TUFTS MEDICAL CENTER/TUFTS UNIVERSITY RESEARCH PROTOCOL

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Study Title: Role of the gut microbiome and the serum metabolome on lean mass and physical function in older adults

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Study Doctor/Co-Investigator:	Christine Liu, M.D.
Study Coordinators:	Brittany Barrett Sarah White

I. Research Plan

40 older adults (70-85y) will be recruited from the local Boston area based on their performance on the short physical performance battery (SPPB), a measure of lower extremity physical function that includes standing balance, a 4-m walk and chair stand tests[1]. The SPPB has been extensively validated as predictive of future disability risk and mortality in older adults[1]. High-functioning (HF; SPPB ≥ 11) and low-functioning (LF; SPPB ≤8)[2] groups will each contain 20 subjects (10 men, 10 women). Gut microbial composition is affected by age[3, 4]. Similarly, sex and BMI are associated with gut microbiome composition[5-7]. With the goal of minimizing gut microbial variability, I propose to recruit HF and LF subjects that are matched for age, sex and BMI but are different in terms of lean mass and physical function.

At baseline, subjects will provide a blood and stool sample that will be subjected to serum metabolomics and fecal sample 16S ribosomal RNA (16S rRNA) gene profiling, respectively. Serum metabolomics provides an analytical description of complex biological media by identifying a large number of small molecule metabolites in a given biological sample[8]. Fecal sample 16S rRNA gene profiling will be used to identify the bacterial relative abundance that is present within each taxonomic level in each fecal sample. Lean mass and physical function will be measured in all subjects. Associations between serum metabolites, fecal bacteria, and between fecal bacteria and serum metabolites with the differentiation of HF from LF, and that are associated with lean mass and specific measures of physical function will be determined. In addition, associations between gut bacteria with circulating markers of insulin resistance and endotoxemia will be identified. Overlapping associations between gut bacteria and serum metabolites with these outcomes is expected to provide an enhanced understanding about the role of gut bacteria on mechanisms that underlie the maintenance of lean mass and physical function in older adults. The causative role of gut bacteria on the maintenance of lean mass and physical function in older adults will be examined by transplanting fecal bacteria from older adults into germ-free mice.

After 1 month, to determine the reproducibility of the baseline gut bacterial associations with lean mass and physical function, study subjects will return to provide a fecal sample that will be subjected to 16S rRNA profiling. Lean mass and physical function will be measured, and associations between fecal bacteria that collectively differentiate HF from LF, and that are associated with lean mass and specific measures of physical function will be identified and compared with baseline findings.

Statistical power: Sample size estimates are based on gut bacterial associations with the GFI[9]. Means ± SD for *Bacteroides*/*Prevotella* of 26.3 ± 13.2 and 14.8 ± 15.1 in the low-frailty and high-frailty groups, respectively, yield an effect size of 0.81, a value that is sufficient to obtain 80% power at a type-I error probability of 0.05 with a study sample size of 40 subjects. To achieve this recruitment goal, up to150 subjects will sign the Preadmission ICF, with the goal of 40 participants completing the study.

Blood and Stool Collection: Following an overnight, 12-hour fast (beginning between 8PM-12AM the day before), eligible candidates will provide a blood sample during the initial visit (baseline) and 1-month study visits. At the screening visit, eligible subjects will be provided with a stool collection kit so that they may provide a stool sample within the 18-hour period that precedes their baseline and 1-month visits. Fasting is optional for providing the stool sample.

Measurement of insulin, glucose, lipopolysaccharide (LPS) and physical function: Serum measurement of insulin and glucose are used to calculate the homeostasis model assessment (HOMA-IR), a circulating index of insulin resistance[10]. LPS is a component of gram-negative bacterial cell walls that can appear in the blood by microbial translocation from the gastrointestinal tract[11], is a circulating marker of metabolic endotoxemia[12]. LPS induces insulin resistance[12], myotube atrophy and decreased muscle function *in vitro*[13, 14], and decreases muscle mass in rodents[15]. Insulin, glucose and LPS will be measured by the Nutrition Evaluation Laboratory (NEL) at the Jean Mayer HNRCA at Tufts University.

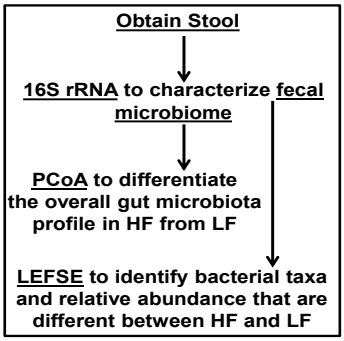
Measures of physical function including the SPPB, leg press one repetition maximum (1 RM) and 400-m will be performed by the NEPS laboratory at the Jean Mayer HNRCA at Tufts University[16]. The importance of measuring the SPPB, the leg press 1 RM and 400-m is illustrated by the finding that reduced performance in each of these measures is associated with increased disability[1, 17, 18] and an increased all-cause mortality risk[1, 18, 19].

Measurement of Dietary Intake: To account for the influence of dietary intake on the gut microbiome, three 24-hour dietary recalls will be collected from enrolled study subjects. Two of these recalls will be collected on the two days prior to the baseline visit. The final dietary recall will be performed on the day of the baseline visit, for a total of three 24-hour dietary recalls.

AIM 1: Identify associations between gut bacteria and circulating metabolites that collectively differentiate HF from LF, and are associated with lean mass and physical function in older adults. Dr. Lustgarten recently reported significant associations between circulating gut bacteria-related metabolites[20-22] with lean and skeletal muscle mass[23] and with measures of physical function[16], evidence that suggests a role for gut bacteria on the maintenance of these outcomes in older adults. To date, studies aimed at identification of associations between gut bacteria and circulating metabolites with lean mass have yet to be published, whereas two studies have reported associations between gut bacteria with function[9, 24]. However, the indices used to measure function were not specific for physical function, as questions about vision, hearing, cognition and psychosocial function were included[25-27]. Therefore, the objective of **AIM 1** is to characterize the association between fecal bacteria and circulating metabolites that collectively differentiate HF from LF, and that are associated with lean mass and physical function in older adults.

Identification of bacterial taxa present in fecal samples: Fecal sample 16S rRNA gene profiling will be used to identify the fecal bacteria relative abundance that is present within each taxonomic level. The Phoenix Laboratory at the Tufts University Medical Center[28] will extract fecal sample DNA, generate 16S rRNA amplicons, and assemble amplicon pools for high-throughput sequencing. The Tufts University Genomics Core Facility[29] will then use an Illumina MiSeq System (Illumina, San Diego, CA) to target the V4 region of the 16S rRNA gene. 16S rRNA sequences will be quality filtered, clustered into Operational Taxonomic Unit (OTUs) and further analyzed using QIIME (quantitative insights into microbial ecology)[30]. Use of 16S rRNA gene profiling allows for the determination of bacterial taxa other than *E. coli* and *Enterobacteriaceae* that may be different in HF when compared with LF older adults. For example, various bacterial species other than *E. coli* are capable of BCAA synthesis[31]. Furthermore, the BCAA catabolic metabolites 2-oxoisovalerate and 4-methyl-2-oxopentanoate are negatively associated with colonic *Lactobacillus spp*. abundance[21].

