Penn Medicine

CLINICAL RESEARCH PROTOCOL

EXPLORATORY PHASE I STUDY IN HEALTHY VOLUNTEERS TO DEFINE CIRCADIAN RELATIONSHIPS BETWEEN SOCIAL BEHAVIOR, BLOOD PRESSURE AND METABOLOMIC SIGNATURES.

Principal Investigators (Regulatory Sponsors):	Carsten Skarke, MD Institute for Translational Medicine and Therapeutics (ITMAT), 8036 Maloney Bldg., 3600 Spruce St Philadelphia, Pennsylvania 19104 Phone: 215-746-8330
Co-Principal Investigators (Regulatory Sponsors):	Aalim Weljie, PhD Institute for Translational Medicine and Therapeutics (ITMAT), Department of Pharmacology, University of Pennsylvania Perelman School of Medicine, 3400 Civic Center Boulevard, Building 421, 10th Floor Phone: 215-573-8085
Admitting Physician / Funding Sponsor:	Garret A. FitzGerald, MD Institute for Translational Medicine and Therapeutics (ITMAT), Department of Pharmacology, University of Pennsylvania Perelman School of Medicine, 3400 Civic Center Boulevard, Building 421, 10th Floor Phone: 215-898-1184
Study Product:	None
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IND Number:	Not applicable
Short Title:	Human Circadian Rhythmicity

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List of Abbreviations and Definitions

ABPM: Ambulatory blood pressure monitoring.
Active Data Collection: Refers to data collection dependent on explicit user input.
BP: Blood pressure.
Background Process: Refers to an operating system service that allows an application to perform an operation without interacting with the user or disrupting other applications.
GBI (Ginger.io Behavior Index): Refers to a set of features (or variables) used by the Ginger.io platform to gather health insights and make inferences.
Ginger.io Behavior Platform: Refers to the mobile application (or app) and the web dashboard.
Mobile Application: Refers to the application installed on the users' phones that allows for data collection. This application includes two components: (1) a background process that is launched automatically by the application (after initial installation and user log-on) to gather data unobtrusively (2) a front end mobile user interface with a survey component that allows the users to receive and submit surveys.
Notification: Refers to an operating system service that allows an application to display a message to the patient to grab their attention.
Passive Data Collection: Refers to data collection from the users' devices without any effort from them.
Web Dashboard: Refers to a web-based study management system that allows investigators and study staff to monitor the data collection process (including passive data collection and survey completion), identify issues and analyze the data.

RESPONSIBILITIES AND SIGNATURES

The responsibilities and signatures below constitute the approval of this protocol, its attachments and amendments, and provide the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH guidelines.

Principal Investigator:

Signed: _____ Date: March 11, 2013

Name: Carsten Skarke
Title: MD

Address: Institute for Translational Medicine and Therapeutics (ITMAT),
University of Pennsylvania School of Medicine,
8036 Maloney Bldg.
3600 Spruce St
University of Pennsylvania,
Philadelphia, Pennsylvania 19104

Telephone: 215.746.8330
Fax: 215. 573.8996

e-mail:
cskarke@mail.med.upenn.edu

Co- Principal Investigator:

Signed: _____ Date: March 11, 2013

Name: Aalim Weljie
Title: PhD

Address: Department of Pharmacology
Institute for Translational Medicine and Therapeutics
Translational Research Center
3400 Civic Center Boulevard, Building 421
10th Floor, Room 10-113
Philadelphia, PA 19104-5158

Telephone: 215-573-8085
Fax: 215-573-9135

e-mail: aalim@upenn.edu

Other Investigators:	Title:	Contact Information:	Responsibilities:
Lavenia Banas	R.N.	8037 Maloney Bldg Hospital of the University of Pennsylvania 3600 Spruce Street Philadelphia, PA 19104 Phone: 215-662-4652 Fax: 215-573-8996 e-mail: banas@itmat.upenn.edu	Clinical Research Coordinator
Lisa Basel-Brown	MS, RD, LDN	Translational Research Center Hospital of the University of Pennsylvania, 1 Dulles Building, 34th and Spruce Streets Philadelphia, PA 19104 Phone: 215-662-7824 Fax: 215-662-2643 e-mail: lisa@spirit.gcrc.upenn.edu	Research Nutritionist
Susanne Fries	MD	8038 Maloney Bldg Hospital of the University of Pennsylvania 3600 Spruce Street Philadelphia, PA 19104 Phone: 215-746-8331 Fax: 215-573-8996 e-mail: fries@upenn.edu	Medical Monitor
John Lawson		Department of Pharmacology, Institute for Translational Medicine and Therapeutics, Smilow Translational Research Center, 3400 Civic Center Boulevard, Building 421, 10th Floor, Room 10-115, Philadelphia, PA 19104-5158 Phone: 215-573-7038 e-mail: jalawson@mail.med.upenn.edu	Lipidomics
Carissa Fuller	CRNP, MSN,	Clinical & Translational Research Center Hospital of the University of Pennsylvania, 1 Dulles Building, 34th and Spruce Streets Philadelphia, PA 19104 Phone: 215.349.8067 Fax: 215-662-2643 e-mail: Carissa.Fuller@uphs.upenn.edu	Nurse Practitioner
Kathleen J. Schumacher	CRNP, MSN, ANP-BC	Clinical & Translational Research Center Hospital of the University of Pennsylvania, 1 Dulles Building, 34th and Spruce Streets Philadelphia, PA 19104 Phone: 215-662-7824	Nurse Practitioner

		Fax: 215-662-2643 e-mail: kathlj@mail.med.upenn.edu	
Cheryl Milhoan	MS, RD	Clinical & Translational Research Center Hospital of the University of Pennsylvania, 1 Dulles Building, 34th and Spruce Streets Philadelphia, PA 19104 Phone: 215-662-3180 Fax: 215-662-2643 e-mail: cheryl@spirit.gcrc.upenn.edu	Research Nutritionist
Lindsey Albenberg	DO	Fellow Division of Pediatric Gastroenterology, Hepatology, and Nutrition The Children's Hospital of Philadelphia Philadelphia, PA 19104 e-mail: AlbenbergL@email.chop.edu	Swab sampling technique
Judith R Kelsen	MD	ASST PROFESSOR C-E, PE-Pediatrics Division of Pediatric Gastroenterology, Hepatology, and Nutrition The Children's Hospital of Philadelphia Philadelphia, PA 19104 Phone: 215-363-9057 e-mail: KELSEN@email.chop.edu	Swab sampling technique
Frederik Bushman	PhD	425 Johnson Pavilion 3610 Hamilton Walk Philadelphia, PA 19104 Office: (215) 573-8732 Fax: (215) 573-4856 e-mail: bushman@mail.med.upenn.edu	Microbiome Studies
Gregory Grant	PhD	Department of Pharmacology, Institute for Translational Medicine and Therapeutics, Smilow Translational Research Center, 3400 Civic Center Boulevard, Building 421, 10th Floor Philadelphia, PA 19104-5158 Office: (215)-898-3736 e-mail: ggrant@pcbi.upenn.edu	Statistical Analyses
Thomas S. Price	PhD	Department of Pharmacology, Institute for Translational Medicine and Therapeutics, Smilow Translational Research Center, 3400 Civic Center Boulevard, Building 421, 10th Floor Philadelphia, PA 19104-5158 Office: (215)-898-1189 e-mail: tsprice@mail.med.upenn.edu	Statistical Analyses
Seth Rhoades		Department of Pharmacology, Institute for Translational Medicine and Therapeutics, Smilow Translational Research Center, 3400 Civic Center Boulevard, Building 421, 10th Floor Philadelphia, PA 19104-5158 e-mail: srhoades@mail.med.upenn.edu	Metabolomics

Annie Curtis, PhD	Inflammation Research Group Trinity Biomedical Science Institute (TBSI) 152-160 Pearse Street Trinity College Dublin Ireland Ph: 353-1-8962449	Transcriptomics Analysis

Study Summary

Title	Exploratory study in healthy volunteers to define circadian relationships between social behavior, blood pressure and urine metabolomics.
Short Title	Human Circadian Rhythmicity
Protocol Number	IRB number 817759
Phase	Clinical study Phase 1
Methodology	Single-arm study design
Study Duration	Between ¾ - 1 year
Study Center(s)	Single-center
Objectives	1) Can the social sensing technology be implemented to explore relationships between patterns of user activity, blood pressure and metabolic signatures? 2) To assess the circadian variation over time for each parameter individually. 3) Can the difference in assessment frequency – unscheduled and random for user activity versus scheduled and timed for blood pressure and metabolites – be integrated to recognize individual circadian patterns? 4) To assess the circadian co-variation over time between user activity, blood pressure and metabolic signatures.
Age	25-35 years of age
Number of Subjects	n=6
Diagnosis and Main Inclusion Criteria	Healthy male volunteers if feasible during recruitment. Key inclusion criteria: 25-35years of age, own a smartphone (Android phones only) which installs the social sensing application ginger.io
Study Product, Dose, Route, Regimen	None included
Duration of administration	Not applicable
Reference therapy	None included
Statistical Methodology	Standard summary statistics; exploring the shape of the curves over time; identifying peaks and troughs.

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.

1.1 Background

Smartphones represent a rich source for user activity data. These social sensing data can be mined to identify patterns of social behavior. Changes therein enable researchers to make inferences about the health status of individuals, e.g. the decrease of social interactions during acute flu outbreaks.

We wish to test in a small pilot study in healthy volunteers whether this social sensing technology can be implemented to explore relationships between circadian patterns of user activity, continuous blood pressure and metabolic signatures in urine samples.

The social sensing technology might provide insight how to integrate quantitatively social behavior into exploring cardiovascular and metabolic functions influenced by the molecular clock.

Blood pressure can easily be monitored as cardiovascular biomarker in healthy volunteers. Circadian variations in blood pressure are well studied in humans. Blood pressure levels in healthy humans vary considerably over the course of a day. Prominent is the steep increase in blood pressure during early morning hours, which gradually decreases throughout the day to reach the lowest levels during nighttime sleep. Here, the mean blood pressure readings from waking versus sleeping hours can differ up to 10-20%. Alterations to this circadian rhythmicity in blood pressure are commonly seen among patients with cardiovascular diseases. The ‘dipping’ phenotype – the substantial decrease in blood pressure during sleeping hours – may change to a ‘non-dipping’ phenotype – the absence of such a drop in blood pressure. This blood pressure characteristic is strongly related to cardiovascular health, e.g. some patients who lack such a nocturnal dip are at increased risk of developing hypertension which in turn often results in end organ damage. The incidence of cardiovascular events in humans has a marked temporal component, such that myocardial ischemia, myocardial infarction, sudden cardiac death, and stroke typically occur in the early morning hours. Above is reviewed in (1-3). A mechanism to consider for these diseases is that environmental cues and behavioral patterns desynchronize body functions. This notion is supported by the recent proposition that an endogenous circadian rhythm causes the blood pressure to peak in the evening at around 9 pm (4).

Metabolomics is becoming a powerful technical approach to assess circadian patterns of metabolomics function in small numbers of participants. Forced de-synchronization or highly standardized protocols in humans revealed that a substantial number of metabolites follow an circadian rhythm, up to 15% in a cohort of n=10 age-matched male subjects (5) and up to 19% in n=8 male volunteers (6). Recently, a timetable of oscillating metabolites has been proposed for the human organism (7). Still a challenge in metabolomics, however, is the variation in noise-versus-signal ratio across different biofluids (8). Dense circadian profiling of the urine metabolome (9) will be enriched with metabolomics data from blood and saliva (10) samples at

selected 'anti-phase' sampling times. A recent study in humans proposed that a few samples collected in 'anti-phase' can determine internal body time in humans (7), a concept developed earlier in mice (11). Furthermore, the relationship between circadian metabolome and transcriptome will be explored as recent evidence suggested that the hepatic circadian clock drives nucleotide synthesis and degradation (12).

