include information about a food or beverage you consume: Time, Food/beverage, Amount, and Description/Preparation.

Date/Day: The Day (and date) will be listed at the top of each diet record to tell you which day(s) we will be asking you to record information

Time: List the time you consumed each food or beverage

Food/Beverage: Write down the name of the food or beverage. Use brand names (e.g. Burger King, Big Mac, Lean Cuisine) whenever possible. Remember to list all additions to foods and beverages such as cream, sugar, butter, jelly, lemon, ketchup, etc. For mixed dishes (e.g. pizza, casseroles, etc) list each food item and try to include the amount for each item. If you use a recipe, divide the total amount used by the number of servings you had. For example, if you used .5 lb of 85% lean ground beef to make a meat loaf that made 4 servings and you had 1 serving, the total amount of group beef you should include is 1/8 of a pound. Or if possible, you may provide the recipe and list what proportion of the entire recipe you ate.

Amount: List the amount eaten or drank in ounces (oz), teaspoons (tsp), tablespoons (tbsp), cups (c) or in units such as 1 slice of whole wheat bread, or 1 orange. When listing meats, list for cooked amounts.

Description/Preparation: Describe methods used to prepare food or beverages (ex: baked, grilled). Include any oil or other products used in the preparing of food (e.g. 1 tsp olive oil to sauté vegetables). Include brand names or fast-food and restaurant names when possible

Reminders List: Please use this list to remind you to include all food and drink that you consume.

1. Record all snacks you ate during each day.
2. Record all beverages such as coffee, tea, water, milk, soda pop, juice, alcohol, etc.
3. Include any items you added to your food or drink such as cream, milk, sugar, lettuce, tomato, ketchup, mustard, pickles, butter, margarine, etc.
4. Record the time of the day when you eat any food or beverage
5. Make sure to describe how each food was prepared (baked, fried, broiled, etc)
6. BE AS DESCRIPTIVE AS POSSIBLE AND CALL Lisa or Cheryl with any questions (215-662-7824/215-662-3180)

The following is an example of a diet record. Please note how detailed it is.

Time	Food/Beverage	Amount	Description
8:00 AM	cereal	1 cup	Cheerios
	milk	½ cup	2%
	bread	2 slices	whole wheat
	jelly	1 tbsp	Smuckers, strawberry
	juice	6 oz	Tropicana, orange, no pulp
12:00 PM	pizza	2 slices	16” pizza from Pizza Hut, thin crust with mozzarella

			cheese, tomato sauce, and pepperoni
	Coke	12 oz	
	Cookies	3	chocolate chip, medium size
7:00 PM	Chicken	3 oz	baked, skinless, boneless
	olive oil	1 tbsp	used to cook chicken
	lemon juice	1 tbsp	drizzled over chicken
	salad	1 cup	ingredients listed below
	lettuce	4 leaves	iceberg
	red tomato	¼ of tomato	raw
	cucumber	4 slices	peeled
	croutons	6 each	onion flavored, Pepperidge
	Farms Dressing	2 tbsp	Low-fat ranch, Ken's brand
	French fries	¾ cup	crispy, baked
10:00 PM	apple	1 each	red and delicious, medium
	coffee	6 oz	milk added (see below)
	milk	1 tbsp	2%

1055

Name: _____ Day: _____

Date: _____

DIET RECORD

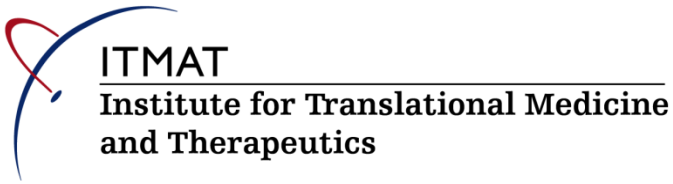
Time	Food/Beverage	Amount	Description

1055

Name: _____ Day: _____

Date: _____

16.8 Flyer for subject recruitment



Doctors at the Institute for Translational Medicine and Therapeutics, University of Pennsylvania are studying variability in response to non-steroidal anti-inflammatory drugs (NSAIDs).

or

"A double-blind, placebo-controlled investigation of inter-individual variability in the pharmacologic response to non-steroidal anti-inflammatory drugs."

If you are a healthy, non-smoking and non-pregnant individual 18 years of age or older, you may qualify for this RESEARCH study.