Bacterial taxa that differentiate HF from LF older adults: Principal co-ordinate analysis (PCoA) of the phylogeny based Unifrac distance metric[32], which are derived from OTUs[33] will be used to determine if the overall gut microbial profile in HF is significantly different when compared with LF older adults. The analysis of similarities function in the statistical software package PRIMER 6 (PRIMER-E Ltd., Lutton, UK[34]) will be used to determine between-group statistical significance ($p \leq 0.05$). To identify bacterial taxa and relative abundance that are significantly different in HF when compared with LF older adults, linear discriminant analysis effect size (LEFSE)[35] will be used. LEFSE is an algorithm for high-dimensional biomarker discovery and explanation that identifies bacterial taxa that characterize the difference between two or more biological conditions.



Test the causative role of human gut bacteria on the maintenance of lean mass and physical function: Although identification of gut bacteria that are associated with lean mass and physical function is an important observation, transplanting intact uncultured human fecal microbiota into recipient germ-free mice permits the donors' communities to be replicated and the causative role of their microbiota on outcome measures to be

discerned. For example, transplantation of human gut microbiota from 4 female twins discordant for kwashiorkor (the condition caused by severe dietary protein deficiency) into germ-free mice resulted in 86-90% of species-level taxa and ~90% of the functions encoded by the donor's microbiome being found in recipient mice, thereby implicating the gut microbiota as a causal factor on kwashiorkor[36].

Transplantation of gut microbiota from a healthy human adult into germ-free mice resulted in all bacterial phyla, 92% of bacterial classes and 88% of genus-level taxa being detected in the feces of recipient germ-free mice[37]. Human gut bacteria-containing mice fed a high-fat western diet gained significantly more adiposity during the two weeks following transplantation than mice that consumed a low-fat, plant polysaccharide-rich diet[37], evidence that demonstrates a causative role for diet-induced increases in adiposity by human gut bacteria. Similarly, transplantation of human gut microbiota from 3 twins (2 female and 1 male) discordant for obesity into germ-free mice resulted in ~75% of family-level bacterial taxa and 99.7% of the functions encoded by the donor's microbiome being represented in recipient mice, thereby resulting in a greater increase in adiposity in mice colonized with fecal microbiota from obese, when compared with its lean co-twin[38].

Collectively, these studies demonstrate that transfer of gut bacteria from humans into germ-free mice is highly efficient, functions encoded by the transferred microbiome are maintained, and phenotypes are transmissible. The objective of **AIM 2** is to test the causative role of human gut bacteria on the maintenance of lean mass and physical function by transferring fecal microbiota from HF and LF older adults into germ-free mice.

Statistical Power: Germ-free mice colonized with fecal bacteria from obese humans gained significantly more adiposity than germ-free mice colonized with fecal bacteria from their lean co-twin (10.0% ± 5.0% compared with 0.1% ± 2.0%), a result that was significant 15 days after colonization[38]. Based on these data, the resulting effect size of 2.6 is sufficient to obtain 99% power at a type-I error probability of 0.05 with a sample size of 12 human subjects. Accordingly, with the goal of identifying a similar change in lean mass and physical function, I propose to transfer fecal bacteria from 12 older adults into germ-free mice.

III.2.a Colonization of germ-free mice with human gut bacteria from HF and LF older adults: Fecal samples from 6 HF (3 males, 3 females) and 6 LF (3 males, 3 females) older adults will be transferred into sex-matched (with the goal of minimizing sex-associated gut microbial variability[6, 7]) C57BL/6J germ-free mice for a duration of 5 weeks at the Germ-Free and Gnotobiotic Microbiology Core at the Harvard Digestive Disease Center (HDDC; Boston, MA). The transmissible effect of host phenotype by gut microbiota has been previously reported by transferring human fecal bacteria into C57BL/6J germ-free mice[36-38]. Furthermore, the homogenous genetic background provided by inbred C57BL/6J germ-free mice allows for a cleaner system to dissect signals from gut bacteria-host interactions, and improves experimental reproducibility, when compared with genetically heterogeneous GFM[39]. One fecal sample from each human donor will be transferred into 5 separate germ-free mice (based on the range of germ-free mice used in human fecal transfer experiments[36, 38]), resulting in 60 mice colonized with human gut microbiota. 5 germ-free mice will not be colonized with fecal bacteria from older adults (germ-free control group). All recipient mice will be fed *ad libitum* with a commercial, sterilized mouse chow[38]. Food consumption will be measured daily to ensure that changes in lean mass or physical function do not result because of differences in food intake.

III.2.b Measurement and analysis of the change in lean mass and physical function in mice colonized with human gut bacteria: Lean mass and physical function in mice colonized with human gut bacteria will be measured on day 0 (prior to colonization), 9, 18, 27 and 35 days post-colonization at the HDDC. Lean mass will be measured with use of quantitative magnetic resonance imaging (EchoMRI-3in1 instrument, Houston, TX)[38]. Muscle strength will be measured using a grip strength meter for mice (Technical and Scientific Equipment GmbH, Bad Homburg, Germany)[40]. Each mouse will firmly grab the pulling bar of the grip strength meter with both forepaws and then pulled gently backwards until it releases its grip. The peak force of each trial is considered the grip strength. A 1-liter beaker filled with water will be used as a swimming pool to test endurance capacity[41].

A weight (10% of body weight) will be attached to the tail of a mouse, which is then placed in the water. The amount of time that the mouse is able to maintain complete buoyancy will be recorded. The EchoMRI, grip strength meter and beaker/weight will be sterilized prior to use to ensure that germ-free conditions at the HDDC are maintained. Because Dr. Lustgarten has previously measured lean mass and physical function in mice[42, 43], he will perform these measurements at the HDDC.

Differences for the change in lean mass and physical function will be calculated relative to initial values. Statistical significance for the change in lean mass and physical function will be compared in mice colonized with fecal bacteria from HF or LF human donors and for germ-free mice that were not colonized with human gut bacteria with use of two-way ANOVA[38].