Dietary intake influences social behavior, circadian rhythms, metabolites and gene expression. Therefore, several instruments to assess dietary intake will be implemented. In addition to the classic instruments such as food frequency questionnaire, innovative approaches via smartphone application will be used as well. To meet the challenge that even nutrition professionals underreport dietary intake in dietary instruments (13), metabolomics will be explored as a strategy to quantify food intake. For example, enterolactone concentrations reflect intake of whole-grain foods (14); concentrations of $\delta^{15}N$ reflect that of fish foods (15).

We have preliminary data from healthy humans that suggest that the composition of the gut bacteria follows a circadian pattern (Carsten Skarke, unpublished observation). Translational studies in humans will rely on study paradigms based on frequent stool sampling techniques not yet in place. Therefore, this protocol includes collecting microbiota by sampling non-invasively the mucosa in the mouth (mouth swabs) as well as in the distal rectum (fecal swabs). This will provide first data on the feasibility of these two frequent sampling techniques.

To limit variability in this pilot data set, the age range for inclusion into the study will be set to 25-35 years of age. Also, male subjects only will be enrolled at this stage of the project, provided this strategy is feasible during the recruitment phase. Otherwise female volunteers will be invited to the study. The menstrual cycle, a supradian rhythm, influences many biological processes in a woman's body, which adds variability to the outcome data, particularly if women are studied at different stages of their menstrual cycle.

1.2 Investigational Agent
None applied in this study.

1.3 Clinical Data to Date
Clinical data to date exists predominantly in the domain of using behavioral patterns, or the change in these, as a predictor of human health or its transition to disease.

Previous research, conducted and compiled by members of the Ginger.io team, has concluded that health-related behavior data from smart phones is predictive of behaviors associated with health outcomes. Described below are three specific examples:

- Common Colds, Influenza, and Stress (16)

In a study involving 80 participants over 3 months using an earlier version of the smart phone app, Ginger.io found that statistical features extracted from phone call data records, SMS records, and movement logs for individuals showed statistically significant differences on the days the person was symptomatic, versus the days that the person was healthy. By designing a Bayesian classifier with 4-fold cross-validation based on these behavior features, it was possible to identify symptomatic days with a recall of 0.6 – 0.9 for different clusters of symptoms. The symptoms data was collected using mobile phone surveys.

- Chronic Inflammatory Bowel Disease (IBD)

In an ongoing study with a leading hospital provider, the Ginger.io mobile platform is being used to understand what behavior changes are correlated with flare-ups of Chronic Inflammatory Bowel Disease (IBD). Clinical investigators are testing an important hypotheses – are stress and IBD flare-ups correlated? A better understanding of the relationship between stress and IBD symptoms can help design tools for early detection and strategies for patients to better manage their condition.

- Obesity and Long-term Weight Gain (17)

An ongoing debate in public policy considers whether unhealthy diet and exercise habits resulting in obesity are "contagious". A study using a previous version of the Ginger.io platform and involving 70 co-located participants over a 6 months, attempted to find links between social exposure to obese and unhealthy individuals and their reported weight changes. It was found that weight changes amongst participants were weakly correlated with exposure to those previously obese or overweight. Further, weight changes amongst participants in the same period were strongly associated with exposure to others that gained weight in the same period.

2 Study Objectives

Based on previous pilot studies by Ginger.io, we expect acceptance for the social sensing technology among participants.

2.1 Primary Objective

To determine the feasibility of the social sensing technology to identify circadian patterns in user activity and to determine its variation over time among healthy volunteers.

Hypothesis:

User activity follows a circadian rhythm among healthy volunteers.

Question:

Can we detect different 'circadian phenotypes' of social behavior among healthy volunteers?

2.2 Secondary Objective

To identify circadian patterns in user activity, blood pressure and metabolomics signatures and to determine the variation over time among healthy volunteers.

Hypothesis:

Social behavior follows a circadian rhythm and correlates with circadian patterns in blood pressure and metabolomics signatures among healthy volunteers.

Question:

Can we link different 'circadian phenotypes' of social behavior to circadian patterns in blood pressure and metabolomics signatures among healthy volunteers?

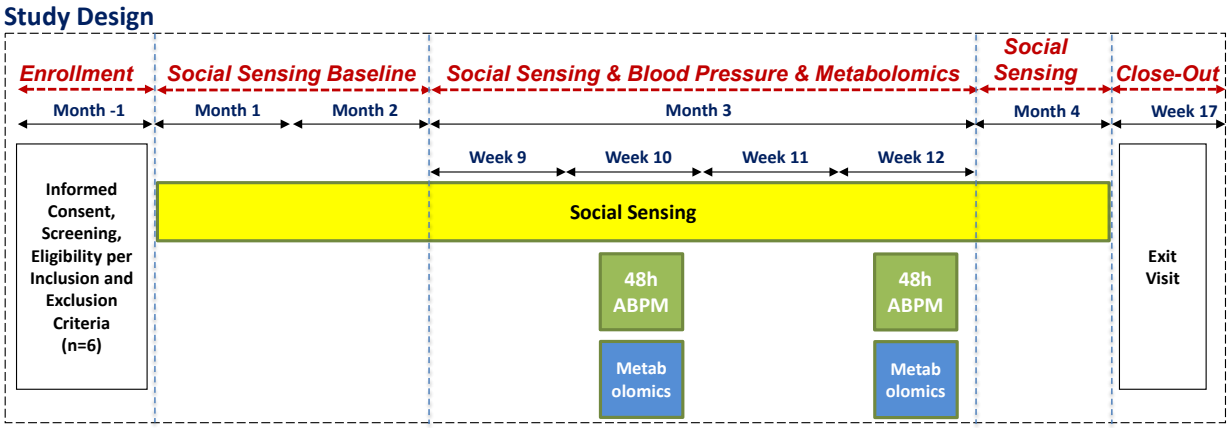
Further secondary objectives are to identify whether dietary and transcriptomic patterns influence circadian patterns in user activity, blood pressure and metabolomics signatures as well as in the microbiome; also, to determine the variation over time among healthy volunteers.

3 Study Design

3.1 General Design

Healthy participants (n=6) will be enrolled in this single-arm Phase I exploratory study. Procedures to obtain informed consent, to assess eligibility per inclusion and exclusion criteria and to conduct the screening clinic visit will be scheduled for the Clinical Translational Research Center (CTRC). Recruitment is facilitated by our database of healthy volunteers and targeted advertisement observing the NIH policy on inclusion of women and minorities in clinical research.

First, baseline data for the user activity will be collected for the duration of a minimum of one month up to a maximum of two months (weeks 1-8). User activity will be continuously recorded for another two month (weeks 9-16), while ambulatory blood pressure (ABPM) will be monitored for the duration of 48 hours twice, i.e. in weeks 10 and 12. Simultaneously to the ABPM, metabolomics will be analyzed in weeks 10 and 12 in urine, blood and saliva samples. mRNA expression will be assessed in timed blood samples. Study close-out will be conducted in week 17 with exit safety labs. Thus, the expected duration of subject participation will be 16 weeks plus the time necessary for the screening and exit visits.



3.2 Primary Study Endpoints

Circadian patterns in user activity and its variability will be identified by visual inspection and exploratory statistical analysis.

3.3 Secondary Study Endpoints

Circadian patterns in user activity, blood pressure, metabolomics and microbiome signatures and its variability will be identified by visual inspection and exploratory statistical analysis.

3.4 Primary Safety Endpoints

Screening and exit laboratory tests will serve as primary safety endpoints.

4 Subject Selection and Withdrawal

4.1 Inclusion Criteria

- Volunteers must be in good health as based on medical history, physical examination, vital signs, and laboratory tests as deemed by PI;
- Volunteers are capable of giving informed consent;
- 25-35 years of age;

- Own a smartphone (Android phones only) which installs the social sensing application ginger.io;
- Non-smoking;
- Male subjects only if feasible during recruitment; and
- In case female volunteers are invited to enroll: non-pregnant, female subjects must consent to a urine pregnancy test.

4.2 Exclusion Criteria

- Recent travel across time zones (within the past month);
- Planned travel across time zones during the planned study activities;
- Volunteers with irregular work hours, e.g. night shifts or becoming a parent.
- Use of illicit drugs;
- Subjects, who have received an experimental drug, 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.
- 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.

4.3 Subject Recruitment and Screening

Healthy volunteers will be recruited from the database of healthy volunteers in the Clinical and Translational Research Center (CTRC), through advertisement (flyer, postings on Craigslist at www.craigslist.org, and the online registry of volunteers at https://www.researchmatch.org/), and by word of mouth. These advertisements will be approved by the IRB for the site; a sample of such information is located in the attachment section of the protocol.

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). As next steps, phone call(s) to next-of-kin if available will be initiated and up to one certified letter will be sent to subjects in order to schedule the exit visit.

5 Study Drug

A study drug will not be administered.

6 Study Procedures

6.1 Medical History / Physical

The medical history and the physical exam will be taken in the CTRC by a nurse practitioner.

6.2 Routine laboratory screens

Screening and exit laboratory screen will consist of:

- Hematology (white blood cell count, red blood cell count, hemoglobin, hematocrit, MCV, MCH, MCHC, RCDW, and platelet count).
- Serum chemistry (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.

6.3 Laboratory screens for compliance

6.3.1 Platelet aggregation

Blood will be collected in Na-Citrate Tubes for aggregation testing. Platelet aggregation will be measured in platelet-rich plasma (PRP) by turbidometry, measuring the change in light transmission during platelet aggregation. Platelets will be stimulated by 20 μmol/L adenosine diphosphate (ADP) (18). Maximum aggregation and the maximum slope of the change in light transmission within 5 minutes post stimulation will be recorded. Additionally, maximum aggregation and slope of secondary aggregation responses will be recorded. On selected days after enrollment, platelet aggregation will be assessed to rule out recent NSAID use (19) by using one or more of the following agents: arachidonic acid (AA), collagen (Coll), thrombin-receptor activating peptide (TRAP) and ADP.

6.3.2 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.

6.3.3 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).

6.3.4 Pregnancy test

A standard pregnancy urine dip test will be used to rule out a pregnancy.

6.4 Questionnaires

6.4.1 Food questionnaires

6.4.1.1 3-day food record

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

6.4.1.2 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 (20). Dietary intake data gathered by interview is governed by a multiple-pass interview approach (21). 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 (22). 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 (23). 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 (24). Standardized, published imputation procedures are applied to minimize missing values (25).

6.4.2 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 appendix.

6.4.3 Activity Diary

a) Subjects will be asked to keep an activity diary during study enrollment to record major changes in the daily activity patterns, e.g. starting to train for a marathon, or a change in jobs which alters the ratio of active/non-active time periods. This data will be used to look for internal consistency with data generated from the actigraphy.
b) Subjects will be asked to fill out an activity diary during each of the 48 hour ABPM session. A template of the diary form from Spacelab Healthcare is provided in the appendix.

6.4.4 Chronotype Questionnaire

The Munich ChronoType Questionnaire will be administered to determine the study participants' chronotype, i.e. their circadian phase preference for mornings or evenings (34) (see Attachments).

This questionnaire divides the population, based on 50,000 participants so far, into seven chronotype groups. These are:

- extreme early types;
- moderate early types;
- slight early types;
- normal types;
- slight late types;
- moderate late types; and
- extreme late types.