Qualified participants will receive, at no cost:

- ***Medical exams***
- ***Blood and urine tests***
- ***Compensation for your commitment based on eligibility***

Clinical Study Coordinator: **Lavenia Banas**

For more information call: **215-662-4652**

NSAID Research Study 215.662.4652	NSAID Research Study 215.662.4652	NSAID Research Study 215.662.4652	NSAID Research Study 215.662.4652	NSAID Research Study 215.662.4652	NSAID Research Study 215.662.4652	NSAID Research Study 215.662.4652	NSAID Research Study 215.662.4652	NSAID Research Study 215.662.4652	NSAID Research Study 215.662.4652	NSAID Research Study 215.662.4652	NSAID Research Study 215.662.4652	NSAID Research Study 215.662.4652	

16.9 Text of electronic advertisement

Doctors at the Institute for Translational Medicine and Therapeutics, University of Pennsylvania are studying variability in response to non-steroidal anti-inflammatory drugs (NSAIDs).

or

“A double-blind, placebo-controlled investigation of inter-individual variability in the pharmacologic response to non-steroidal anti-inflammatory drugs.”

If you are a healthy, non-smoking and non-pregnant individual 18 years of age or older, you may qualify for this RESEARCH study.

Qualified participants will receive, at no cost:

- Medical exams
- Blood and urine tests
- Compensation for your commitment based on eligibility

Clinical Study Coordinator: Lavenia Banas

For more information call: 215-662-4652

16.10 Text of screening questionnaire invitation email

Subject: Clinical Research Study Screening Questionnaire

Thank you for your interest in participating in our clinical study of variability in the response to non-steroidal anti-inflammatory drugs (NSAIDs):

"A double-blind, placebo-controlled investigation of inter-individual variability in the pharmacologic response to non-steroidal anti-inflammatory drugs"

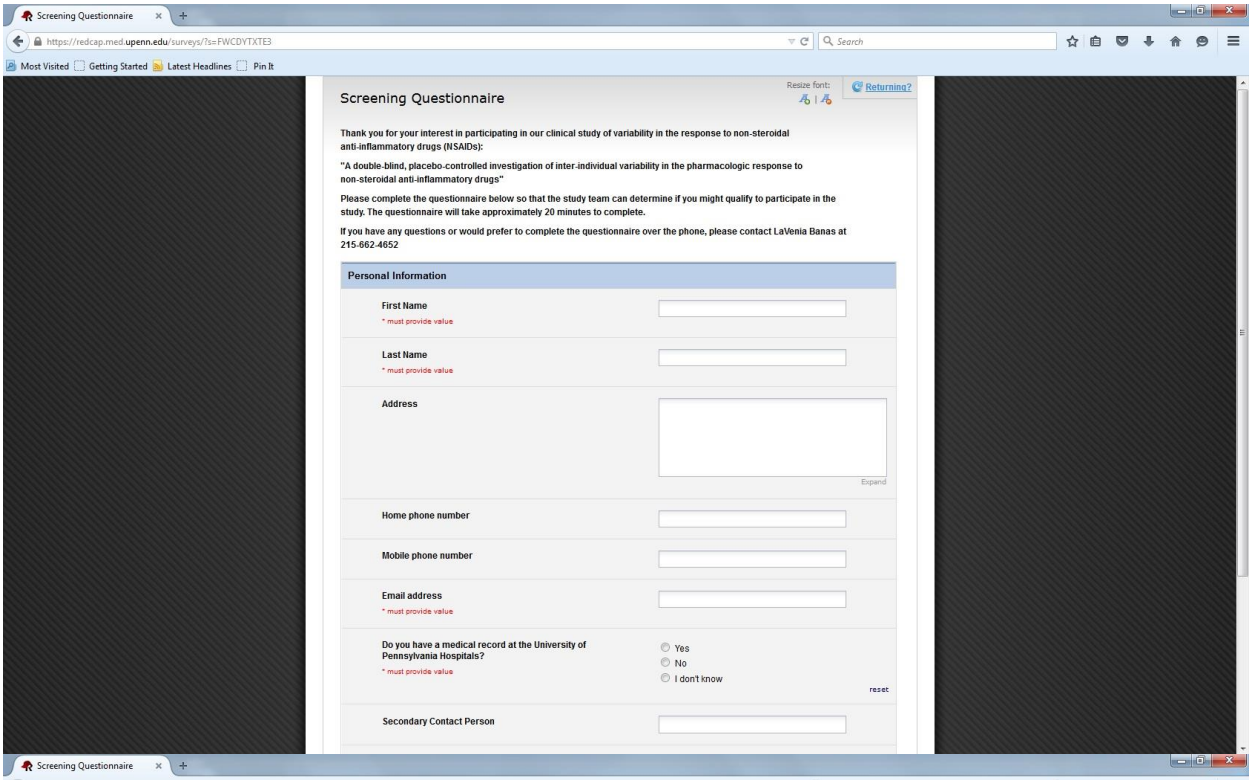
Please go to the link below to complete the screening questionnaire so that the study team can determine if you might qualify to participate in the study. The questionnaire will take approximately 20 minutes to complete.