III.2.c Bacterial taxa identification and association with the change in lean mass and physical function in mice colonized with human gut bacteria: Fecal pellets will be obtained from 5 germ-free mice at baseline, which will not be colonized with human gut bacteria. Five weeks post-colonization with human gut bacteria, fecal pellets will be collected from 60 mice (30 each from the HF and LF donors) and from the 5 germ-free mice that did not receive human gut bacteria. 16S rRNA gene profiling on these 70 fecal pellets will be performed as described in **Section III.1.a**.

Associations between gut bacteria-containing principal components and individual bacterial taxa with the 35-day change in lean mass, mouse grip strength and endurance capacity will be identified as described in **Section III.1.b**. Supervised machine learning (random forest) will be used to predict whether a sample came from a mouse colonized with HF or LF donor microbiota[44].

The gut bacterial profile that is associated with the change in lean mass and physical function in mice colonized with HF or LF human fecal bacteria is expected to be similar to the gut bacterial profile that is associated with differentiation of HF from LF older adults. CIA will be used to identify associations between circulating metabolites with gut bacteria that collectively differentiate mice colonized with HF or LF gut bacteria.

A. Subject Characteristics

1) Subject eligibility criteria:

a) Inclusion criteria:

i) Willing and able to sign the IRB approved informed consent form
ii) Male and Female: males, 70-85y; females, 70-80y
iii) BMI: males < 35, females <30 kg/m^2
iv) Willing to come to the HNRCA laboratory for baseline and 1-month follow-up study visits
v) SPPB ≥ 11 ("High-Functioning", HF; 20 subjects: 10 males, 10 females)
vi) 4 ≤ SPPB ≤ 8 ("Low-Functioning", LF; 20 subjects: 10 males, 10 females)

b) Exclusion Criteria:

i) Non-English speaker
ii) Acute or terminal illness
iii) Surgery in the past 6 months
iv) Lower extremity fracture within the past 6 months
v) Myocardial infarction in the past 6 months
vi) Coronary artery disease, peripheral vascular disease, previous stroke, or history of transient ischemic attacks
vii) Cognitive impairment (MMSE score < 23)
viii) Uncontrolled hypertension (> 160/100 mmHg)
ix) Neuromuscular disease or drugs affecting neuromuscular function
x) Androgen therapy in males
xi) Estrogen therapy in females

xii) Significant immune disorder
xiii) Kidney Failure
xiv) Pancreatic disease
xv) Diabetes
xvi) Gastrointestinal or malabsorption diseases
xvii) History of cholecystectomy
xviii) Use of probiotics, prebiotics or antibiotics in the past 3 months
xix) The subject has any other condition, which in the opinion of the Investigator, precludes the subject's participation in the trial.
xx) With the goal of matching the HF and LF groups for age, sex,and BMI, subjects may be excluded because their age, sex or BMI puts them outside the range needed for this study.

c) **Withdrawal/Termination criteria:** Subjects may stop participating in this study at any time for any reason. If an enrolled subject decides to withdraw from this research study, they will be asked to inform the Principal Investigator (Dr. Lustgarten), the Lab Director (Dr. Roger Fielding), or his representative. The principal investigator may stop participation in this research study without the subject's permission for any of the following reasons:
- The participant did not adhere to any or all HNRCA volunteer rules and regulations
- Participant does not follow the study procedures
- There has been a change in the subject's health that would make the subject ineligible

B. Risk/benefit assessment:
Following an overnight 12-hour fast (beginning between 8PM-12AM the day before), study subjects will provide a blood and stool sample, and will have their lean mass measured. Subjects will then be provided breakfast. Study subjects will complete two 24-hour dietary recalls on the two days prior to the baseline visit, with a third dietary recall completed during breakfast of the baseline visit. Following breakfast, subjects will perform physical function testing. Prior to the baseline and 1-month study visits, all subjects will be instructed to refrain from any strenuous exercise or physical activity for 48 hours prior to the start of the study protocol.

1) **Physical risk**:
a. Fasting: After an overnight fast, you will be asked to provide a blood sample. Fasting is optional for the provision of the stool sample. Risks associated with overnight fasting may include fainting and dizziness.

b. Blood draw: There may be a slight discomfort during blood drawing and there is the possibility of a small bruise forming at the puncture site. There is also the remote possibility of a superficial inflammation (phlebitis) of the vein.

c. Stool collection: There are no risks to this procedure.

d. Measurement of Dietary Intake: There are no risks to this procedure.

e. Memory test (MMSE): You may become frustrated from the memory test.

f. Measurement of lean mass: Total body lean mass will be measured by dual energy X-ray absorptiometry (DXA). Each DXA scan causes a whole-body exposure that is equivalent to 1.5 days of natural background radiation exposure. This is generally regarded as safe.

g. Physical function testing: The risk of injury from supervised physical function testing is very rare. Subjects will have direct supervision during all activities. There is the possibility of transient muscle discomfort resulting from the 1RM testing. There is also a small chance that the participant may have

a heartbeat irregularity, symptoms of chest pain, or abnormality of their blood pressure during testing and exercise sessions. Detailed emergency procedures have been submitted with this protocol and briefly explained in section E.2 under "Adequacy of Protection Against Risk". The participant will be assured that they may stop the session at any time, at which point the physical function assessment will be terminated.

a. **Psychological risk**: N/A

b. **Social risk**: N/A

c. **Economic risk**: N/A

d. **Benefits of participating in the study:** There are no direct benefits to the volunteers of this study. However, they may be helping others benefit in the future from a potentially improved understanding of the role of gut bacteria on mechanisms that may underlie the maintenance of lean mass and physical function in older adults.

C. Specific methods and techniques used throughout the study:

1. **Study Procedures**:

i) *Health status*: Each subject will complete a screening history review and medical history questionnaire during study screening.

ii) *Cognitive function*: Cognitive function will be assessed using the mini–mental state examination (MMSE)[45] during the screening period. If your MMSE score is less than 23, the principal investigator (Dr. Lustgarten) will inform you that you are not eligible for the study. It is then recommended that you discuss your MMSE score with your primary care physician.

iii) *Anthropometric Data:* Height and weight will be measured at the preadmission screening visit, and at baseline and 1-month follow-up visits. Height will be measured in duplicate to the nearest 0.1 cm using a stadiometer. Body weight will be measured using a calibrated digital scale to the nearest 0.1 kg.

iv) *Blood draw*: At baseline and 1-month follow-up study visits, fasting blood samples (40mL) will be obtained from all subjects. Serum will be derived from whole blood via centrifugation, and frozen in 250 uL aliquots until used for serum metabolomics, and for measurement of circulating markers of insulin resistance (glucose, insulin) and metabolic endotoxemia (LPS). Serum metabolomics will be performed by the Emory University Metabolomics Core. A 20 mL portion of each sample will also be saved for future exploratory analyses, which may include additional metabolomic profiling. Consent for tissue storage will be obtained through a separate consent form. Subjects who initially consent to tissue banking but later decide to withdraw their blood samples from storage and future use may sign and return a section of the consent form pertaining to this or verbally notify the Principal Investigator or study coordinator at any time. For subjects that decline participation in the optional tissue banking process, a total of 20 mL of blood will be collected.