In the online version available at https://www.bioinfo.mpg.de/mctq/core_work_life/core/introduction.jsp?language=eng, the "*chronotype is calculated from the sleep times on both work and free days and is given as the time of mid-sleep (half way between onset and end) on days when the subject would not be restricted by social obligations and would not suffer from any accumulated sleep debt. The duration of sleep is independent of the timing of sleep (i.e., Chronotype). For example, a subject who goes to sleep at 11 p.m. and wakes at 7 a.m. has the same chronotype as one who sleeps between midnight and 6 a.m. The distribution of chronotypes in the population is similar to that for height: there are few extremely short or tall people, with the majority being of average height. In analogy, there are few extreme early and late subjects and many in between*."

6.4.5 Questionnaires delivered through the Ginger.io platform

- Ginger.io Demographic Questionnaire (only at download)
- Ginger.io Sleep Questionnaire (daily in the morning)
- Ginger.io App Satisfaction Questionnaire (only at completion)

Copies are provided in the appendix.

6.5 Remote Food Photography

Dr. Martin and colleagues developed and validated the Remote Food Photography Method® (RFPM) (26, 27). RFPM data are collected with the SmartIntake© smartphone app, which is used to streamline data collection and minimize participant burden. When using the SmartIntake© app, participants place a reference card next to their food and capture images of

their food selection and plate waste. The app has barcode scanning capabilities and a Price Look-up (PLU) number entry system to easily identify foods in the image. The app also has text and voice message capability, allowing participants to easily record food descriptors that are automatically tagged to the food image. These data and food images are wirelessly sent by the app to the server-based Food Photography Application©, which is used to manage the data collection process and analyze the food images to estimate energy and nutrient intake. To facilitate data quality and completeness, the SmartIntake© app includes Ecological Momentary Assessment (EMA) (28) methodology to remind participants to capture images of the foods and beverages that they consume. These reminders are text messages that are scheduled for delivery at the personalized meal times of the participants. The responses to EMAs are tracked in near real-time, which allows the research team to quickly identify when data collection problems occur. In such cases, a back-up method is used (e.g., a food record or food recall conducted by phone).

The SmartIntake© app sends participants food images and accompanying food identifier data (e.g., barcodes, PLU numbers, food descriptions) to a Pennington server where the Pennington team analyzes the images to estimate food intake. The analysis process relies on a computer program that was built by H. Ray Allen, Ph.D. and team at PBRC called the Food Photography Application©. The program allows the operator to identify a match for each food from the Food and Nutrient Database for Dietary Studies 5.0 (29) and other sources, such as manufacturer's information and Nutrition Fact Panels, to calculate energy and nutrient intake. Additionally, the operator uses the program to estimate portion size by visually comparing participants' food images to images of foods with a known portion size (i.e., standard portion images). This process relies on existing and validated methodology (26, 27, 30, 31).The standard portion images are contained in a searchable "archive" that includes over 8,000 standard portion images. This process results in estimates of food selected and plate waste, which is used to calculate food intake by difference. The RFPM has been found to accurately measure the energy (26, 27) and nutrient intake of adults (26).

Key Personnel at the Pennington Biomedical Research Center are:

Corby K. Martin, Ph.D. Associate Professor Director, Ingestive Behavior Laboratory, Pennington Biomedical Research Center 6400 Perkins Rd., Baton Rouge, LA 70808 Phone: 225-763-2585 FAX: 225-763-3022 email: Corby.Martin@pbrc.edu	**Mandy Cowley, M.A.** Lab Manager Ingestive Behavior Laboratory Pennington Biomedical Research Center 6400 Perkins Road Baton Rouge, LA 70808 Mandy.Cowley@pbrc.edu 225-763-2832	**Rebecca Schulin, RD, LDN** Research Dietitian/Interventionist Ingestive Behavior Laboratory Pennington Biomedical Research Center 6400 Perkins Road Baton Rouge, LA 70808 (225)-763-3058 rebecca.schulin@pbrc.edu

Installing the SmartIntake application on a subject's Droid-based smartphone does not require any personal information about the phone. The application is installed from a password protected website. The only PHI being transferred to the Pennington Biomedical Research Center during this study is the email the subject uses to send the food photos.

6.6 Urine Collections for Metabolomics Analyses

Prior to the start of timed urine collections, subjects are advised to void their bladder. For each collection interval, subjects are advised to void their bladder at the end of each interval into labeled opaque urine containers. Subjects will be advised to store urine containers under cold

conditions (fridge). Urine samples will be aliquoted and frozen at -80°C immediately after drop-off in the CTRC. An overview of corresponding hour intervals and 24-hour clock is provided in the appendix.

6.7 Blood Collections for Metabolomics Analyses

Blood collections will be done in Na-Heparin plasma tubes as well as serum tubes. Please see the appendix for the blood sampling schedule and volumes of total blood drawn throughout this study.

6.8 Saliva Collections for Metabolomics Analyses

Saliva will be collected using Sarstedt Salivette®, a standardized hygienic saliva collection system. Saliva is an easily obtained biofluid which can be collected by chewing on a roll-shaped saliva collector. The manufacturer Sarstedt indicates that an average volume of saliva collected is 1.1±0.3 mL.

6.9 Nasal brush to collect epithelial cell for transcriptomic analyses

Epithelial cells will be collected from the right/left inferior turbinate with a standard cytology brush, Bionix Medical Technologies, Toledo, OH or other supplier/manufacturer. Alternatively, a flocked nasal swab or a nasal mucosal curette can be used. Brushings will be facilitated by using a nasal speculum. Epithelial cell brushings will be immediately placed into RNA lysis buffer and snap frozen in liquid nitrogen. Storage at -80°C until use.

6.10 Blood Collections for Transcriptomics Analyses

Blood collections will be done in Qiagen 2.5 mL PAXGene Blood RNA Tubes. After inverting gently 8x-10x, PAXgene Blood RNA Tubes will be stored upright at -80°C.

6.11 Mouths Swabs for Microbiome Analyses

Suitable mouths swabs are for example the "DenTek Slim Brush Cleaner (Ultra Thin Soft Brush)", available from Henry Schein Inc. Furthermore, sterile tubes, e.g. BD Falcon 15mL High-Clarity Polypropylene Conical Tube (17x120 mm style) Sterile, are necessary to store mouths swabs.

The procedure to take a mouth swab for microbiome analysis is as follows:

1. Have participant swish mouth with water to remove any residual food particles.
2. Remove DenTek brush from package and hold participant's cheek away from gums.
3. Slide brush along the base of the gum line, repeat several times with each brush to get lots of bacteria.
4. Swab in the same manner for the left side, right side, and for the front gums. A total of 6 swabs (2 in each section) will be used.
5. Place swabs in 1x 15mL conical tube directly after swabbing. Label the tube appropriately.
6. Store entire 15mL conical tube in -80°C freezer as soon as possible after collecting sample (this will ensure that the bacteria do not get degraded over time by saliva, heat, etc.).

The lab of Dr. Gary Wu, Penn Gastroenterology, has established this sampling method and is available to assist in these efforts.

6.12 Fecal Swabs for Microbiome Analyses

Rectal swab samples will be obtained by inserting a sterile swab 2-3 cm into the rectum. The swab will be turned 360 degrees, removed, and placed into a sterile tube and frozen until analysis.
The lab of Dr. Gary Wu, Penn Gastroenterology, has established this sampling method and is available to assist in these efforts.

6.13 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 (35) provided in the appendix.

As an exploratory analysis, stool water will be prepared for fecal water metabolomics (36). Stool samples (5-8 g) will be homogenized, then ultracentrifuged at 4 °C at 50 000 rpm (171 500g) for 2 h. The supernatant will be separated and 2 μL of NaN3 (100 mg/mL) as an antimicrobial agent per gram of fecal water will be added. The fecal water samples will be stored at -80 °C as 1 mL aliquots.

6.14 Water Intake

To facilitate timed urine collections, a constant oral water intake of ≥237 ml/hour (1 cup/hour) during waking hours, and *ad libitum* water intake during sleeping hours is advised during the hours of scheduled urine collections.

6.15 Vital Signs

Blood pressure (CTRC sphygmomanometer), heart rate and temperature will be assessed as vital signs.

6.16 Social Sensing Technology

Ginger.io describes its platform as follows:

The Ginger.io Platform consists of two major components: 1) the mobile application and 2) a web dashboard.

The mobile application has two elements:

> Passive data collection: Behavior data is gathered unobtrusively through a background process on the mobile device. The background process is launched automatically by the application once the user logs in. After the initial logon, the process continues to be automatically launched whenever it is not already running (on phone restart or other events that terminate the process).
>
> Active data collection: Surveys and other self-report data are gathered through user input. This is achieved in two ways: (i) the user receives a notification that a survey is available and upon clicking the notification, they are transferred to the survey page,

(ii) the user can manually launch the application and they are shown all the surveys available at that time.

The web dashboard is available at https://data.ginger.io and provides additional functionality for researchers and study administrators to manage the participants and the data collection process. Researchers can create studies directly on the dashboard and invite participants. Researchers can also monitor the data collection process through the dashboard and take adequate measures to reach out to customers who are not submitting surveys or whose phones are problematic. Further, researchers also have access to basic analytics through this dashboard.

Currently, the Ginger.io platform allows the collection of communication data as well as mobility data from smartphones using the Android operating system, while the Apple iPhone operating system only allows the collection of mobility data. Therefore, we will only include subjects at this point who own and use a smartphone with the Android operating system.

6.16.1 Data Collection (through Mobile Phone) and Transmission

Upon invitation from the researcher or study administrator, users install the application and log in with a password provided to them. The data collection process begins soon after as detailed above. Passive data is gathered through a background process on the mobile phone and users actively submit surveys when notified through the application.

The data is stored securely on the mobile device temporarily. At regular intervals, the application checks for an available connection to the WWW. If such a connection is available, the data is transmitted over a secure connection to the server and deleted from the device. If a connection is not available or if a transmission is not successful, the data continues to be stored on the device until it is transmitted successfully.

The data collected through the device includes data such as call and sms logs (including call/sms time, call duration, sms length, and phone number), location and device usage. Private information such as actual content of voice calls or sms messages or emails is never read, recorded or transmitted.

6.16.2 Data Storage, Encryption and Server Security

The data described above is encrypted and transmitted to the server over a secure 128-bit SSL 3.0 connection using the HTTPS protocol. Extra care is taken to ensure that the request cannot be spoofed or imitated by an attacker through the use of unique keys that are generated dynamically for every API request through a unique identifier and client secret that is embedded inside the application and cannot be accessed by any user or application.

Ginger.io linux-based servers are protected using a firewall and access control lists (ACLs), and access is restricted to Ginger.io employees and contractors. Superuser activity and researcher activity on the server is logged for security and auditing. The servers are regularly updated and patched with latest security updates to ensure that there are no known threats.

These servers host the database where the data is stored. Linux---based servers and access to the servers is restricted to a few users responsible for maintaining and testing the database. For additional security, all the data that has personally identifiable information about the participant (such as e-mail) is stored in a separate database from the one that has the data collected from the users and data related to the studies. The passively collected data from the phones as well as the actively-reported survey information is stored in the second database. Phone numbers and other such private information stored in this database are anonymized by hashing over the identifiers so that there is no threat to privacy because the data is not human-readable anymore.

6.16.3 Data Access (through Web Dashboard)

Researchers and study staff have access to the web dashboard through an account on the site. Information pertaining to a study is only available to researchers and study staff associated with that study. Participants or researchers not involved in the study cannot access the data through the dashboard. Data accessed through the dashboard is also transmitted through 128-bit SSL 3.0 connection using the HTTPS protocol. HTTP access is disabled.