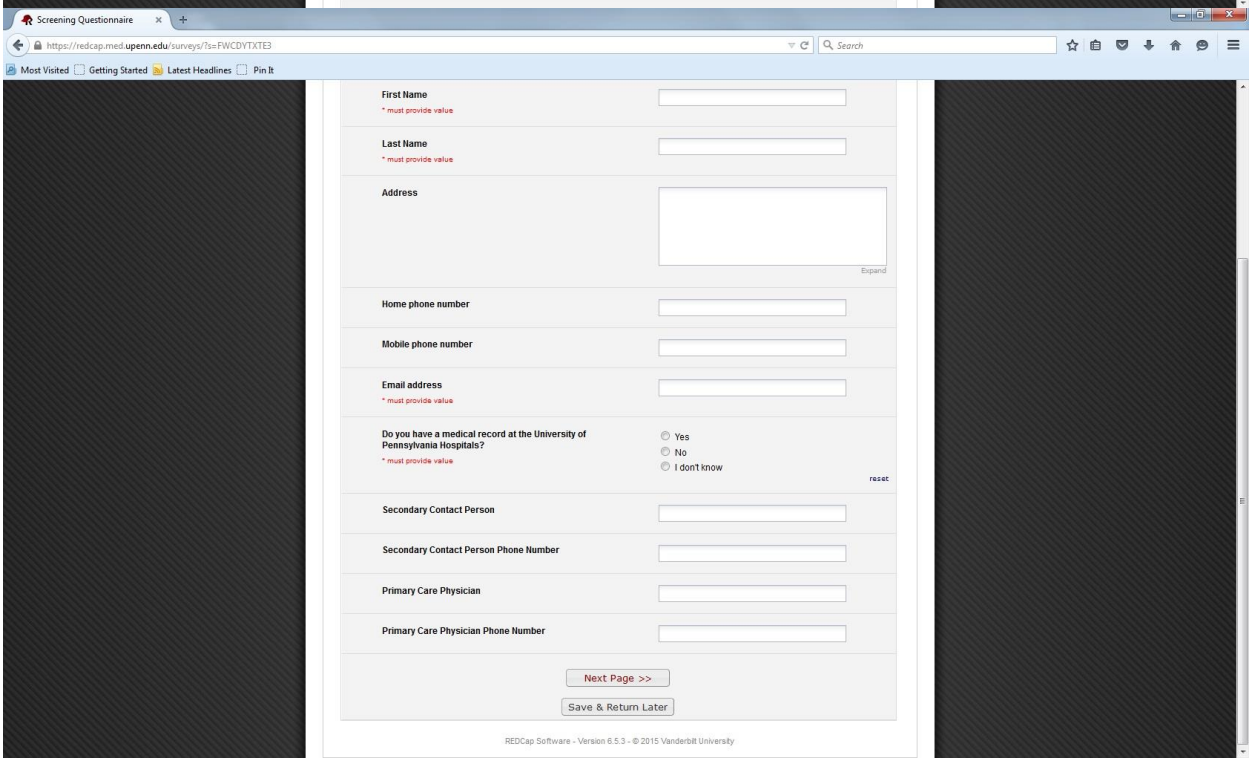
If you are eligible, a member of the study team will contact you to give you more information about the study and answer any questions you may have.