v) *Stool Collection*: Stool samples will be obtained at the baseline and 1-month study visits. Stool samples will be collected (see Supplementary documents) and stored as specified in the Manual of Procedures by the Human Microbiome Project[46]. Freshly obtained stool samples will be transported to the Phoenix Laboratory at Tufts Medical Center, where the stool samples will be homogenized, aliquotted into ten 200mg lots into pyrogen-free Eppendorf tubes, and frozen at -80°C. Five additional 200 mg aliquots will also be saved for future exploratory analyses, which may include additional gut bacterial profiling. Consent for tissue storage will be obtained through a separate consent form. Subjects who initially consent to tissue banking but later decide to withdraw their stool samples from

storage and future use may sign and return a section of the consent form pertaining to this or verbally notify the Principal Investigator or study coordinator at any time.

vi) *DXA:* Total body lean mass will be measured at baseline and 1-month follow-up visits using DXA (Discovery A, Hologic Inc., Waltham, MA) at the Boston Nutrition Obesity Research Center Body Composition Unit at Tufts University. The DXA system generates photons at two principal energy levels (40 and 70 KeV) which allow measurement of bone mineral and soft tissue. Lower extremity skeletal muscle mass will be estimated using the DEXA-SM model developed by Wang *et al*. (1999)[47]. The reliability of this method for estimating regional skeletal muscle mass is high (C.V. < 4%).

vii) *Measurement of Dietary Intake:* If you are enrolled into the study you will complete 3 record-assisted, 24-hour dietary recalls. A member of the Tufts University Dietary Assessment Unit will provide instructions and a log, and will ask you to write down everything you eat and drink over the 24-hr period during the 2 days prior to your baseline visit. The final dietary recall will be completed in-person at the baseline visit, for a total of three 24-hour dietary recalls. You will be given a two-dimensional measuring aid (Food Amounts Booklet) to assist with estimating portion size. A member of the Dietary Assessment Unit will complete dietary recalls by phone with you over the two days prior, and including on the day of your baseline visit.

viii) *Short Physical Performance Battery* (SPPB): Study subjects will have their SPPB measured at the screening visit, and at baseline and 1-month study visits by a NEPS lab study coordinator (*i.e.* Brittany Barrett). The SPPB test consists of timed standing balance, gait speed, and timed chair-rise assessments[1]. Performance for each of these tasks is scored between 0 and 4, with a summary score of 0–12.

ix) *Four hundred-meter(m) walk* (400-m): Study subjects will have their 400-m measured at baseline and 1-month study visits by a NEPS lab study coordinator (*i.e.* Brittany Barrett). Study participants will walk 10 laps of a 20-m course at their usual pace. Rest intervals will be permitted while standing, for up to 60 seconds. Gait speed will be calculated from the time and distance completed for each participant.

h) *Lower extremity 1RM muscle strength (LP 1 RM)*: Study subjects will have their lower extremity 1RM muscle strength measured (K400, Keiser Sports Health Equipment Inc., Fresno, CA) at baseline and 1-month study visits by a NEPS lab study coordinator (*i.e.* Brittany Barrett). The 1RM is defined as the maximum load that can be moved one time only throughout the full range of motion (ROM) while maintaining proper form[48]. The examiner will progressively increase the resistance for each repetition until the subject can no longer move the lever arm one time through the full ROM[49]. To account for the strong correlation between the LP 1 RM with total lean mass, the LP 1 RM will be divided by total lean mass (LP/Lean) to determine muscle quality[50].

2. **Subject Timeline**:
Screening Visit (***approximately 1 hour***):
- Screening History Questionnaire
- Medical History Questionnaire
- Preadmission Informed Consent
- Measurement of height, weight
- MMSE
- Assessment of inclusion/exclusion criteria
- SPPB, to enroll subjects to HF (SPPB ≥11) or LF (SPPB ≤ 8) groups

Baseline Visit (***approximately 3 hours***):

- Study Informed Consent
- HNRCA Building Behavior form
- Tissue Banking Informed Consent
- Measurement of height, weight
- Vital Signs
- Blood draw
- Stool Sample
- DXA
- In-person dietary assessment
- SPPB
- 400-m
- LP 1 RM

1-month follow-up visit (***approximately 3 hours***)***:***

- Measurement of height, weight
- Vital Signs
- Blood draw
- Stool Sample
- In-person dietary assessment
- DXA
- SPPB
- 400-m
- LP 1 RM

This schedule is subject to change in order to accommodate unforeseen delays or complications. For example, participants require a longer rest break when determining their 1RM. If this happens, 1RM assessment may take longer than 45 minutes.

D. Assessment of Subject Safety and Data and Safety Monitoring Plan

1. SAEs are defined as:
    - Death
    - A life-threatening adverse experience
    - Inpatient hospitalization or prolongation of existing hospitalization
    - A persistent or significant disability/incapacity or a congenital anomaly/birth defect
    - Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious adverse experiences if they might jeopardize the participant or might require medical or surgical intervention to prevent one of the outcomes in the definition

An *Unanticipated Problem* is an incident, experience, or outcome that meets all of the following criteria:

- The nature, severity, or frequency is unexpected for the subject population or research activities as described in the current IRB approved protocol, supporting documents, and the ICF(s)
- It is related or possibly related to participation in the research
- It suggests the research may place the subject or others at a greater risk of harm then was previously recognized

An AE is defined as any untoward or unfavorable medical occurrence in a human subject, including any abnormal physical exam or laboratory finding, symptom, or disease, temporally associated with a subject's participation in the research. Examples of AEs that will be summarized and reported at the time of continuing review include, but are not limited to: anxiety, fatigue, decreased appetite, insomnia, dizziness, muscle or joint stiffness, muscle strain or soreness, ankle or knee pain, foot pain, and other minor symptoms that may have restricted the participant's usual activities for at least ½ day like flu or allergy problems.

2. Reporting timeframe for SAEs and AEs: Unanticipated Problems, SAEs, and AEs will be reported to the Principal Investigator and the study physician. Unanticipated problems will be reported to the IRB within 5 business days. Any SAE classified as "not related" will be reported to the IRB within 15 business days. Clinically significant AEs classified as "not related" will be summarized and submitted to the IRB at the time of continuing review or study closure if this occurs before the next continuing review.

3. The study physician will act as the medical monitor throughout this pilot study. Due to the limited sample size and minimally invasive procedures that will be utilized during this investigation, a data safety monitoring board will not be used for this pilot study.