Researchers or study staff can invite participants through the web dashboard. They are provided an option to use the study participants' name or a coded identifier as is suitable for their study protocol. The coded identifier (or name) is used to refer to the participant on the dashboard pages and in communication with the participants.

Data available on the dashboard includes aggregated or processed data about the participants related to the amount of data being collected (number of location samples, number of phone calls), time-related information such as when surveys were taken, and processed data such as GBI features (from passive data) or survey scores (for surveys that have scoring associated with them). Researchers are also notified when passive data is not being collected from a participant or when participant is not answering surveys.

6.16.4 HIPAA Compliance

Ginger.io provides clients with a platform that is HIPAA-compliant from a technology standpoint as detailed in this document. It is the responsibility of the client user to remain HIPAA-complaint when using the Ginger.io technology within their medical practices.

Ginger.io technology, security and privacy policies comply with HIPAA standards --- such as encryption (SSL), system---user identifiers (login, passwords), high---end server security, frequent backups, strong privacy policies, and strong internal business and employee policies. All patient personal and health information that may be included in the Ginger.io database will be treated in compliance with all applicable laws and regulations. Additional security and privacy safeguards can be enabled upon request of the client.

6.16.5 Backups

Rotating backups are implemented weekly, monthly and daily through full server snapshots. Such precautionary measures make Ginger.io more resistant to hardware/software failures as well as human errors by enabling us to revert to a previous state in case of any issues and ensuring that Ginger.io does not lose any important data.

6.16.6 Ginger.io Privacy Philosophy

The goal at Ginger.io is to provide a platform for patients, health care providers, and researchers to collect and analyze data to better understand patients' behavior in order to improve their health. Ginger.io takes their users' privacy very seriously. Ginger.io's philosophy rests on a few key principles of data ownership:

1. Users own their data. They opt-in to the system, have control over the use of any data that is generated, and can request to have their data removed from the system.
2. If data is shared, it is done in an aggregated and anonymous fashion. Ginger.io is working with leading enterprise partners and academic researchers to explore ways of leveraging aggregate anonymous data to improve patient health. Ginger.io respects the privacy and anonymity of their users and never shares specific individual data unless explicitly asked by their users to do so or otherwise required by law.
3. Users should get value in exchange for sharing their data.

Ginger.io has seen a great willingness amongst users to share their data to collectively advance the state of health care for themselves and for others. Ginger.io is committed to continue working closely with the health and data communities to promote these innovations.

6.16.7 Set-up and removal of the Ginger.io smartphone application

Supervision will be provided to install and remove the Ginger.io smartphone application by the volunteer. Its use will be explained using a demonstration device.

6.16.8 Potential Risks and Discomforts

The primary risk to participants is loss of privacy. The following measures have been put in place to mitigate such risk:

- The mobile data is exclusively linked to coded identifiers for the participants during the collection and transmission process.
- All server transactions are transferred over a public-private key encrypted channel, based on the HTTPS/TLS web protocol.
- Content of phone calls and SMS messages is never captured. Raw audio data is never captured.
- Behavior data is only stored on the device until the data is uploaded to the secure server. In the event of a loss or misplaced phone, only data that was never uploaded is available on the phone.
- Coded data stored on the secure server is only accessible to trained researchers, and secured with a firewall and other security measures.
- Participants have the right to withdraw from the study at any time by uninstalling the application and informing the researchers. Participants can also request Ginger.io to have their personal data permanently deleted from our databases. However, this task may take 2-3 months to ensure that all traces of their behavior data are removed from backups. Further, it will not be possible to identify user data from aggregated population and study-level statistics.

6.17 Actigraphy

Actigraphy will noninvasively assess and log the sleep, wake and activity patterns for each participant. In detail, data capture entails 24 hour sleep/wake measurements including total sleep time (TST), sleep latency, wake after sleep onset (WASO), sleep efficiency, energy expenditure, metabolic rates, steps taken, physical activity intensity, heart rate R-R intervals, subject position, and ambient light levels.

This device will be applied as a wrist watch which facilitates incorporation into the clinical study setting. Actigraphy devices will be sources from e.g. ActiGraph, Pensacola, FL, http://www.actigraphcorp.com/company/ or similar suppliers.

Battery life on the actigraphy devices is indicated as 22-25 days. Therefore, batteries will be periodically exchanged by new ones during brief CTRC visits.

6.18 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 (37). 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	42-52	13-17
Extra Large Adult	48-50	15-20

* The bladder of the cuff should encircle at least 80% of the arm circumference (37).

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 (38, 39).

6.19 Metabolomics

A dual analytical approach combining NMR and LC-MS analysis will be conducted to provide both quantitative and sensitive exploratory metabolomics analysis as previously described (40). Samples for NMR will be ultrafiltered to remove protein components while samples for mass spectrometry will be separated into organic and aqueous components.

Ultrafiltration of samples: Ultrafiltration will be carried out as previous reported. Briefly, a 3 kDa MW 500 µL maximum volume cutoff filter (Pall Life Sciences) will be used to separate macromolecular components from the metabolites of interest. The filters will be rinsed with 4x600 µL of distilled water to remove preservative components and centrifuged for 4x8 min (all centrifugation at 4^{o}C at 10,000 rpm). 300 µL of sample will be filtered for 60 min followed by a final wash with 100 µL of D_2O for an additional 45 minutes. The filtrate will be dried using a vacuum centrifuge and the final solution contained the metabolites of interest.

Organic extraction: 200 µL of cold methanol/chloroform (2:1; v/v) will be added to 50 µL of each sample, or the retentate of ultrafiltration to characterize residual lipids and/or metabolites. 100 µL of each layer of 1:1 chloroform/water will be added and the samples will be centrifuged for 7 minutes at 13,300 rpm. The upper fraction (aqueous), lower fraction (organic) will be separated prior to analysis.

NMR and MS Analysis: NMR analysis of organic extracts and ultrafiltered samples will be conducted using a Bruker AVANCE III 700 MHz NMR equipped with a TXI triple resonance cryoprobe. Metabolites from NMR samples will be quantified using the 'Targeted Profiling' methodology (41). Mass spectral analysis will be conducted using a Waters UPLC-ESI-qTOF MS for profiling and LC-ESI-MS/MS for metabolite identification. LC-MS data will be analyzed by a combination of XCMS (42), public databases (43-45) and/or vender specific software and databases such as the Waters MarkerLynx package (Waters Corp).

This analytical approach will adapted to analyze fecal water samples.

6.20 Transcriptomics

RNA will be extracted from blood using the Qiagen PAXgene blood RNA kit (Qiagen, Valencia, CA). RNA-seq will be performed as paired end RNA sequencing using, adapters from Illumina's Genomic DNA Sample Prep Kit. In previous experiments, we determined that 80 million reads

approach the maximum coverage depth, while >20 million read pairs, is sensitive to over 85% of the limiting value. We have privileged access to the Penn Genomics Frontiers Institute's (PGFI) two HiSeq2000 instruments which currently generate >100 million reads per lane but will be upgraded to generate in excess of 300 million reads per lane in the near future. Thus, sample multiplexing using DNA barcoding has become feasible and will reduce the costs of this rapidly developing methodology. Read alignment is deceptively complicated for RNA-seq experiments of this size and the standard methods have required improvement for our purposes. Resolving the location of reads which do not span exon/exon junctions, or which span known junctions, is relatively straightforward as long as the genes being sequenced do not have significant polymorphisms with respect to the reference sequence. The introduction of polymorphisms, novel splice junctions, and aberrant splicing, all present considerable challenges but are common factors frequently encountered in data sets. The ITMAT bioinformatics group has developed a novel analysis pipeline that starts by processing the reads which are easy to map by using Bowtie (46) to align against the genome and against the transcriptome. A module is then employed which leverages the information from the genome and transcriptome mappings and adds considerable accuracy. The remaining reads are mapped with BLAT and processed with a complex set of filters designed to isolate the correct mapping.
The analyses will be performed on the PGFI compute cluster, which is equipped to handle these data volumes. Response networks for dietary effects will be constructed using Bayesian methods to estimate the likelihood that detected exons/genes are to participate in the response to our dietary interventions. The dietary intervention response networks will be used to filter the human subject data so that only the most variable components of this network will be interpreted as candidate nodes for introducing variation in the clinical response to dietary perturbations. The importance of such nodes can then be followed-up in validation experiments.

6.21 Genetic Analyses

Blood samples will be stored to run future exploratory genetic analysis. These 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. The human samples collected for this analysis will be de-identified and will not contain any Protected Health Information (PHI) prior to in-house sample distribution or shipment to external collaborators.

6.22 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.

6.23 Proteomics Analyses

Circadian protein synthesis contributes to blood pressure control (47). Therefore, plasma samples will be submitted to a proteomics platform for analysis, e.g. SomaLogic (http://www.somalogic.com/Technology.aspx)

6.24 Additional Analyses

Urine electrolytes will be determined since some evidence suggests that the circadian clock modulates renal sodium handling (48).
Also, cortisol, renin activity, aldosterone, creatinine clearance, GFR, and BUN will be measured.

6.25 Distribution and shipment of human specimens

This research project relies heavily on collaborative efforts. The human specimens collected for this study will be de-identified and will not contain any Protected Health Information (PHI) prior to in-house sample distribution or shipment to external collaborators.

6.26 Clinical Study Visits

Please see flow-chart for the study schedule as well as the blood draw schedule in the appendix of this protocol. The appendix also provides a comparison between hour intervals of urine collection and corresponding 24-hour clock time.

6.26.1 Screening Visit

The subject will be admitted to the outpatient unit of the Clinical and Translational Research Center (CTRC) at the Hospital of the University of Pennsylvania at 9 am ± 2 hours. The following procedures will be performed:

1. Explain study rationale and study procedures, address questions, document in writing and obtain written informed consent from participant.
2. Discuss study inclusion and exclusion criteria.
3. Assign a subject number.
4. Record vital signs (blood pressure, heart rate, temperature).
5. Select correct cuff size for ABPM measurements and obtain blood pressure every 10 minutes over one hour to assess tolerability during screening visit (≥ 6 BP measurements); point out to subject that blood pressure will be assessed in intervals of 20 minutes during waking hours and in intervals of 30 minutes during sleeping hours.
6. Record demographics.
7. Record medical history (including medications) using the health questionnaire in the appendix.

8. CTRC Nurse Practitioner: Perform full physical examination including weight, height, and vital signs; for women, record the following menstrual cycle parameters: overall cycle length, start and end of menstrual flow, menstrual intensity, and other associated symptoms;
9. Collect venous blood and urine samples for hematology (white blood cell count, red blood cell count, hemoglobin, hematocrit, MCV, MCH, MCHC, RCDW, and platelet count), serum chemistry (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, drug, cotinine [for nicotine] screening, urinary electrolytes, and self-reporting upon screen for HIV and hepatitis B/C.
10. Collect venous blood sample for genomic analyses (provided that the subject has agreed and signed the informed consent form appropriately).
11. Perform screening tests to detect drug use and smoking.
12. Perform urine pregnancy test for women of childbearing potential.
13. Hand out questionnaires (food frequency; physical activity), give instructions and retain for study binder.
14. Explain how to take pictures of everything consumed including food, beverages and all snacks for the 24 hours prior to Visit V1.
15. Schedule the next visit within 1-4 weeks from the screening visit. Once confirmed schedule all remaining study visits.

6.26.2 Ginger.io Set-up Visit (Visit V1)

1. Record vital signs.
2. Help to set-up of Ginger.io application on subjects' smartphone.
3. Record AEs/SAEs.
4. Set up dietary assessments including SmartIntake App to record everything consumed including food, beverages and all snacks.
5. Schedule a test-run for the SmartIntake App.
6. Administer Munich ChronoType Questionnaire.
7. Set up activity diary.
8. Re-confirm dates and times of the following study visits.