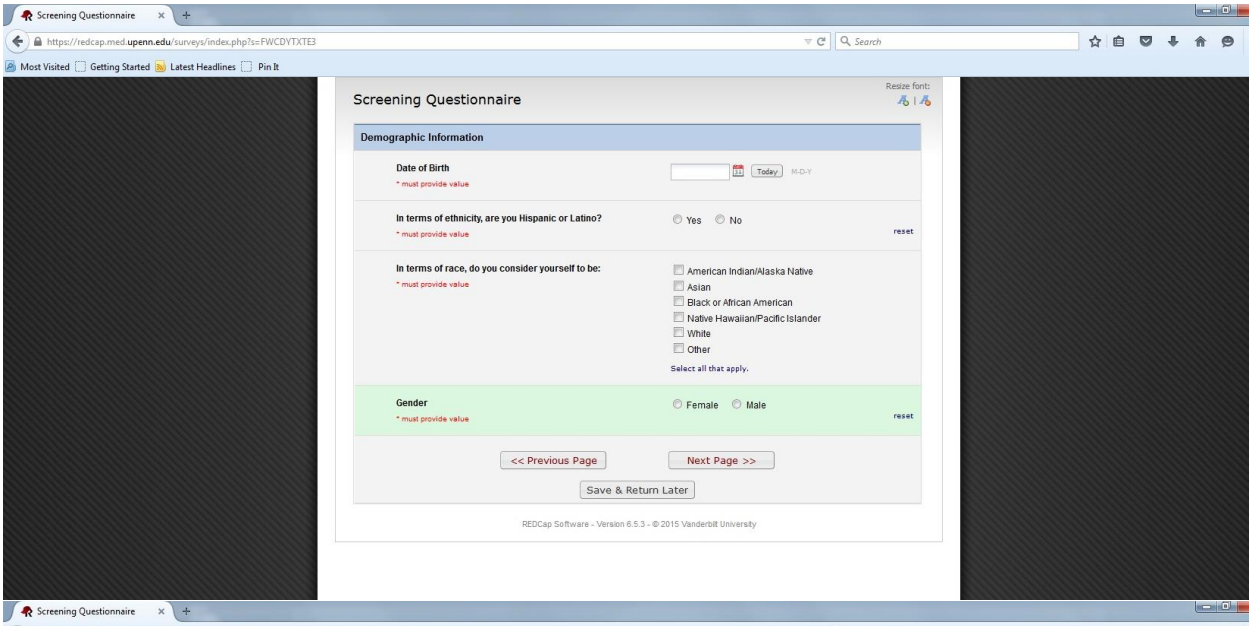
If you have any questions or would prefer to complete the questionnaire over the phone, please contact LaVenia Banas at 215-662-4652