4. The Principal Investigator (Dr. Lustgarten) in consultation with the study physician will classify the relationship of the SAE/AE to the study protocol as follows:

- *Not Related*: The event is clearly related to factors such as the subject's clinical state, not to interventions associated with the study protocol

- *Remote*: The event is most likely related to factors such as the subject's clinical state, not to interventions associated with the study protocol

- *Possible*: The event follows a reasonable temporal sequence from interventions associated with the study protocol, but is possibly related to such factors as the subject's clinical state

- *Probable*: The event follows a reasonable temporal sequence from interventions associated with the study protocol and cannot be reasonably explained by factors such as the subject's clinical state

- *Highly Probable*: The event follows a reasonable sequence from interventions associated with the study protocol and cannot be reasonably explained by factors such as the subject's clinical state

Potential AEs for study related activities and interventions will be explained to each participant by trained study personnel during the informed consent process. Each participant will be instructed to report the occurrence of an AE or SAE at scheduled data collection times. Participants also have access to study personnel at other times to report SAEs or concerns about the safety of participating in this study.

Participation in this study is completely voluntary. Participants may elect to withdraw from this investigation at any time without penalty or coercion, or without any requirement that they provide an explanation of their decision to withdraw.

The Principal Investigator reserves the right to cease an individual's participation in this study for any reason, including but not limited to: participant not following study procedures, or a change in the participant's health.

At the discretion of the study physician, stopping rules for subject participation in this pilot study will be if any SAE is determined to be "probable" or "highly probable", as related to the study participation.

The Principal Investigator will submit to the IRB any updated information that may affect the conduct of this study or subject safety, rights, welfare or willingness to take part in the research.

E. Subject Participation

1) **Recruitment and consent process**: Subjects who are in the Recruitment Database for the Nutrition, Exercise Physiology, and Sarcopenia Laboratory (IRB#11603) will be utilized to recruit for this study. Subjects that meet the age inclusion criteria will be sent a direct mailing letter and contacted by telephone. We will also contact previous research subjects who have given permission to be contacted regarding new studies. Subjects will be recruited as part of health promotion program of municipals, day-care service units, and university. The participants will be recruited through local advertisements, flyers, and recommendation by care-givers of social intuitions. Participants will be either free-living or assisted living elderly. Our laboratory has been successful in recruiting older adults for a large number of research studies using similar enrollment criteria and screening procedures. No individuals will be excluded from participation in the study due to racial or ethnic origin. Employees of Tufts University and the HNRCA will not be specifically recruited. However, employees who respond to general advertisements may be enrolled, as long as they are not employees under the supervision of the investigators. The Principal Investigator or research assistant will interview the subjects initially over the phone using a prescreening telephone questionnaire. If a potential subject meets the inclusion criteria and is interested in participating, he/she will be invited to our laboratory for an initial evaluation. A preadmission screening informed consent will be obtained prior to an initial physical exam. Study staff will interview the subject, review the study, including inclusion/exclusion criteria. If the individual is eligible to participate they will informed by telephone, and will be asked to meet with the Principal Investigator (Dr. Lustgarten) or Study Coordinator (Brittany Barrett) at the initial study visit to review and sign the study informed consent document. Subjects will be given as much time as they require to review all study documents and to ask questions about the study. The subject will then be asked to summarize the study back to the Study Coordinator to confirm their understanding. Subjects will be reminded that they do not have to sign the consent form or take part in the study and can leave the study anytime, even after signing the consent form. Subjects will designate their consent through signing an IRB approved study consent form. NEPS laboratory study coordinators have been trained in the procedures needed to obtain informed consent and to perform study assessments by the Principal Investigator. The Study Coordinators have extensive experience in a variety of studies in older adults in obtaining informed consent and performing the study procedures that are proposed in the current project.

1) **Registration**: This study will not be registered on clinicaltrials.gov, as this study design is not required to be posted.

2) **Transportation**: Transportation will not be provided by the HNRCA for this study. However, we will encourage our participants to use public transportation.

3) **Non-English speaking persons**: Non-English speaking subjects will be excluded from this investigation, as this study has a limited sample size and funding. This study requires participants to follow precise instructions. This will not enable the investigation to support non-English speaking participants without compromising the integrity of the study or the safety of non-English speaking subjects.

4) Location where study will be performed:
   USDA HNRCA
   711 Washington St.
   Boston, MA 02111

   Body Composition DXA Lab at Tufts University
   136 Harrison Avenue

Boston, MA 02111

5) **Personnel who will conduct the study, including**:
   i) Present during study procedures and their proximity during the study:
   Principal Investigator: Michael S. Lustgarten, PhD

   ii) Obtaining informed consent:
   Principal Investigator: Michael S. Lustgarten, PhD
   Coordinators: Brittany Barrett, Sarah White. NEPS lab coordinators have completed CITI-certification training, and have extensive experience with obtaining subject's informed consent by their participation in a number of NEPS lab studies.

   iii) Providing on-going information to the study sponsor and the IRB:
   Principal Investigator: Michael S. Lustgarten, PhD
   iv) Maintaining participant's research records:
   Principal Investigator: Michael S. Lustgarten, PhD
   Coordinators: Brittany Barrett, Sarah White

ii. **Subject fees**: None

iii. **Confidentiality**: Medical information produced by this study will not become part of the subject's medical record, unless they request it to be. Every effort will be made to maintain the confidentiality of your research records for this study by the investigators. The information will be stored in the investigator's file and identified by a code number only. Information contained in subjects' research records will not be given to anyone unaffiliated with the HNRCA in a form that could identify them, without their written consent or as specified by law. Subjects have the option for their private physician to receive their test results from the current study. If subjects do not wish for their private physican to receive their results, subjects will inform the principal investigator for this study, Dr. Lustgarten.

It is possible that their medical research record, including sensitive information or identifying information, may be inspected and/or copied by the study monitor and sponsor, federal or state government agencies such as the Office of Human Research Protection, or hospital accrediting agencies, in the course of carrying out their duties. If their record is inspected or copied by the study sponsor or by any of these agencies, the HNRCA will use reasonable efforts to protect their privacy and the confidentiality of their medical information.