6.26.3 Brief Visits to exchange actigraphy device (Visit V2, V3 and V12)

1. Record vital signs.
2. Record AEs/SAEs.
3. Download actigraphy data.
4. Provide fully charged actigraphy device.

6.26.4 Preparatory Visits (Visit V4 and V8)

1. Record vital signs.
2. Explain again the procedures for the ABPM sessions and sequential urine collections in the upcoming visits.
3. Explain again the SmartIntake App for taking pictures of everything consumed including food, beverages and all snacks. Explain the importance to include a standard measure

such as a coin or a plastic card in the size of a typical credit card with each picture. Confirm that timestamp and GPS tag are provided for each picture.
4. Hand out urine collection container for interval (06:00-09:00, Day 1) hours prior to start of ABP recordings.
5. Record AEs/SAEs.
6. Re-confirm dates and times of the following study visits.

6.26.5 Assessment Visits – Day 1 (Visit V5 and V9)

This visit consists of two parts, a morning and evening visit to the CTRC, exactly 12 hours apart.

1. Record vital signs (morning and evening).
2. Download actigraphy data (only morning).
3. Equip actigraphy device with new batteries (only morning).
4. Only Visit V5: Have subjects' void their bladder into urine collection container for hour interval (06:00-09:00, Day 1).
5. Only Visit V9: Have subjects' void their bladder into urine collection container for hour interval (06:00-09:00, Day 3).
6. Apply ABPM instrumentation (target time: 9 am) to start the 48 hour measurement cycle; instruct subject how to handle ABPM equipment; hand out instruction sheet.
7. Only Visit V5: Start urine collection for hour interval (09:00-12:00, Day 1).
8. Only Visit V9: Start urine collection for hour interval (09:00-12:00, Day 3).
9. Only Visit V5: Discharge subject with urine collection container and information how to obtain sequential urine collection for hour intervals (12:00-15:00, Day 1); (15:00-18:00, Day 1); (18:00-21:00, Day 1); (21:00-24:00, Day 1); (24:00-6:00, Day 1); and (06:00-09:00, Day 1).
10. Only Visit V9: Discharge subject with urine collection container and information how to obtain sequential urine collection for hour intervals (12:00-15:00, Day 3); (15:00-18:00, Day 3); (18:00-21:00, Day 3); (21:00-24:00, Day 3); (24:00-6:00, Day 3); and (06:00-09:00, Day 3).
11. Take the first blood and saliva sample (morning, target time: 9-9:30 am) and a second blood and saliva sample exactly 12 hours later (evening, target time: 9-9:30 pm) for metabolomics and transcriptomics analyses.
12. Collect mouth and fecal swabs during morning and evening visits.
13. Collect nasal brushings during morning and evening visits.
14. Hand out stool collection container to collect each single bowel movement during Day 1.
15. Collect urine containers of past collection intervals.
16. Collect the stool containers of bowel movements since last visit.
17. Perform urine pregnancy test for women of childbearing potential.
18. Record AEs/SAEs (morning and evening).
19. Re-confirm dates and times of the following study visits.

6.26.6 Assessment Visits – Day 2 (Visit V6 and V10)

This visit consists of two parts, a morning and evening visit to the CTRC, exactly 12 hours apart.

1. Record vital signs (morning and evening).
2. Collect the urine containers with sequential urine collection since the past study visit.
3. Only Visit V6: Have subjects' void their bladder into urine collection container for hour interval (06:00-09:00, Day 2).

4. Only Visit V10: Have subjects' void their bladder into urine collection container for hour interval (06:00-09:00, Day 4).
5. Control ABPM instrumentation (target time: 9 am) to continue the 48 hour measurement cycle.
6. Only Visit V6: Start urine collection for hour interval (09:00-12:00, Day 2).
7. Only Visit V10: Start urine collection for hour interval (09:00-12:00, Day 4).
8. Only Visit V6: Discharge subject with urine collection container and information how to obtain sequential urine collection for hour intervals (12:00-15:00, Day 2); (15:00-18:00, Day 2); (18:00-21:00, Day 2); (21:00-6:00, Day 2); and (06:00-09:00, Day 2).
9. Only Visit V10: Discharge subject with urine collection container and information how to obtain sequential urine collection for hour intervals (12:00-15:00, Day 4); (15:00-18:00, Day 4); (18:00-21:00, Day 4); (21:00-6:00, Day 4); and (06:00-09:00, Day 4).
10. Take the first blood and saliva sample (morning, target time: 9-9:30 am; i.e. 12 hours after the last blood/saliva sample) and a second blood and saliva sample exactly 12 hours later (evening, target time: 9-9:30 pm) for metabolomics and transcriptomics analyses.
11. Collect mouth and fecal swabs during morning and evening visits.
12. Collect nasal brushings during morning and evening visits.
13. Hand out stool collection container to collect each single bowel movement during Day 2.
14. Collect urine containers of past collection intervals.
15. Collect the stool containers of bowel movements since last visit.
16. Record AEs/SAEs (morning and evening).
17. Re-confirm dates and times of the following study visits.
18. Schedule face-to-face interview with bionutritionist.

6.26.7 Assessment Visits – Day 3 (Visit V7 and V11)

1. Record vital signs.
2. Collect the urine containers with sequential urine collection since the past study visit.
3. Collect the stool containers of bowel movements since last visit.
4. Take a blood and saliva sample (morning, target time: 9-9:30 am; i.e. 12 hours after the last blood/saliva sample) for metabolomics analyses.
5. Collect mouth and fecal swabs.
6. Collect nasal brushings.
7. Collect ABPM instrumentation (target time: 9 am) to finalize the 48 hour measurement cycle.
8. Have subjects list all activities and comments with approximate times over the past 48 hours into the subject diary recall form.
9. Record AEs/SAEs.
10. Re-confirm date and time of the exit visit.

6.26.8 Exit Visit (Visit V13)

1. Record vital signs.
2. CTRC Nurse Practitioner: Perform full physical examination including weight, height, and vital signs; for women, record the following menstrual cycle parameters: overall cycle length, start and end of menstrual flow, menstrual intensity, and other associated symptoms;

3. Collect venous blood and urine samples for hematology (white blood cell count, red blood cell count, hemoglobin, hematocrit, MCV, MCH, MCHC, RCDW, and platelet count), serum chemistry (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), and for urinalysis.
4. Record AEs/SAEs.
5. Remove actigraphy device.
6. Assist in removing the Ginger.io application from the subjects' smartphone.
7. Assist in removing the SmartIntake application from the subjects' smartphone.
8. Assist in resetting the timestamp and GPS tag function to pre-study conditions.
9. Address any unanswered question.

7 Statistical Plan

7.1 Sample Size Determination

Preliminary data on the relationship between social behavior, blood pressure and metabolomics signatures does not exist. Therefore a formal sample size calculation was not conducted. To collect first pilot data, the sample size is set at n=6. Since the number of measures obtained from the social sensing technology as well as the metabolomics approach easily outnumbers any feasible number of subjects enrolled in such an exploratory study, the emphasis is on the fast generation of first data. This strategy is commonly used for high-dimensionality data.

7.2 Statistical Methods

Standard summary statistics including means, standard deviations, proportions, and 95% confidence intervals will be produced for all measures. The focus of the data exploration will be on exploring the shape of the curves over time, as well as identifying peaks and troughs.

The data generated in this study are multimodal and dispersed across time and space. Outcomes are **i)** behavioral features (call/SMS/missed interactions count [number/hour], call duration [minutes/ hour], SMS length [characters/hour], interaction diversity [unique individuals/hour], aggregate communication [minutes/hour] and mobility radius [miles/hour]); **ii)** 48-hour blood pressure (mean arterial pressure, pulse pressure, systolic/diastolic blood pressure [mmHg], heart rate [beats/minute], average real-time variability (ARV) [%]); **iii)** metabolomics (urine [ng/mg creatinine] and plasma/serum metabolites [ng/mL]).

For the Primary Study Endpoint, the diurnal behavior of each of the relevant measurements that are provided by the Ginger.io platform will be identified and characterized. Standard methods will be applied (49). We will identify individual-specific behavioral patterns and population-wide signatures in a behavioral data set spanning three months of collecting social activity data. We will investigate the extent to which records of sleep patterns and/or other metrics can be used to normalize the multi-dimensional data across subjects. This will establish a universal time scale in order to make their diurnal patterns comparable and hence, enable a proper replicate analysis as needed for the Secondary Study Endpoint.

For the Secondary Study Endpoint, we will identify the relationships between the social and biological factors that are not confounded due to their mutual circadian behavior but which hypothesize a causal link between them. This will be achieved by compiling comparable

measurements (using universal time determined in the Primary Study Endpoint) across all individuals at fixed universal time points. With n=6 subjects, the total observational period of 96 hours for the biological measurements will enable robust correlation analyses between all pairs of relevant factors for this pilot investigation.

7.3 Subject Population(s) for Analysis

Any subject randomized into the study will be included in subject population whose data will be subjected to analysis.

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 <u>any one of the following</u> 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.

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.

8.4 Reporting of any breaches of confidentiality to the Ginger.io system

Details on how Ginger.io will be reporting any breaches of confidentiality to their system back to the study site are as follows:
1) The designated person at Ginger.io reports any breaches of confidentiality to their system back to the study site within 5 business days after becoming first aware of the breach;
2) The designated person at Ginger.io will characterize the extent of breach by providing in writing i) what kind of data has been compromised, ii) who of the users/subjects has been

compromised, iii) how long the breach existed (start and end times and dates), iv) what immediate measures have been taken to remedy the breach;
3) Recipients of Ginger.io's reports on the side of the study site is i) a designated investigator of the study team, and ii) a designated member of the UPENN IRB to be determined;
4) The designated investigator and the designated IRB member discuss and agree on a course of action within 5 business days.

Names and contact information of the designated persons are:

Ginger.io
Sasank Mudunuri
965 Mission St, Suite 730, San Francisco, CA 94103
Telephone: 1-855-446-4374
e-mail: sasank@ginger.io

Investigator UPENN
Carsten Skarke, MD
Institute for Translational Medicine and Therapeutics (ITMAT),
University of Pennsylvania School of Medicine,
8036 Maloney Bldg., 3600 Spruce St
Philadelphia, PA 19104
Telephone: 215.746.8330
e-mail: cskarke@mail.med.upenn.edu

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
A designated, qualified medical monitor from the Institute of Translational Medicine and Therapeutics will be reviewing subjects' safety labs, SAE/AE reporting and subjects' overall study course to monitor for missed AEs/SAEs. The medical monitor will use the template provided in the appendix.

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.

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. 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". 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 Data Management

The UPenn-based data management software REDCap (http://project-redcap.org/) will be deployed to collect and store study data. REDCap (Research Electronic Data Capture) is a secure web application for building and managing online surveys and databases. Using REDCap's stream-lined process for rapidly developing projects, one may create and design projects using 1) the online method from your web browser using the Online Designer; and/or 2) the offline method by constructing a 'data dictionary' template file in Microsoft Excel, which can be later uploaded into REDCap. Both surveys and databases (or a mixture of the two) can be built using these methods. REDCap provides audit trails for tracking data manipulation and user activity, as well as automated export procedures for seamless data downloads to Excel, PDF, and common statistical packages (SPSS, SAS, Stata, R). Also included are a built-in project calendar, a scheduling module, ad hoc reporting tools, and advanced features, such as branching logic, file uploading, and calculated fields.

9.5 Records Retention

Study essential documents will be retained for 7 years after completion of research.