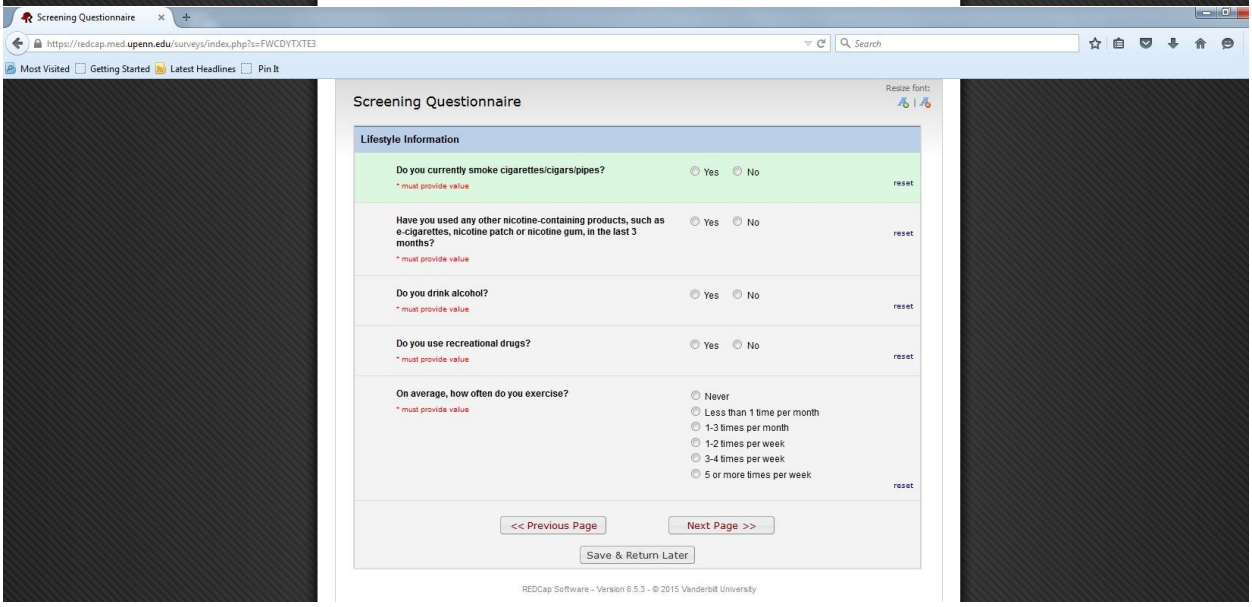
16.11 Screening questionnaire

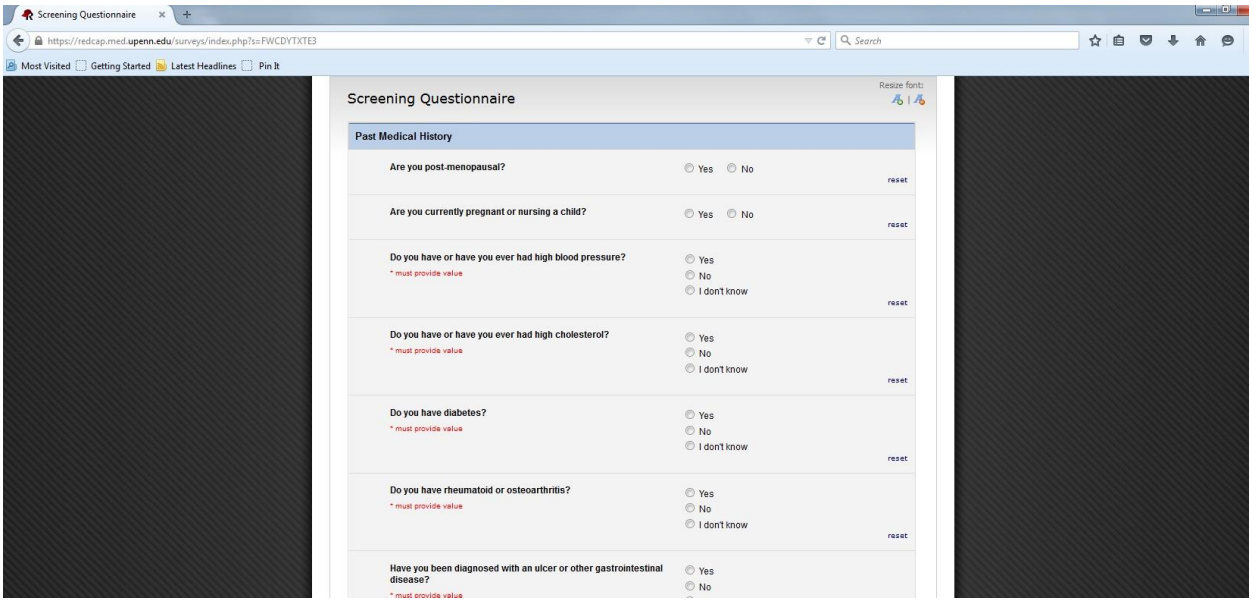
The screening questionnaire can be accessed at https://redcap.med.upenn.edu/surveys/?s=FWCDYTXTE3 . Screenshots of the online questionnaire follow.

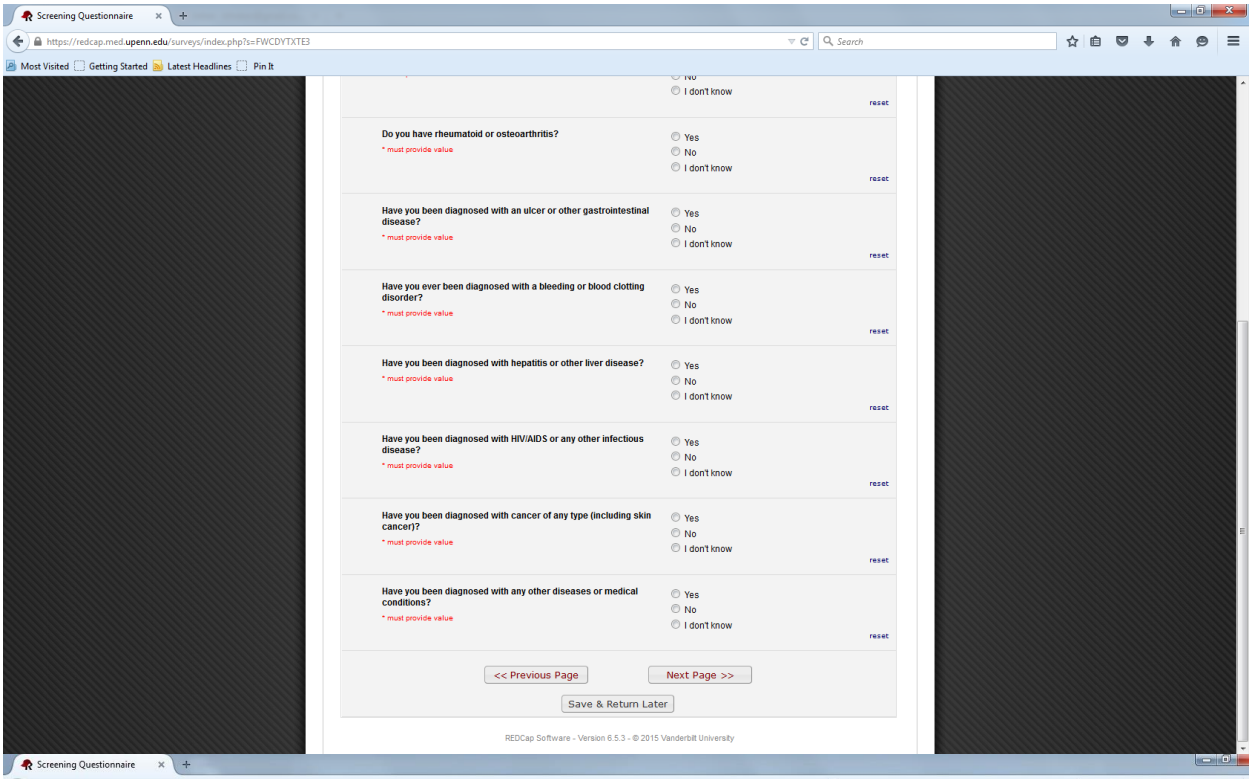


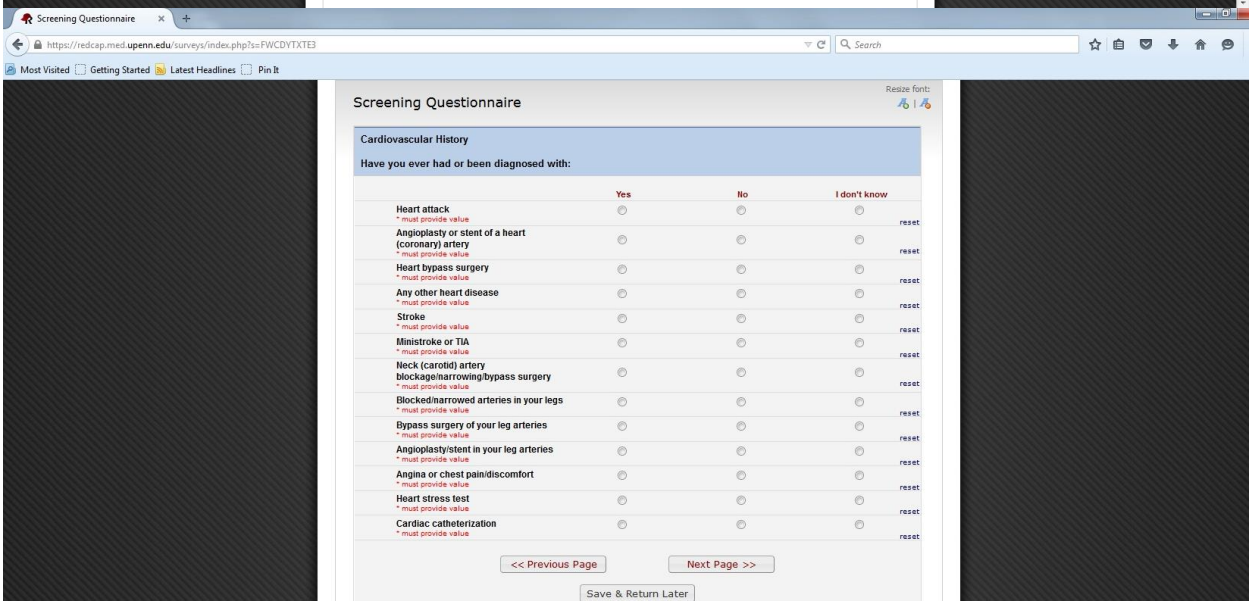


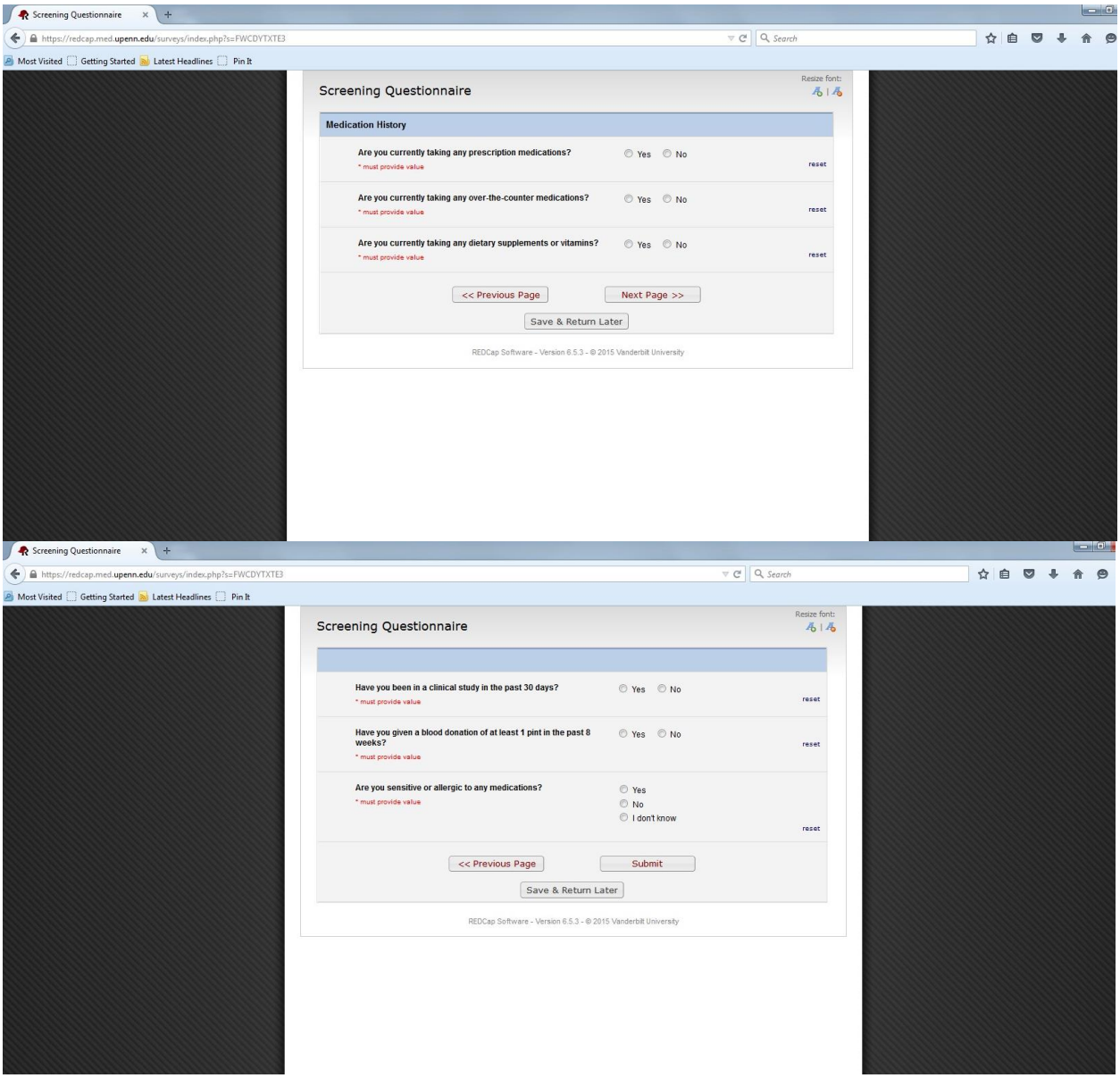











Screening Questionnaire
https://redcap.med.upenn.edu/surveys/index.php?s=FWCDYTXTE3
Most Visited
Getting Started
Latest Headlines
Pin It
Search
Resize font:
Screening Questionnaire
Medication History
Are you currently taking any prescription medications?
* must provide value
Yes
No
reset
Are you currently taking any over-the-counter medications?
* must provide value
Yes
No
reset
Are you currently taking any dietary supplements or vitamins?
* must provide value
Yes
No
reset
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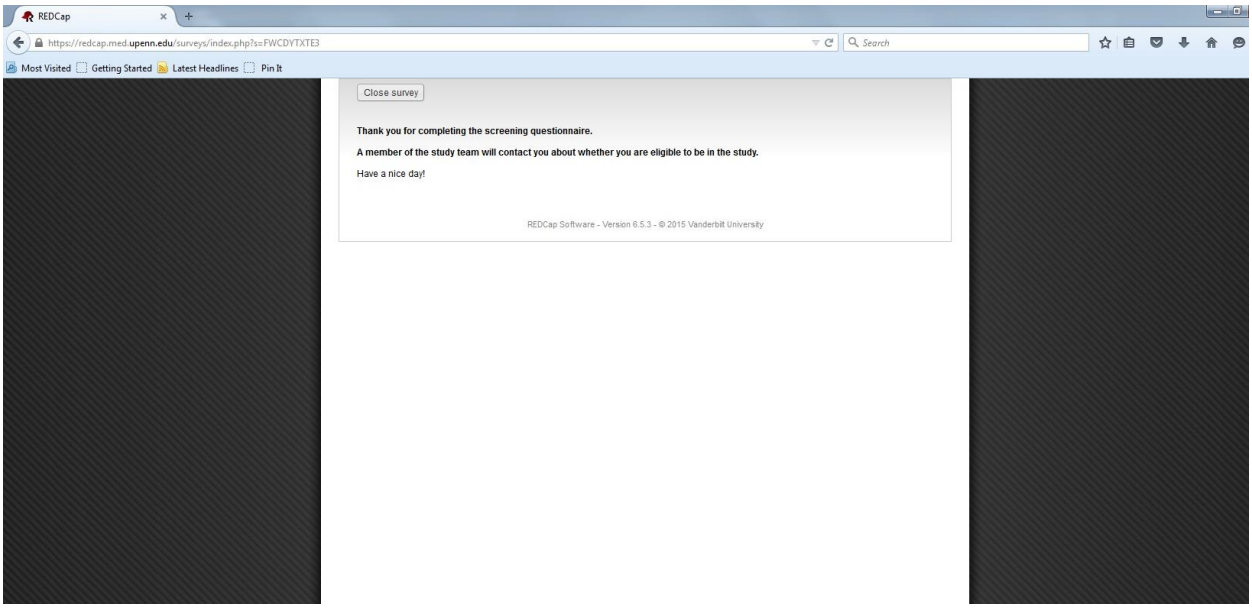
Save & Return Later
REDCap Software - Version 6.5.3 - © 2015 Vanderbilt University
Screening Questionnaire
https://redcap.med.upenn.edu/surveys/index.php?s=FWCDYTXTE3
Most Visited
Getting Started
Latest Headlines
Pin It
Search
Resize font:
Screening Questionnaire
Have you been in a clinical study in the past 30 days?
* must provide value
Yes
No
reset
Have you given a blood donation of at least 1 pint in the past 8 weeks?
* must provide value
Yes
No
reset
Are you sensitive or allergic to any medications?
* must provide value
Yes
No
I don't know
reset
<< Previous Page
Submit
Save & Return Later
REDCap Software - Version 6.5.3 - © 2015 Vanderbilt University



16.12 Sample text of email reminders

Subject: Clinical Research Study Treatment Day 2 Morning Dose

This is a reminder to take your morning dose of study medication for Treatment Day 2.

You should take the study medication at approximately 8 AM with a full glass of water. Please complete the survey to record the time you take the study medication.

If you forget to take the study medication at 8 AM, you may take it up to 6 hours late (2 PM). If more than 6 hours have passed, you should skip this dose and take your next dose as scheduled (8 PM). If you skip a dose, please indicate this on the survey.

If you have any questions or concerns, please contact LaVenia Banas at 215-662-4652 or reply to this email.

Thank you!

Subject: Clinical Research Study Treatment Day 6 Evening Dose and Appointment Reminder

This is a reminder to take your evening dose of study medication for Treatment Day 6.

You should take the study medication at approximately 8 PM with a full glass of water. Please complete the survey to record the time you take the study medication.

If you forget to take the study medication at 8 PM, you may take it up to 6 hours late (2 AM). If more than 6 hours have passed, you should skip this dose and take your next dose as scheduled (8 AM). If you skip a dose, please indicate this on the survey.

This is also a reminder that you are scheduled for a study visit tomorrow morning at the CTRC. Please arrive at 7:30 AM. You should fast from 9 PM tonight. You may drink plain water throughout the night and in the morning before your visit.

If you have any questions or concerns, please contact LaVenia Banas at 215-662-4652 or reply to this email.

Thank you!

16.13 Text of text message reminders

Clinical Research Study Reminder: It is time to take your study medication. Reply OK to confirm that you have taken the dose.