Data are used only in aggregate and no identifying characteristics of individuals are published or presented. Results of testing are sent to participant's private physicians if participants agree to this. The participant will have a research record with the HNRCA. Every effort will be made to maintain the confidentiality of their research records for the study by the investigators

i. **How data will be coded, recorded, and stored to protect confidentiality**: Confidentiality of data is maintained by using research identification numbers that uniquely identify each individual. Safeguards are established to ensure the security and privacy of participants' study records. The information collected from participants in this study has a low potential for abuse, since the data do not address sensitive issues. Nevertheless, appropriate measures are taken to prevent unauthorized use of study information. The research ID number is used. The research records are kept in a locked room at the HNRCA. The files matching participants' names and demographic information with research ID numbers are kept in a separate room and are stored in a locked file that uses a different key from that of all other files. Only study personnel have access to these files. After the study is completed, local

data are stored with other completed research studies in a secured storage vault for a minimum of 7 years after the study has been closed out by the IRB.

iii) **Parties who will have access to the data, including the key to the identity code**: The Principal Investigator and research team will have access to study data and the identity key code.

iv) **Parties who will have access to research records**: The Principal Investigator, research team, and applicable HNRCA staff will have access to research records.

9. **Alternatives**: The alternative to participating in this research study is to decide not to participate. The decision to not participate will not affect the way the subject will be treated at Tufts Medical Center or Tufts University.

10. **How new information will be conveyed to the study subject and how it will be documented**: Any information collected during this investigation that may impact the subject's health will be communicated to the participant by the study physician. Documentation will be made in the participant's file and this information will be reported to the IRB, as necessary.

    Any new information about the study that might affect subjects' willingness to continue to participate in the research study will be provided to subjects as either a letter or updated informed consent form.

11. **Payment, including a prorated plan for payment**: All potential subjects who attend the screening visit, whether or not they choose to participate or are ineligible will receive a screening payment of $15. Payments will be mailed by check approximately two weeks after your visit.

    Subjects that complete the baseline but not 1-month visit will be paid $75. Subjects that complete the 1-month visit will receive an additional $75, for a total stipend of $150. Including the screening visit, participants can receive a total of $165.

Table 2. Payments

Stipend	**Per Visit**	**Number of Visits**	**Compensation**
Screen	$ 15.00	1	$ 15.00
Study Visit	$ 10.00	2	$ 20.00
Blood Draw	$ 10.00	2	$ 20.00
Fecal Sample	$ 10.00	2	$ 20.00
DXA	$ 20.00	2	$ 40.00
Physical Function	$ 25.00	2	$ 50.00
Total			**$165**

Participants will receive payment three times throughout this investigation. Participants will be paid following the screening visit, and following the baseline and 1-month study visits.

12. **Payment for a research-related injury**: Emergency medical treatment will be given to participants if they are hurt or get sick as a direct result of this study. However, the study volunteer is still responsible for payment. The study volunteer or their insurance carrier will have to pay for any such medical care. Any needed medical care is available at the usual cost. All needed facilities, emergency treatment and professional services are available to the subject, just as they are to the general public. There are no plans to pay for treatment if a study volunteer gets hurt or sick because of this study. The institution has not set aside any money to pay for a research-related injury or illness.

v) **Tissue banking considerations**: All of the information collected during the course of this study will remain in a secure location. The coded vials will be analyzed at the HNRCA and the information will be sent to the Principal Investigator (Dr. Lustgarten). The samples may be retained for unspecified future use; however, they will not be used for genetic testing.

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MRU STUDY # 2875 IRB approval date: August 22, 2017

JEAN MAYER USDA HUMAN NUTRITION RESEARCH CENTER ON AGING
TUFTS UNIVERSITY

INFORMED CONSENT to PARTICIPATE in RESEARCH

Study Title: Role of the gut microbiome and the serum metabolome on lean mass and physical function in older adults

Principal Investigator: Michael S. Lustgarten, Ph.D.

Co-Investigator: Roger A. Fielding, Ph.D.
Anne Kane, Ph.D.

Study Doctor/Co-Investigator: Christine Liu, M.D.

Study Coordinators: Karan Bhatia
Brittany Barrett

Study Telephone Number: (617) 556-3399

INTRODUCTION

You are being invited to take part in this research study because based on your preadmission screening you meet the study entry eligibility criteria set by the study investigators.

Taking part in this research study is totally your choice. You can decide to stop taking part in this research study at any time for any reason. If you stop being in this research study, it will not affect how you are treated at Tufts Medical Center at Tufts University or the Jean Mayer USDA Human Nutrition Research Center on Aging (HNRCA) at Tufts University.

Please read all of the following information carefully. Ask Michael Lustgarten, Ph.D., or his representative, to explain any words, terms, or sections that are unclear to you. You should also ask any questions that you have about this research study. Do not sign this consent form unless you understand the information in it and have had your questions answered to your satisfaction. Your questions will be answered in words, or if you prefer, in writing. You should talk about this research study and the information in this informed consent form with whomever you want before you sign it.

If you decide to take part in this research study, you will be asked to sign this form. You will be given a copy of the signed form. You should keep your copy for your records. It has important information, including names and telephone numbers, to which you may wish to refer in the future.

New things might be learned during this study that you should know about. The investigators will tell you about new information that may affect your willingness to stay in this study.

As a participant in this study, your identity, medical records, and data relating to this study will be kept confidential, except as required by law. The principal investigator, Dr. Lustgarten may also look at records that identify you if applicable to the study.

If you have question about your rights as a research study subject, call the Tufts Medical Center and Tufts University Health Sciences Institutional Review Board (IRB) at (617) 636-7512. The IRB is a group of doctors, nurses, and non-medical people who review human research studies for safety and protection of people who take part in the studies. Federal law requires the IRB to review and approve any research study involving humans. This must be done before the study can begin. The study is also reviewed by the IRB on a regular basis while it is in progress.

This research study has been reviewed and approved by the IRB at Tufts Medical Center and Tufts University Health Sciences.

PURPOSE OF STUDY

This research study will examine the role of intestinal bacteria on muscle mass and physical function in older adults. This study will help us better understand if gut bacteria are involved, and if they are, how gut bacteria may affect muscle function. Older adults that are different in terms of how much lean mass and in their physical function are specifically recruited for this study.

Results from this study are intended as the basis for future studies aimed at alteration of intestinal bacterial composition, an approach that may be a means for improving muscle mass and physical function in older adults.

Up to 150 subjects will be enrolled in this study at the HNRCA. This study is supported by a grant from the Jean Mayer Human Nutrition Research Center on Aging (HNRCA) at Tufts University.

PROCEDURES TO BE FOLLOWED/STUDY ASSESSMENTS

Height and weight will be measured, blood will be drawn, stool collected, your dietary intake will be recorded, and total body lean mass and physical function will be measured in eligible participants at the HNRCA's Nutrition, Exercise Physiology, and Sarcopenia (NEPS) Laboratory. These assessments will be performed at the baseline and 1-month follow-up visits. The actual procedures are explained below:

Anthropometric Data: Height will be measured in duplicate to the nearest 0.1 cm using a stadiometer. Body weight will be measured using a calibrated digital scale to the nearest 0.1 kg.