9.6 Data Handling, Record Keeping, and Confidentiality in relation to the Ginger.io smartphone application

Please refer to section 6.8.

10 Study Monitoring, Auditing, and Inspecting

10.1 Study Monitoring Plan

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.).

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 in 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 Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the 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.

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 for a copy of the Subject Informed Consent Form. This consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of a subject, using the 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 departmental funds. Ginger.io will provide all services pertaining to the social sensing technology at no cost to the University of Pennsylvania principal investigators.

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 Payments

Subjects will receive $840. This payment will be prorated as follows: $50 for completing the screening visit; $300 for completion of each of the two 48-hour sessions of combined ambulatory blood pressure monitoring and urine, blood and saliva collection, mouth and fecal swabs, nasal brushings, as well as food photography; $25 for completion of each of the four brief study visits to the CTRC; $30 for completion of each of the three months of daily sleep surveys and incidental entries into the activity diary. In addition, up to $40 may be compensated for costs related to the increased data transfer when using the apps Ginger.io and SmartIntake. Subjects are asked to provide an invoice showing the additional costs.

13 Master License Agreement

With regard to the Ginger.io smartphone app, this research will be conducted with a master license agreement in place between The University of Pennsylvania and Ginger.io.
With regard to the SmartIntake app, this research will be conducted with a master license agreement in place between The University of Pennsylvania and the Pennington Biomedical Research Center.

14 Security and Privacy Information Impact Assessment

For the Ginger.io platform, a security and privacy impact assessment (SPIA) has been conducted by the University of Pennsylvania Information Security Office. The resulting SPIA and the no-revisions letter are provided as copies in the appendix.

For the SmartIntake platform, a security and privacy impact assessment (SPIA) has been conducted by the University of Pennsylvania Information Security Office. The resulting SPIA and the no-revisions notification to the IRB occurred on June 20, 2014 executed by Mr. Michael Moran.

15 Publication Plan

Drs. Garret A. FitzGerald, Carsten Skarke and Aalim Weljie hold jointly the primary responsibility for publication of the results of this study.

16 Trial Registration

This study will be registered in ClinicalTrials.gov.

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18 Appendix

18.1 Study Flow-Chart

Visit No. to CTRC	**SCR**	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**	**V7**	**V8**	**V9**	**V10**	**V11**	**V12**	**V13-Exit**
Month	-1	1	1	2	3	3	3	3	3	3	3	3	4	4
Week		1	4	7	9	10	10	10	11	12	12	12	15	18
Day	Mon-Fri	Wed±2	Wed±2	Wed±2	Wed±2	Tues±1	Wed±1	Thur±1	Wed±2	Tues±1	Wed±1	Thur±1	Wed±2	Mon-Fri
Hours in CTRC	2	1	0.25	0.25	0.5	1.5	1.5	1.5	0.5	1.5	1.5	1.5	0.25	1
Demographics	**X**													
Informed Consent	**X**													
In- / exclusion criteria	**X**													
Compliance[1]	**X**					**X**				**X**				
Medical History / Physical	**X**													X
Routine laboratory screen	**X**													X
Questionnaires (3-day food record; Physical Activity; chronotype)	**X**													
Questionnaires (24-hour dietary recall)						X				X				
Face-to-face interview w/ bionutritionist		**X**												
Taking pictures of everything consumed[2]		**X**				**X**	**X**	**X**		**X**	**X**	**X**		
Actigraphy		**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	
Actigraphy battery change			**X**	**X**		**X**				**X**			**X**	
Set-up & removal of Ginger.io technology		**X**												**X**
Monitoring User Activity		**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	X	
Sleep questionnaire[3]		**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	X	
Ambulatory blood pressure monitoring[4]		**X**[7]				**X**	**X**	**X**		**X**	**X**	**X**		
Sequential urine collections[5]						**X**	**X**	**X**		**X**	**X**	**X**		

Blood and saliva samples[6]		**X**[7]				**X**	**X**	**X**		**X**	**X**	**X**		
Nasal brushings[6]		**X**[7]				**X**	**X**	**X**		**X**	**X**	**X**		
Metabolomics						**X**	**X**	**X**		**X**	**X**	**X**		
Transcriptomics						**X**	**X**	**X**		**X**	**X**	**X**		
Proteomics						**X**	**X**	**X**		**X**	**X**	**X**		
Microbiome analyses (stool, mouth, rectum)						**X**	**X**	**X**		**X**	**X**	**X**		
Genetic analyses	**X**													
Vital Signs	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Assessment AEs/SAEs		**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Activity Diary[8]	**(X)**	**(X)**	**(X)**	**(X)**	**(X)**	**(X)**	**(X)**	**(X)**	**(X)**	**(X)**	**(X)**	**(X)**	**(X)**	**(X)**

Footnotes:

1) Urine pregnancy, urine drug and urine cotinine test.

2) SmartIntake App on smartphone: Capturing picture of everything consumed including food, beverages and snacks starts shortly before the 48 hour period of ABPM

3) Sleep questionnaire consists of the single question: “How many hours did you sleep last night?" which will be administered through the smartphone app on a daily basis. Alternatively, if the Ginger.io technology can provide this, the exact hours of in-bed and out-of-bed will be surveyed. Options to realize this on the smartphone include for example a free text entry or a dial.

4) 9 am as target start time.

5) Sequential 3-hour urine collection intervals during waking hours plus a 6-hour collection interval during sleeping hours, i.e. 0-3, 3-6, 6-9, 9-12, 12-15, 15-21, 21-24, 24-27, 27-30, 30-33, 33-36, 36-39, 39-45, and 45-48. Start of urine collections coincides with start of the 48 hour period of ABPM; corresponding 24-hour clock urine collection intervals are 09:00-12:00; 12:00-15:00; 15:00-18:00; 18:00-21:00; 21:00-24:00; 24:00-6:00; 06:00-09:00. Subject will be familiarized with ABPM measurements and equipment for $\geq$ 2 hours during the screening visit.

6) Blood and saliva samples in exactly 12-hour ‘anti-phase’ intervals at time points 0, 12, 24, 36, and 48 after the start of urine collections and ABPM recordings.

7) Test-run of procedures to assess how participants tolerate ambulatory blood pressure monitoring and specimen collection via swabs and brushes.

8) Subjects will be asked to keep an activity diary during study enrollment to record major changes in the daily activity patterns, e.g. starting to train for a marathon, or a change in jobs which alters the ratio of active/non-active time periods.

18.2 Blood sample Schedule

Human Circadian Rhythmicity	**SCR**	**V1** morning	**V2** morning	**V3** morning	**V3** evening	**V4** morning	**V4** evening	**V5** morning	**V6** morning	**V7** morning	**V7** evening	**V8** morning	**V8** evening	**V9** morning	**V10-Exit** morning
Week		1	9	10	10	10	10	10	11	12	12	12	12	12	17
Day	Mon-Fri	Wed± 2	Wed± 2	Tues± 1	Tues± 1	Wed± 1	Wed± 1	Thur±1	Wed± 2	Tues± 1	Tues± 1	Wed± 1	Tues± 1	Thur±1	Mon-Fri
Laboratory Screens															
CBC, EDTA-Plasma, 1x 4.0 mL Purple Top Tube	4														4
Chemistry, Serum, 1x 6.0 mL Gold Top Tube	6														6
Platelet Aggregation, Na-Citrate, 1 x 4.5 mL Blue Top Tube	4.5			4.5						4.5					
Metabolomic & Proteomics Analyses															
Metabolomics, Na-Heparin-Plasma, 2x 6.0 mL Green Top Tube				12	12	12	12	12		12	12	12	12	12	
Metabolomics, Serum, 2x 6.0 mL Red Top Tube				12	12	12	12	12		12	12	12	12	12	
Clinical Labs															
EDTA-Plasma, 2x8.0 mL Lavender Top Tube						16						16			
Serum, 1x 6.0 mL Red Top Tube				6	6	6	6	6		6	6	6	6	6	
Serum, 2x 6.0 mL Red Top Tube						12						12			
Transcriptomic/genetic Analyses															
Transcriptomics, PAXgene Blood RNA Tubes, 2x 2.5 mL				5	5	5	5	5		5	5	5	5	5	
Genetic analyses, Buffy Coat, EDTA, 1x 4.0 mL Purple Top Tube	4														
Subtotals [ml] per subject	**18.5**	**0**	**0**	**39.5**	**35**	**63**	**35**	**35**	**0**	**39.5**	**35**	**63**	**35**	**35**	**10**
Total [ml] per subject	**443.5**														
Blood [mL] allocated for repeat blood draws only if necessary due to e.g. processing error	**36**														
Maximum volume of blood draws [mL]	**479.5**														

18.3 Hour intervals versus 24-hour clock

Day #	1 or 3	1 or 3	1 or 3	1 or 3	1 or 3	1 or 3	1 or 3
Daytime vs Nighttime	Daytime	Daytime	Daytime	Daytime	Daytime	Nighttime	Daytime
24-hour clock	09:00-12:00	12:00-15:00	15:00-18:00	18:00-21:00	21:00-24:00	24:00-06:00	06:00-09:00
Hour Interval	0-3	3-6	6-9	9-12	12-15	15-21	21-24

Day #	2 or 4	2 or 4	2 or 4	2 or 4	2 or 4	2 or 4	2 or 4
Daytime vs Nighttime	Daytime	Daytime	Daytime	Daytime	Daytime	Nighttime	Daytime
24-hour clock	09:00-12:00	12:00-15:00	15:00-18:00	18:00-21:00	21:00-24:00	24:00-06:00	06:00-09:00
Hour Interval	24-27	27-30	30-33	33-36	36-39	39-45	45-48

18.4 Health Questionnaire

Health Questionnaire

Personal Information:

Name:_____ Home phone:_____

Address:_____ Work phone: (optional):_____

City, State, Zip:_____ Email:_____

Occupation:_____ Fax #: _____

Contact Person:_____ Phone:_____

Physicians to Receive Letters:

1) Primary MD :_____ Address:_____

_____

Phone:_____ Fax #:_____

2) Other MD:_____Address:_____

Specialty: _____ _____

Phone:_____ Fax #: _____

Demographics

Gender: M / F In terms of ethnicity, do you consider yourself Hispanic or Latino? Yes/No

Date of Birth:_____

Age:_____

Height:_____

Weight:_____

In terms of race, do you consider yourself?

1-American Indian/Alaska Native Yes / No

2-Asian Yes / No

3-Black or African American Yes / No

4-Native Hawaiian/Pacific Islander Yes / No

4-White Yes / No

5-other_____

Heart Risk Assessment:

1. Do you currently smoke cigarettes/cigars/pipe? Yes / No

If yes, how may per day or week? Number: _____ Per: day / week (circle)

If no, have you ever smoked? Yes / No

For how many years did you/have you smoked? _____

2. Do you drink alcohol? Yes / No

If yes, How much do you drink?

a) >1 drink per day

b) 1 drink per day

c) <1 drink per day but > 1 drink per week

d) < 1 drink per week

If yes, what kind of alcohol beverages do you drink?