Blood Draw

To perform the blood draw, you will be asked to not eat or drink anything (except water) on the previous evening between 8-12 PM onwards. At baseline and 1-month follow-up study visits, fasting blood samples (40mL) will be obtained. Serum will be derived from whole blood and frozen until used to measure: the amount and different types of chemicals present in your blood, markers of your ability to process blood sugar (glucose, insulin), and a marker of bacteria that is present in your blood, LPS). A 20 mL portion of each 40 mL sample will be saved for future exploratory analyses, which may include additional metabolite and bacterial analyses. A separate consent form

about sample storage will ask you permission to allow us to keep some of your blood to perform potential future analyses. You do not have to sign the separate consent for tissue banking to participate in this main study. The amount of blood drawn at each blood collection will equal approximately 3 tablespoons. If you decline to have your blood samples stored for potential future use, a total of 20 mL of blood will be collected.

Stool Collection

A stool collection kit (see Supplementary documents) will be provided to you, should you qualify, after the screening visit. You may provide a stool sample during the 18-hour period that precedes the baseline and 1-month follow-up study visits. If you are unable to provide a stool sample during that 18-hour period, stool will be collected upon your attendance to the HNRCA during the baseline and 1-month study visits. After following the directions for providing the stool sample, you will return it to the NEPS lab as soon as possible, where it will then be processed to achieve the study goals. The amount and different types of bacteria present in your samples will be determined and associated with your measures of lean mass and physical function. With the goal of determining the impact of your intestinal bacteria on muscle mass and physical function, a portion of your stool sample will be transplanted into mice that do not have any intestinal bacteria (germ-free mice). A separate consent form about sample storage will ask you permission to allow us to keep some of your feces to perform potential future analyses. You do not have to sign this separate consent for tissue banking to participate in this main study.

Please note that you may choose to have blood and stool samples taken but not stored for future unspecified tests.

Measurement of Dietary Intake

If you are enrolled into the study you will complete three 24-hour dietary recalls. A member of the Tufts University Dietary Assessment Unit will provide instructions and a log, and will ask you to write down everything you eat and drink over a 24-hr period during the 2 days prior to your baseline visit. The final dietary recall will be completed in-person at the baseline visit. You will be given a two-dimensional measuring aid (Food Amounts Booklet) to assist with estimating portion size. A member of the Dietary Assessment Unit will

complete dietary recalls by phone with you over the two days prior, and including on the day of your baseline visit.

Lean mass measurement
Total body lean mass will be measured at baseline and 1-month follow-up visits using DXA (Discovery A, Hologic Inc., Waltham, MA) at the Boston Nutrition Obesity Research Center Body Composition Unit at Tufts University. Each DXA scan causes a whole-body exposure that is equivalent to 1.5 days of natural background radiation exposure. This is generally regarded as safe. You cannot eat or drink before this assessment. It will be taken early in the morning. You will be asked to not eat or drink anything (except water) the previous evening from 8 PM onwards.

Physical Function Testing
You will be assessed and supervised with a trained, NEPS laboratory exercise physiologist from the research team. This person will ensure that the proper form and degree of difficulty are maintained.

1. *Short Physical Performance Battery* (SPPB): The SPPB test consists of timed standing balance, measurement of your speed in completing a timed walk, and timed chair-rise assessments. Study subjects will have their SPPB measured at baseline and 1-month study visits by a trained exercise physiologist (*i.e.* NEPS lab study coordinators. These coordinators have extensive experience in conducting these tests in older adults.

2. *Four hundred-meter (m) walk* (400-m): Study subjects will have their 400-m measured at baseline and 1-month study visits by a NEPS lab study coordinator. Study participants will walk 10 laps of a 40-m course at their usual pace. Rest intervals will be permitted while standing, for up to 60 seconds. Gait speed will be calculated from the time and distance completed for each participant.

3. *Lower extremity 1RM muscle strength (LP 1 RM)*: Study subjects will have their lower extremity 1RM muscle strength measured (K400, Keiser Sports Health Equipment Inc., Fresno, CA) at baseline and 1-month study visits by a trained exercise physiologist (*i.e.* NEPS lab study coordinators). The 1RM is defined as the maximum load that can be moved one time only

throughout the full range of motion (ROM) while maintaining proper form. This test will determine the heaviest weight you can lift through a full range of motion in good form. The examiner will progressively increase the resistance for each repetition until you can no longer move the lever arm one time through the full ROM. To help us determine your 1 RM we will use a numbered subjective rating scale that will tell us how hard or heavy the weight feels to you.

You will warm-up on a stationary bicycle for 5 to 10 minutes. The test will be started with a light 8 repetition warm-ups with your legs, after which a resistance (weight) will be chosen that is estimated to be just below your 1 RM strength. You will then lift the weight 1 time. If you are able to lift the set amount of weight through the full range of motion using proper form and technique, more weight will be added and another attempt will be made after a rest of about 15 -120 seconds. This process will continue until you can no longer lift the weight. The highest weight lifted will be recorded as your 1 RM value. Your 1 RM will be assessed during the first week of the study, and it will take about 30-45 minutes to complete. However, since you will also be given the research study consent form at this visit, please allow a total of 3 hours for this visit.

You cannot start any new exercise or rehabilitation program during the study.

TIMELINE OF STUDY PROCEDURES

You will be asked to undergo measurement of lean mass and physical function testing assessments. These assessments will take place at the baseline and 1-month study visits at the NEPS laboratory. Your study participation will include only these 2 visits.

Before the baseline and 1-month study visits, please refrain from any strenuous exercise or physical activity for 48 hours before the start of the study protocol.

Baseline Visit (3 hours):

On the day before the baseline visit, the study coordinator will call you to remind you not to eat anything for 12 hours before your study visit on the following day.

- Review and complete consent form
- Review and complete the HNRCA Building Behavior form
- Measure height, weight, and vital signs
- Fasting blood sample collection
- Stool sample collection
- Measurement of lean mass by DXA

In-person dietary assessment (performed during breakfast). Note: Dietary recalls will be additionally be recorded by you on each of the 2 days prior to this in-person assessment. Instructions for completing these dietary recalls are included with this document.

- Breakfast consisting of: a bagel, toast, or English muffin that is topped with cream cheese, jam, or peanut butter, a piece of fruit: apple or banana, and choice of beverage, including coffee, tea, or juice will be provided to you before:
- Physical function testing (SPPB, 400-m, LP 1 RM)

1-month follow-up (Visit 2, 3 hours):
On the day before the 1-month follow-up visit, the study coordinator will call you to remind you not to eat for 12 hours before your study visit on the next day.

- Measure height, weight, and vital signs
- Fasting blood sample collection
- Stool sample collection
- Measurement of lean mass by DXA
- Breakfast consisting of: a bagel, toast, or English muffin that is topped with cream cheese, jam, or peanut butter, a piece of fruit: apple or banana, and choice of beverage, including coffee, tea, or juice will be provided to you before:
- Physical function testing (SPPB, 400-m, LP 1 RM)

This schedule is subject to change in order to accommodate unforeseen delays or complications. For example, you may require a longer rest break when determining your 1RM. If this happens, assessment of your 1RM may take longer than 45 minutes.

POTENTIAL RISKS
Fasting

You will be asked to provide a blood sample after an overnight fast. Fasting is optional for providing the stool sample. Risks associated with overnight fasting may include feeling faint and dizziness.

Blood Draw
There may be a slight discomfort and pain during blood drawing. There is the possibility of a small bruise forming at the puncture site. There is also the remote possibility of swelling of the vein and infection.

I.

II. *Stool collection*

III. There are no risks to this procedure.

IV. Measurement of Dietary Intake

V. There are no risks to this procedure.

VI. *Measurement of lean mass*

VII. Total body lean mass will be measured by dual energy X-ray absorptiometry (DXA). Each DXA scan causes a whole-body exposure that is equivalent to 1.5 days of natural background radiation exposure.

VIII. *Physical function testing*

The risk of injury from supervised physical function testing is very rare. Subjects will have direct supervision during all activities. There is the possibility of transient muscle discomfort resulting from the 1RM testing. There is also a small chance that the participant may have a heartbeat irregularity, symptoms of chest pain, or abnormality of their blood pressure during testing and exercise sessions. The participant will be assured that they may stop the session at any time, at which point the physical function assessment will be terminated. The researchers might also end your exercise session if they believe it is in your best interest.

In the event of an emergency, the researchers will call the study doctor or, for emergencies, will call 911. All research staff performing exercise testing and assessments are trained in CPR. There will be at least two CPR trained, NEPS laboratory exercise physiologists from the research team and/or research staff members in the lab at all times to assist you. A doctor will be available either at the Metabolic Research Unit at the HNRCA or by telephone.

Other Risks
Other risks include anxiety prior to participating in the study, fatigue, decreased appetite, insomnia, dizziness, muscle or joint stiffness, muscle strain or soreness, ankle or knee pain, foot pain that result from the physical function testing, and other minor symptoms that may have restricted the participant's usual activities for at least ½ day like flu or allergy problems.

POTENTIAL BENEFITS
This research study is not designed to benefit you. Results from your blood test and DXA can be shared with your primary care physician, upon your request.

ALTERNATIVES
You have the alternative to not to participate in this research study.

X. WITHDRAWAL AND STUDY TERMINATION
If you decide to withdraw from this research study, you must inform the Principal Investigator, Dr. Lustgarten. The investigator or study sponsor may stop your participation in this research study without your permission for any of the following reasons:

- **You do not adhere to any and all HNRCA volunteer rules and regulations**
- **You do not follow the study procedures**
- **There has been a change in your health**
- **The principal investigator (Dr. Lustgarten) has ended the study**

If you withdraw or are withdrawn from the study, any data collected from you before your withdrawal will still be used for the study.

WHOM TO CONTACT
If you have any problems or questions about the study you may speak with the researchers at any time. Their telephone numbers are:

Principal Investigator: **Michael Lustgarten**, Ph.D.
HNRCA: (617) 556-3399 (Monday thru Friday, 9:00am-5:00pm)
Cell: (646) 600-0124 (Anytime)

Lab Director, Co-Investigator: **Roger Fielding, Ph.D.**
Office: (617) 556-3016 (Monday thru Friday, 9:00am-5:00pm)
Cell: (617) 304-4369 (Anytime)

Study Doctor: **Christine Liu, M.D.**
Office: (617) 556-3074 (Mondays and Tuesdays 9:00am-5:00pm)
Cell: (617) 680-7728 (Anytime)

Study Coordinators: **Brittany Barrett, Karan Bhatia**
HNRCA: (617) 556-3399 (Monday thru Friday, 9:00am-5:00pm)

You may also direct your questions or concerns about your rights as a research subject to the Tufts Medical Center and Tufts University Health Sciences Institutional Review Board (IRB) by calling (617) 636-7512.

RESEARCH RELATED INJURY

Emergency medical treatment will be given to you if you are hurt or get sick as a direct result of being in this research study. You or your insurance carrier will be required to pay for any such medical care. Any needed medical care is available at the usual cost. All needed facilities, emergency treatment, and professional services are available to you, just as they are to the general public. The institution will not pay for your treatment if you become ill or injured as part of this study.

COSTS

There is no cost to you for participation in this study.

PAYMENT

To participate in this study you will need to provide your Social Security number. This is necessary so that we can give you your payment for this study. You will receive a total payment of $165 if you complete the entire study. You will be paid $15 for the screening visit, and $75 each, after completion of the baseline and 1-month study visits. Checks will be mailed and you should receive them in approximately two weeks. In the event that you discontinue your participation in the study, you will be paid an amount proportional to the time you have spent in the study.

CONFIDENTIALITY

Information produced by this study will not become part of your hospital medical record, unless you request it to be. The information will be stored in the investigator's file and identified by a code number only. Information contained in your research records will not be given to anyone unaffiliated with the HNRCA or this study, in a form that could identify you, without your written consent or as specified by law.

It is possible that your research record (and not your medical record) including sensitive information or identifying information may be inspected and/or copied by the Tufts Medical Center and Tufts University Health Sciences IRB, federal or state government agencies such as the Office of Human Research Protection, or hospital accrediting agencies, in the course of carrying out their duties. If your research record is inspected or copied by the principal investigator, Dr. Lustgarten, or by any of these agencies, the HNRCA will use reasonable efforts to protect your privacy and the confidentiality of your research information.

Information collected on you during the course of the study will remain in a secure location at the HNRCA. Most of the information relating to your participation in this study (such as the results from your exercise testing, completed questionnaires, etc.) will be analyzed at the HNRCA. Your data will be de-identified, so that your identity will remain unknown, and to ensure confidentiality.

The results of this study may be published in a medical book or journal or used for teaching purposes. However, your name and any other identifying information will not be used in any publication or teaching materials.

You will have a research record with the HNRCA. Every effort will be made to maintain the confidentiality of your research records for this study by the investigators.

Documentation of Consent

I have been given a copy of this form. I have read it or it has been read to me. I understand the information and have had my questions answered to my satisfaction. I agree to take part in this study.

I understand that I will be informed of any new findings developed during the course of this research study that may affect my willingness to stay in this research study.

_____	_____
Date	Participant's Signature

I have fully explained to _____ the nature and purpose of the above-described study and the risks that are involved in its performance. I have answered all questions to the best of my ability.

_____	_____
Date	Principal Investigator or Representative's Signature