Please describe: _____

_____

_____

_____

_____

_____

3. How often do you exercise?

a) every day

(for example walking, running, biking, swimming or gym work-out)

b) three times a week

c) once a week

d) once a month or less

e) not at all

Past Medical History

1. Do you have or did you ever have High Blood Pressure?
   Yes / No / Don't know Year of Diagnosis:_____
2. Do you have or did you ever have High Cholesterol?
   Yes / No / Don't know Year of Diagnosis:_____
3. Do you have diabetes?
   Yes/No/Don't know Year of Diagnosis: _____
4. Have you been diagnosed with rheumatoid or osteoarthritis?
   Yes / No / Don't know Year of Diagnosis:_____
5. Have you been diagnosed with hepatitis or any other liver disease? Yes / No / Don't know
   Diagnosis:_____ Year of Diagnosis: _____
6. Have you been diagnosed with HIV / AIDS or any other infectious disease? Yes / No / Don't know
   Diagnosis:_____ Year of Diagnosis:_____
7. Have you ever been diagnosed with cancer of any type (including skin cancer)?
   Yes / No / Don't know
   Diagnosis:_____ Year of Diagnosis: _____
8. If Female: Postmenopausal?
   Yes / No / Don't know Year of last menstrual period:_____
   Both ovaries surgically removed?
   Yes / No / Don't know Year removed:_____
   Are you taking hormone replacement therapy?
   Yes / No How many years?_____
   Did you ever take hormone replacement therapy?
   Yes / No How many years?_____

Family History:

Do you have a family history of heart attack or stroke? Yes No Don't know

		Age at time of heart attack or stroke?	Current Age?	Alive?
Biological mother	Yes / No / Don't know	_____	_____	Yes / No / Don't know
Biological father	Yes / No / Don't know	_____	_____	Yes / No / Don't know
Biological brothers	1) Yes / No / Don't know	_____	_____	Yes / No / Don't know
or half-brothers	2) Yes / No / Don't know	_____	_____	Yes / No / Don't know
	3) Yes / No / Don't know	_____	_____	Yes / No / Don't know
	4) Yes / No / Don't know	_____	_____	Yes / No / Don't know
Biological sisters	1) Yes / No / Don't know	_____	_____	Yes / No / Don't know
or half-sisters	2) Yes / No / Don't know	_____	_____	Yes / No / Don't know
	3) Yes / No / Don't know	_____	_____	Yes / No / Don't know
	4) Yes / No / Don't know	_____	_____	Yes / No / Don't know

*if >8 siblings, list on back of this sheet

Cardiovascular History

1. Have your doctors ever diagnosed you with:

heart disease Yes / No / Don't know

What?_____

heart attack Yes / No / Don't know
angioplasty or stent of a heart (coronary) artery Yes / No / Don't know
heart bypass surgery Yes / No / Don't know
stroke Yes / No / Don't know
ministroke or TIA Yes / No / Don't know
neck (carotid) artery blockage/narrowing/bypass surgery Yes / No / Don't know

blocked/narrowed arteries in your legs Yes / No / Don't know
bypass surgery of your leg arteries Yes / No / Don't know
angioplasty/stent in arteries in your legs Yes / No / Don't know

2. a) Has your doctor ever told you that you have angina? Yes / No / Don't know
b) Do you have a history of chest pain/discomfort brought on by exertion and relieved by rest? Yes / No / Don't know

c) Do you have a history of chest pain relieved by medication? Yes / No / Don't know
What medication ? 1- nitroglycerine
2- other -name_____

3. Have you ever had;

a) Heart stress test? Yes / No

Was the result abnormal? Yes / No / Don't know

b) Have you ever had a cardiac catheterization? Yes / No

Was the result abnormal? Yes / No / Don't know

8) Have you ever had any surgical procedures? Yes/No

If yes, what type: Date/Year:

_____

_____

_____

_____

Medications and dietary supplements you are currently taking:

Medication or Dietary Supplement:	**Dose, How Often, and Start Date:**

Other Past Medical History Not Covered:

Condition Year of diagnosis

Diet

1. Do you follow a specific diet? Yes / No
   a) Ovo-lacto vegetarian (you do eat eggs, milk and cheese)? Yes / No
   b) Vegan (you do not eat any animal products)? Yes / No
   c) other Yes / No If yes, please describe the diet?

18.5 Flyer for subject recruitment

ITMAT
Institute for Translational Medicine and Therapeutics

Doctors at the Institute for Translational Medicine and Therapeutics, University of Pennsylvania are studying the patterns of social behavior (recorded by cell phone use), blood pressure and metabolites in urine.

or

"Exploratory study to define circadian relationships between social behavior, blood pressure and metabolomics signatures."

If you are a healthy, non-smoking and non-pregnant individual between 18 – 65 years of age and own a smartphone, 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**

Circadian Research Study 215.662.4652

18.6 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

18.7 Template Medical Monitoring

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: _____

18.8 Sample subject Diary Recall Form

To The Patient

The portable automatic blood pressure unit that you are wearing measures and records your blood pressure and heart rate at predetermined intervals. Be sure that the monitor is comfortably positioned before you leave the office, then go about your normal daily activities. The heart rate data should be used for reference only, not as clinical diagnostic data.

To obtain maximum information from the monitor, it is important that you record in this diary the initial TIME when the cuff inflates, the ACTIVITY in which you are involved when the reading is taken and the time at which you take your MEDICATIONS. It is unnecessary to list redundant activities and the time of day when the cuff inflates; just list the activities when they change (i.e., work to leisure activity). In addition, if symptoms occur between readings, push the START/STOP button on the monitor and an additional reading will be taken. Readings will then continue on a normal cycle. Be sure to describe the symptoms in the diary. Should you wish to abort a reading in progress, simply push the START/STOP button.

Important Notes

DO	once the tone sounds, avoid unnecessary movement while the blood pressure reading is being recorded. Try to keep your arm still.
DO NOT	remove the blood pressure monitor from the carrying case.
DO NOT	flip the ON/OFF switch as this will turn the blood pressure monitor off. Turn the machine off only in emergencies.
DO NOT	get the monitor wet; however, should the monitor get wet, there is no electrical shock hazard from the monitor.
DO NOT	worry if the blood pressure monitor cannot take a blood pressure reading every time it cycles. If it can't take a reading, remain still and the monitor will make one more attempt to measure your blood pressure in 60 seconds. (If a reading cannot be recorded, an event code will appear on the display.)

Spacelabs Medical

An OSI Systems Company

AMBULATORY BLOOD PRESSURE MONITOR

PATIENT DIARY

Patient Name _____

Address _____

City _____ *State* _____

Physician _____

Phone Number _____

Age _____ *Height* _____

Weight _____ *Sex* _____

Medication(s) _____

_____

_____

_____

000-0027-02 Rev. F

Log Information

TIME RECORDING STARTED _____

TIME RECORDING COMPLETED _____

TIME INTERVAL

00:00-06:00 Hrs. _____ Min.

06:00-12:00 Hrs. _____ Min.

12:00-18:00 Hrs. _____ Min.

18:00-24:00 Hrs. _____ Min.

DISPLAY ON/OFF _____

ALARM ON/OFF _____

MONITOR # _____

Afternoon NOON to 6 PM

TIME	ACTIVITY/ MEDICATION	COMMENTS/ SYMPTOMS

Morning 6 AM to NOON

TIME	ACTIVITY/ MEDICATION	COMMENTS/ SYMPTOMS

Evening 6 PM to MIDNIGHT

TIME	ACTIVITY/ MEDICATION	COMMENTS/ SYMPTOMS

000-0027-02 Rev. F

18.9 Munich ChronoType Questionnaire (MCTQ)

Munich ChronoType Questionnaire (MCTQ)

Please enter your age, gender, etc.. This information is important for our evaluations

Age: _____ **female** **male** **Height** _____ **Weight** _____

On work days ...

I have to get up at... _____o'clock
I need... _____min to wake up
I regularly wake up... before the alarm with the alarm
From... _____o'clock I am fully awake
At around... _____o'clock, I have an energy dip
On nights before workdays, I go to bed at _____o'clock...
... and it then takes me... _____min to fall asleep
If I get the chance, I like to take a siesta/nap ...
correct I then sleep for..._____ min
not correct I would feel terrible afterwards

On free days (please only judge normal free days, i.e., without parties etc.) ...

My dream would be to sleep until... _____o'clock
I normally wake up at... _____o'clock
If I wake up at around the normal (workday) alarm time, I try to get back to sleep...
correct not correct
if I get back to sleep, I sleep for another... _____min
I need ... _____min to wake up
From... _____o'clock I am fully awake
At around... _____o'clock, I have an energy dip
On nights before free days, I go to bed at... _____o'clock...
... and it then takes me... _____min to fall asleep
If I get the chance, I like to take a siesta/nap ...
correct I then sleep for..._____ min
not correct I would feel terrible afterwards

---

once I am in bed, I would like to read for ... _____ min, ...
... but generally fall asleep after no more than ... _____ min.

I prefer to sleep in a completely dark room correct not correct
I wake up more easily when morning light shines into my room correct not correct

---

How long per day do you spend on average outside (really outside) exposed to day light?
On work days: ___ hrs. ___min. On free days: ___ hrs. ___min.

Self assessment

After you have answered the preceding questions, you should have a feeling to which chronotype (time-of-day-type) you belong to. If for example, you like (and manage) to sleep quite a bit longer on free days than on workdays, or if you cannot get out of bed on Monday mornings, even without a Sunday-night-party, then you are more a late type. If, however, you regularly wake up and feel perky once you jump out of bed, and if you would rather go to bed early than to an evening concert then you are an early type. In the following questions, you should categorise yourself and your family members.

Please tick only one possibility!

Description of categories:

extreme	early type = 0
moderate	early type = 1
slight	early type = 2
	normal type = 3
slight	late type = 4
moderate	late type = 5
extreme	late type = 6

I am...	0	1	2	3	4	5	6
as a child, I was ...	0	1	2	3	4	5	6
as a teenager, I was ...	0	1	2	3	4	5	6
In case you are older than 65: in the middle of my life, I was ...	0	1	2	3	4	5	6
My parents are/were...							
Mother ...	0	1	2	3	4	5	6
Father ...	0	1	2	3	4	5	6
My siblings are/were ... (please underline Brother or Sister)							
Brother/Sister	0	1	2	3	4	5	6
Brother/Sister	0	1	2	3	4	5	6
Brother/Sister	0	1	2	3	4	5	6
Brother/Sister	0	1	2	3	4	5	6
Brother/Sister	0	1	2	3	4	5	6
Brother/Sister	0	1	2	3	4	5	6
Brother/Sister	0	1	2	3	4	5	6
My partner (girl/boy friend, spouse, significant other) is/was ...	0	1	2	3	4	5	6

18.10 No-Revisions Letter – Security and Privacy Impact Assessment

Bob DeSilets
Information Security Officer
Penn Medicine Academic Computing Services
University of Pennsylvania
desilets@mail.med.upenn.edu
(215)746-5578

Ms. Meghan Blair
IRB Administrator
IRB #3
University of Pennsylvania
Office of Regulatory Affairs
3624 Market St., Suite 301 S
Philadelphia, P A 19104-6006
Ph: 215-573-2540/ Fax: 215-573-9438

PRINCIPAL INVESTIGATORS:	Garret A Fitzgerald, MD & Carsten Skarke, MD
TITLE:	Exploratory Phase I Study In Healthy Volunteers To Define Circadian Relationships Between Social Behavior, Blood Pressure And Metabolomic Signatures.
PROTOCOL#:	817759
REVIEW BOARD:	IRB #3

University of Pennsylvania, Perelman School of Medicine Information Security Office: Protocol Review

June 19, 2013

Dear Ms. Meghan Blair,

I have conducted a Security and Privacy Impact Assessment (SPIA) review of the Ginger.Io platform that has been proposed for use in clinical study protocol #817759. I am not requesting any revisions to their procedures.

The SPIA review is attached to this document for your reference.

Best regards,

Robert L. DeSilets, Jr.
Perelman School of Medicine Information Security Officer
University of Pennsylvania

18.11 Security and Privacy Information Impact Assessment (SPIA)

SPIA for Vendors

Summary:

Ginger.io Overview
The vendor, Ginger.io, provides technology to harvest and integrate user data from smartphones to generate behavioral features about its users. Smartphones represent a rich source for user activity data. These social sensing data can be mined to identify patterns of social behavior. Changes therein enable researchers to make inferences about the health status of individuals, e.g. the decrease of social interactions during acute flu outbreaks. We wish to test in a small pilot study in healthy volunteers whether this social sensing technology can be implemented to explore relationships between circadian patterns of user activity, continuous blood pressure and metabolic signatures in urine samples. The social sensing technology might provide insight how to integrate quantitatively social behavior into exploring cardiovascular and metabolic functions influenced by the molecular clock.
Temporal storage on participants' smartphones using the mobile application 'Ginger.io' installed; Ginger.io Behavior Platform which refers to the mobile application (or app) and the web dashboard. The Web Dashboard refers to a web---based study management system that allows investigators and study staff to monitor the data collection process (including passive data collection and survey completion), identify issues and analyze the data.
The data collected through the device includes data such as call and sms logs (including call/sms time, call duration, sms length, and phone number), location and device usage. Private information such as actual content of voice calls or sms messages or emails is never read, recorded or transmitted.
The data described above is encrypted and transmitted to the server over a secure 128-bit SSL 3.0 connection using the HTTPS protocol. Extra care is taken to ensure that the request cannot be spoofed or imitated by an attacker through the use of unique keys that are generated dynamically for every API request through a unique identifier and client secret that is embedded inside the application and cannot be accessed by any user or application.
The data is stored securely on the mobile device temporarily. At regular intervals, the application checks for an available connection to the WWW. If such a connection is available, the data is transmitted over a secure connection to the server and deleted from the device. If a connection is not available or if a transmission is not successful, the data continues to be stored on the device until it is transmitted successfully. Data is recorded in 10 min intervals and stored on the phone. Ginger.io attempts to upload every hour.
Ginger.io linux---based servers are protected using a firewall and access control lists (ACLs), and access is restricted to Ginger.io employees and contractors. Superuser activity and researcher activity on the server is logged for security and auditing. The servers are regularly updated and patched with latest security updates to ensure that there are no known threats. These servers host the database where the data is stored. Linux---based servers and access to the servers is restricted to a few users responsible for maintaining and testing the database. For additional security, all the data that has personally identifiable information about the participant (such as e-mail) is stored in a separate database from the one that has the data collected from the users and data related to the studies. The passively collected data from the phones as well as the actively-reported survey information is stored in the second database. Phone numbers and other such private information stored in this database are anonymized by hashing over the identifiers so that there is no threat to privacy because the data is not human-readable anymore.

For Vendor:

1. Do you have a SAS 70 Type II certification or other third party certification of your information security controls? How recently was the review performed? How regularly are reviews performed? Can we get a copy?

   We have not undergone certification by any third-party certification groups. However, we've successfully gone through rigorous security reviews with several major healthcare

organizations, and we regularly review our own security practices to ensure that they remain up-to-date.

2. Do you have any certifications for any compliance frameworks such as FISMA, HIPAA, PCI, etc.?

   Ginger.io technology, security and privacy policies comply with HIPAA standards - such as encryption (SSL), system-user identifiers (login, passwords), high-end server security, frequent backups, strong privacy policies, and strong internal business and employee policies. All patient personal and health information that may be included in the Ginger.io database will be treated in compliance with all applicable laws and regulations. Additional security and privacy safeguards can be enabled upon request of the client.

3. Please describe controls to address the threat of information being compromised by an external hacker or malicious software.

   Your response should refer where applicable to safeguards such as intrusion detection, anti-virus, firewalls, vulnerability scanning, penetration testing, encryption, authentication and authorization protections and policies, including those involving passwords, removal of unnecessary network services, limiting of administrative access, code review, logging, employee training and other relevant safeguards.

   General:

   Ginger.io has a standard Corporate Information Security Policy (CISP) and can provide details if needed. In particular, Section 14 of our CISP outlines the different steps that occur as part of developing new features. Our change management protocol includes the following steps:

   - ***Customer requirements generation***
   - ***Feature-level specification***
   - ***User-experience mocks and review***
   - ***Code development***
   - ***Pre-commit code reviews***
   - ***Testing, including automated and manual***
   - ***Deployment to a staging environment***
   - ***Full release***

   The Ginger.io application has been penetration-tested by partners as part of security reviews by other partners.
   Ginger.io's servers are protected using TripWire as an IDS.
   For additional security, all the data that has personally identifiable information about the participant (such as e-mail) is stored in a separate database from the one that has the data collected from the users and data related to the studies. The passively collected data from the phones as well as the actively-reported survey information is stored in the second database. Phone numbers and other such private information stored in this database are anonymized by hashing over the identifiers so that there is no threat to privacy because the data is not human-readable anymore.

   ***Passwords*:**

   The rules to create passwords are as follows:A minimum of 8 characters and two complexity cases are required. This is automatically enforced by the system. There are four possible complexity cases: small letters [a-z], capital letters [A-Z], numerals[0-9] and special characters[e.g. -,#!]

   Currently, our system does not allow password policy changes on a per study basis. We do not currently lock accounts, but all failed logins are logged in our system in order to identify any malicious activity.

Mobile Device Security:

The data is stored securely on the mobile device temporarily. At regular intervals, the application checks for an available connection to the WWW. If such a connection is available, the data is transmitted over a secure connection to the server and deleted from the device.

Data security on the phone is handled at the OS level. At a high level, the data is encrypted at rest automatically, and each app is sandboxed from other apps in order to prevent unauthorized access. Once the data is successfully transmitted to the server, it is wiped from the device.

4. Please describe controls to address the threat of information being intercepted in transit by unauthorized persons.

   Your response should refer where applicable to safeguards such as encryption during transmission, encrypting wireless traffic, physically securing devices in transit, network traffic segregation, and other relevant safeguards, and include descriptions of encryption protocols and algorithms used.
   Researchers and study staff have access to the web dashboard through an account on the site. Information pertaining to a study is only available to researchers and study staff associated with that study. Participants or researchers not involved in the study cannot access the data through the dashboard. Data accessed through the dashboard is also transmitted through 128-bit SSL 3.0 connection using the HTTPS protocol. HTTP access is disabled.

5. Please describe controls to address the threat of information being mistakenly disclosed to unauthorized persons.

   Your response should refer where applicable to issues of awareness and training, removal of unnecessary data (electronic and paper), use of screen savers and lockouts, limiting storage of confidential data on remote devices, verification of identity of individuals requesting access, and other relevant safeguards that enforce “need to know”.
   Access to the web dashboard is granted to individual participants by researchers. They are provided an option to use the study participants’ name or a coded identifier as is suitable for their study protocol. The coded identifier (or name) is used to refer to the participant on the dashboard pages and in communication with the participants.

6. Please describe controls to address the threat of information knowingly being misused by your workforce and contractors.

   Your responses should refer where applicable to issues of strong sanctions policy and practice, background checks, role-based access to information, oversight of data authorization by supervisor, terminating access to data for terminated employees and employees changing job functions, prohibition on sharing passwords, and other relevant safeguards.
   Section 4 of our CISP details employee responsibilities with respect to privacy of patient data. As part of on-boarding, the importance of this is stressed. Any violation of this policy is treated very seriously.

7. Please describe controls to address the threat of physical theft or loss of data.

   Your responses should refer where applicable to policies on the storage of confidential data on laptops, PDAs, USB drives and other portable devices, encryption of data on portable devices,

two factor authentication, removal of unnecessary information, physical protection of desktops and servers, and other relevant safeguards.

Data is stored on our servers which are hosted by third-party vendor Linode. Linode takes a number of steps to ensure physical security: Security at each facility is made up of several layers. There is either a manned checkpoint or keycard access to get into the building itself (in some places, even the parking lot). Once inside the building, there are several layers of physical security before actually reaching computer equipment, including biometric fingerprint, handprint, retina scan, key card, and PIN numbers. All equipment is in locked cages, so it requires an additional key, thumbprint, or keycard to get into the locked cage.

8. Please describe controls to address community concerns regarding privacy practices.

Your responses should refer where applicable to privacy statements, opt-in or opt-out consents, compliance with applicable privacy rules, and other relevant safeguards.
Our goal at Ginger.io is to provide a platform for patients, health care providers, and researchers to collect and analyze data to better understand patients' behavior in order to improve their health. We take our users' privacy very seriously. Our philosophy rests on a few key principles of data ownership:

1. ***Users own their data. They opt-in to the system, have control over the use of any data that is generated, and can request to have their data removed from the system.***

2. ***If data is shared, it is done in an aggregated and anonymous fashion. We are working with leading enterprise partners and academic researchers to explore ways of leveraging aggregate anonymous data to improve patient health. We respect the privacy and anonymity of our users and never share specific individual data unless explicitly asked by our users to do so or otherwise required by law.***

3. ***Users should get value in exchange for sharing their data.***

We have seen a great willingness amongst users to share their data to collectively advance the state of health care for themselves and for others. We are committed to continue working closely with the health and data communities to promote these innovations. For a complete copy of the Ginger.io privacy policy please download it from our website (http://ginger.io/).
The data collected through the device includes data such as call and sms logs (including call/sms time, call duration, sms length, and phone number), location and device usage. Private information such as actual content of voice calls or sms messages or emails is never read, recorded or transmitted.

9. Please describe controls to address the use, handling, protection and sharing of confidential data shared with subcontractors.

Your responses should state any relevant relationships that may induce additional risk to the safe storage of sensitive data (such as outsourcing of key services, use of sub-contractors or cloud services for hosting, etc.) and refer where applicable to contractual safeguards and reviews of security programs / practices.
Ginger.io has subcontracted Linode for data storage; please see safety specifications described in paragraph 7.

10. Please describe controls to address threats to the availability of data based on inadequate business continuity procedures.

Your responses should refer to business continuity and disaster recovery plans and procedures, regular testing, routine data backups and offsite storage.
Rotating backups are implemented weekly, monthly and daily through full server snapshots. Such precautionary measures make us more resistant to hardware/software failures as well as human errors by enabling us to revert to a previous state in case of any issues and ensuring that we do not lose any important data.

18.12 Surveys delivered through the smartphone app Ginger.io

18.12.1 Ginger.io Demographic Questionnaire (only at download)

Gender
- Male
- Female

Age
- 18-24
- 25-34
- 35-44
- 45-54
- 55-64
- 64+

Ethnicity
- Hispanic or Latino
- Not Hispanic or Latino

Race
- American Indian/ Alaska Native
- Asian
- Native Hawaiian or Other Pacific Islander
- Black or African American
- White

Highest degree or level of school you completed
- Primary School
- High School
- Associate degree
- Bachelor's Degree
- Advanced Degree (ex. Master's, Professional, Doctorate)

Marital Status
- Single, Never Married
- Married
- Separated
- Divorced
- Widowed

18.12.2 Ginger.io Sleep Questionnaire (daily in the morning)

"How many hours did you sleep last night?"
Alternatively, if the Ginger.io technology can provide this, the exact hours of in-bed and out-of-bed will be surveyed. Options to realize this on the smartphone include for example a free text entry or a dial.

18.12.3 Ginger.io App Satisfaction Questionnaire (only at completion)

Choices to respond to questions below are:
- 1 Strongly disagree
- 2
- 3 Neutral

4
5 Strongly agree

Question #1: Ginger.io surveys are easy to complete?
Question #2: Completing a survey every day is not burdensome?
Question #3: Overall, the Ginger.io experience is what I expected?
Question #4: What motivates you to complete the Ginger.io surveys?
Question #5: Please share any comments for your answers above or additional feedback: