Clinical Trials.GOV: NCT04377126

PROTOCOL TITLE: Unacylated **G**hrelin to **I**mprove **F**unc**T**ioning in PAD: The **GIFT** Trial: Phase II

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STUDY SUMMARY:

Investigational Agent(s) (Drugs or Devices)	Unacylated Ghrelin
IND #	130513
Sample Size	30
Funding Source	National Institute on Aging (NIA)
Indicate the type of consent to be obtained	☒Written ☐Verbal/Waiver of Documentation of Informed Consent ☐Waiver of HIPAA Authorization ☐Waiver/Alteration of Consent Process
Research Related Radiation Exposure	☐Yes ☒ No
DSMB / DMC / IDMC	☒Yes ☐No

OBJECTIVES:
Our work and that of others shows that people with lower extremity peripheral artery disease (PAD) have greater functional impairment, faster functional decline, and higher rates of mobility loss compared to people without PAD (1-8). In people with PAD, ischemia of calf skeletal muscle results in calf muscle injury that includes loss of myofibers and calf muscle mitochondrial dysfunction (9-14). Reduced calf muscle mitochondrial activity and smaller calf muscle size are associated with greater functional impairment and increased mobility loss in people with PAD (14-18). Therapies that regenerate calf skeletal muscle cells, increase mitochondrial activity, and increase calf muscle capillary density may improve functioning and prevent mobility loss in people with PAD. Currently, few medical therapies exist for improving functioning in PAD. Preclinical evidence shows that unacylated ghrelin promotes skeletal muscle regeneration, increases muscle mitochondrial activity, and stimulates angiogenesis in the setting of ischemia (19-24). This pilot study will investigate the therapeutic potential of unacylated ghrelin to improve functional impairment in people with PAD.

Ghrelin is a peptide and hormone that is primarily produced by P/D1 cells of the gastric fundus and circulates in both acylated and unacylated forms. Acylated ghrelin acts on the Growth Hormone Secretagogue Receptor (GHSR), increasing growth hormone levels, appetite, and insulin resistance (25-29). Unacylated ghrelin was originally thought to be an inactive metabolite, because it does not act on GHSR and does not increase appetite, or cause insulin resistance (20,30-32). However, recent evidence shows that unacylated ghrelin promotes muscle cell differentiation in vitro (19), blocks muscle wasting in several mouse models of muscle atrophy (21), and improves the dystrophic muscle phenotype in a mouse model of muscular dystrophy (mdx mice) (22). In other tissues, unacylated ghrelin improves mitochondrial ATP production and reverses abnormal mitophagy (20,23,24). In a murine hind-limb ischemia model, unacylated ghrelin increased hind limb muscle satellite cells, promoted skeletal muscle regeneration, induced angiogenesis, and improved hind limb muscle function (33). Unacylated ghrelin also improves endothelial function and has been studied in more than 70 healthy humans without adverse effects (25,29,34-39). We have already obtained an Investigational New Drug (IND) approval (IND #130513), and we successfully completed a dose finding study in nine patients with PAD. Results of the dose finding study were used to design this pilot randomized trial.

This pilot trial will gather preliminary evidence to test our hypothesis that unacylated ghrelin improves walking performance and prevents mobility loss in older patients with PAD. We hypothesize that the favorable effect of unacylated ghrelin on walking performance will be mediated by increases in calf muscle satellite cell abundance, muscle fiber regeneration, capillary density, and calf muscle perfusion. If results of the GIFT Trial support our hypotheses, findings will be used to design a large randomized trial of unacylated ghrelin, in subsequent study, to improve functioning and prevent mobility loss in older people with PAD.

BACKGROUND:

PAD affects 10-15% of men and women age 65 and older (1,40-42) and will be increasingly common as the U.S. population survives longer with chronic disease. Our prior work demonstrates that people with PAD have greater functional impairment and more rapid functional decline compared to those without PAD (1-8). The functional impairment documented in PAD is associated with disability, increased mortality, and poorer quality of life (6,43-45). Only two medications, pentoxifylline and cilostazol, are FDA-approved for treating PAD-associated walking impairment. Of these, pentoxifylline is typically ineffective and benefits from cilostazol are modest (46-50). Effective therapies are urgently needed for PAD.

Calf skeletal muscle fibers in PAD are damaged. Electron microscopy demonstrates pathologic changes in myofibrils, mitochondria, nuclei, and sarcolemma of calf muscle myofibers in patients with PAD (9). In 34 participants with PAD and 21 without PAD who underwent calf muscle biopsy, PAD participants had 30% higher protein carbonyl content ($P<0.001$) and 40% higher 4-hydroxy-2-nonenal (HNE) levels ($P<.001$), indicating greater calf muscle oxidative stress abundance in the PAD compared to the non-PAD muscle (9). More severe calf muscle oxidative stress is associated with more severe myofiber damage and fewer myofibers (9). Our preliminary work shows that muscle fiber atrophy, measured by fiber size on microscopy, is associated with slower walking velocity in PAD (14). In summary, people with PAD have ischemia-related pathophysiologic changes in calf muscle that are associated with functional impairment and decline (14-18).

Calf muscle mitochondria are damaged in PAD. Skeletal muscle mitochondria from PAD patients demonstrates a quantitative mitochondria dysfunction, with reduced energy production (18,51,52). One study used magnetic resonance spectroscopy to compare the efficiency of

mitochondrial oxidative phosphorylation in 12 men with PAD and 14 without PAD who engaged in submaximal plantarflexion exercise. After submaximal exercise, and controlling for blood flow, the PAD participants had poorer phosphocreatine recovery time (137 seconds ± 41 vs. 44 seconds ± 3, P=0.02) and poorer adenosine triphosphate (ATP) recovery time (60 seconds ± 10 vs. 29 seconds ± 2, P=0.02) than patients without PAD (18). These and other results (51,52) demonstrate an intrinsic defect in calf muscle mitochondrial function in patients with PAD.

Overview of unacylated ghrelin as a therapeutic intervention. Ghrelin is a peptide and hormone that is produced by P/D1 cells of the gastric fundus and circulates in both acylated and unacylated forms. Acylated ghrelin acts on the Growth Hormone Secretagogue Receptor (GHSR), increasing growth hormone, appetite, and insulin resistance (26-29). Unacylated ghrelin was originally thought to be an inactive metabolite, because it does not act on GHSR, increase appetite, or cause insulin resistance (20,30-32). However, recent studies in humans and animals show that unacylated ghrelin improves endothelial function, regenerates muscle cells, and increases mitochondrial activity and capillary density (33,53-56).

In a mouse model of hind-limb ischemia, unacylated ghrelin increased skeletal muscle regeneration, angiogenesis, and limb strength. Togliatto and colleagues induced hindlimb ischemia in 81 mice and randomized them to intra-peritoneal injections of unacylated ghrelin, acylated ghrelin, or saline for 21 days (33). Mice who received unacylated ghrelin had significantly more skeletal muscle satellite cells (measured by Pax7/MyoD markers), more regenerating skeletal muscle fibers, and better plantarflexion function in the ischemic limb than mice who received acylated ghrelin or saline. Mice who received unacylated ghrelin had higher capillary density in skeletal muscle of their ischemic limb, compared to the other two groups. The greater abundance of satellite cell number in mice who received unacylated ghrelin was mediated by P38/MAPK activation (33), consistent with prior reports that satellite cell proliferation depends on P38/MAPK protein activation. Separately, a dystrophic Duchenne Muscular Dystrophy mdx model showed that unacylated ghrelin maintained satellite cell abundance, reduced muscle degeneration, and improved muscle function (22).

Unacylated ghrelin promotes myocyte regeneration. In an *in vitro* study, unacylated ghrelin induced differentiation of proliferating skeletal myoblasts and promoted their fusion into multi-nucleated syncytia (21). A high affinity binding site for deacylated ghrelin was identified on C2C12 myoblasts that is distinct from the (GHSR)-1a receptor to which acylated ghrelin binds (21). This preliminary evidence supports our hypothesis that unacylated ghrelin may improve functioning by promoting myocyte regeneration and differentiation in calf muscle damaged by PAD-related ischemia-reperfusion injury.

Unacylated ghrelin increases mitochondrial activity in preclinical models. First, in the hind limb ischemia mouse model by Togliatto et al (33), unacylated ghrelin favorably affected oxidative stress in ischemic muscle, by increasing the mitochondrial specific enzyme, superoxide dismutase-2 (SOD-2), suggesting improved mitochondrial function. Second, in an in vitro model, unacylated ghrelin upregulated expression of Cox subunit IV and increased cellular ATP content (23). Third, in a rat liver model of ischemia reperfusion, ischemia reduced oxygen consumption in liver cells, but this effect was completely reversed after unacylated ghrelin treatment (23). For these reasons, we hypothesize that unacylated ghrelin will improve mitochondrial oxidative metabolism in PAD participants.

Unacylated ghrelin is likely to improve skeletal muscle perfusion. In rats, unacylated ghrelin induces endothelium-dependent vasodilation of the mesenteric vascular bed (57). In separate study, unacylated ghrelin inhibited apoptosis of endothelial cells (56). In 12 humans

with metabolic syndrome, intravenously administered unacylated ghrelin improved endothelium-dependent brachial artery flow-mediated dilation (FMD) within one hour after ghrelin infusion (25). By increasing FMD and promoting angiogenesis, we hypothesize that unacylated ghrelin will improve arterial perfusion of calf muscle in people with PAD.

Scientific Premise. Basic and preliminary human evidence show that unacylated ghrelin has therapeutic properties likely to improve walking performance in PAD (19-25,33,56,57). First, in a hind limb ischemia model and in a mouse model of Duchenne Muscular Dystrophy, unacylated ghrelin increased satellite cells, promoted myofiber regeneration, and improved muscle strength or function (22,33). Second, unacylated ghrelin promoted skeletal muscle myoblast differentiation in vitro (21). Third, in a hind-limb ischemia model, unacylated ghrelin promoted angiogenesis and reduced oxidative stress, consistent with improved mitochondrial activity (33). Fourth, unacylated ghrelin reversed ischemia related mitochondrial damage in a rat model of ischemia/reperfusion (23). Fifth, in animals and humans, unacylated ghrelin promoted endothelium-dependent vasodilation and improved endothelial function (25,56,57-59,) Together this evidence supports our hypothesis that unacylated ghrelin will increase calf muscle regeneration, promote mitochondrial activity, increase angiogenesis, and enhance calf muscle perfusion, thereby improving walking ability in PAD.

Dose-finding study of unacylated ghrelin performed to prepare for the GIFT Trial. An IND has been obtained for the GIFT Trial (IND # 130513, sponsor: MM McDermott) to perform a dose finding study, which was completed. In this dose finding study, we studied the pharmacokinetics of unacylated ghrelin, by administering three doses of unacylated ghrelin subcutaneously on three different days: 10 ug/kg, 20 ug/kg, and 40 ug. Each dose was administered to six people with PAD (a total of nine people with PAD received one or more doses). Each dose was administered in the morning, followed by measurement of unacylated ghrelin levels at defined time points during the 24 hours after the injection. The PAD participants had a mean age of 71.0 (standard deviation (SD): 4.7), and mean ankle brachial index (ABI) of 0.69 (SD: 0.13). Three (33.3%) were African American and 3 (33.3%) had diabetes mellitus. Higher doses of unacylated ghrelin were associated with higher peak unacylated ghrelin levels. Twelve hours after each injection, values remained 6-7 times higher than baseline. Twenty-four hours after each injection, values had returned to baseline. All doses were determined to be safe. Brachial artery flow-mediated dilation (FMD) was measured before each unacylated ghrelin injection and at 6 hours and 24 hours after each injection.

STUDY ENDPOINTS:

Primary Aim. Among 30 participants with PAD age 55 and older, we will determine whether those randomized to subcutaneously administered unacylated ghrelin for four months have greater improvement or less decline in six-minute walk distance at 4-month follow-up, compared to placebo. *We hypothesize that PAD participants randomized to unacylated ghrelin will achieve greater improvement or less decline in six-minute walk distance at 4-month follow-up, compared to those randomized to placebo.*

Secondary Aim. Among 30 participants with PAD age 55 and older, we will determine whether those randomized to subcutaneously administered unacylated ghrelin for four months have greater improvement or less decline in maximal treadmill walking time and greater increase or less decline in calf muscle perfusion at four-month follow-up, compared to placebo. *We hypothesize that PAD participants randomized to unacylated ghrelin will achieve greater increases or less decline in maximal treadmill walking time and calf muscle perfusion at four-month follow-up, compared to those randomized to placebo.*

Exploratory Aim. Among 30 participants with PAD, we will determine whether subcutaneously administered unacylated ghrelin for four months increases calf muscle fiber type and size, satellite cell number, the number of capillaries per fiber, and succinate dehydrogenase (SDH) mitochondrial activity at 4-month follow-up, compared to placebo. *We hypothesize that PAD participants randomized to unacylated ghrelin will achieve greater improvement in each of these skeletal muscle outcomes at four-month follow-up, compared to those randomized to placebo.*

EXPLORATORY AIM. Among 30 participants with PAD age 55 and older, we will determine whether those randomized to subcutaneously administered unacylated ghrelin for four months have greater improvement or less decline in the WIQ distance score and in the PROMIS mobility questionnaire at 4-month follow-up, compared to placebo.

STUDY INVESTIGATIONAL AGENTS:
Participants will be randomized to subcutaneously administered unacylated ghrelin or placebo. Synthetic unacylated ghrelin powder will be supplied by the CS Bio Company and prepared for subcutaneous injection by the Investigational Pharmacy at the University of Chicago. Because Northwestern University's Investigational Pharmacy does not perform sterility filtering that is currently required by federal regulations, the study drug and placebo will be dispensed from the University of Chicago pharmacy after randomization. Up until approximately December 2022, 5he University of Chicago pharmacist dispensed study drug according to group assignment. Northwestern University's Investigational Pharmacy dispensed placebo vials for the study run-in. Update May 2023: Participants who begin study injections after May 2023 will have study drug prepared at Mark Drugs (384 E Irving Park Rd, Roselle, IL 60172) and placebo prepared at Northwestern University's Investigational Pharmacy.

Study drug is dispensed in individual vials and stored in a freezer at -15 degrees Celsius or colder at the participant's home. If the participant's freezer is not -15 degrees Celsius or colder, the vials may be stored in the refrigerator and dispensed every 14 days. Participants will be taught to draw up the study drug in a syringe (one vial per day). Participants who are not able to self-administer or have the study drug administered subcutaneously at home will not be eligible. Dispensed vials will be labeled with the participant's name, date of birth, expiration date, and instructions for administration. Vials will NOT be labeled with the group assignment, ensuring that both the research team collecting data and study participants are blinded to group assignment (i.e. double blinded status). Study drug is stored and handled according to the University of Chicago Research Pharmacy or Mark Drugs SOP.

Dr. Mary McDermott is the holder of the IND for the study (IND#130513).

PROCEDURES INVOLVED:
Initial eligibility criteria will be assessed by telephone. Potential participants who remain eligible after the telephone screening will be scheduled for a baseline visit, where they will undergo additional testing to determine their eligibility for randomization.

Participants will be recruited at both Northwestern and at the University of Chicago Medical center. However, participants recruited at the University of Chicago will undergo study measures at the University of Chicago, except for MRI testing and muscle biopsy which will be performed at Northwestern.

Baseline testing. Participants will provide written informed consent at baseline. An ankle brachial index (ABI) will be performed. Questionnaires will be administered and physical

functioning tests, including the six-minute walk, will be performed. A treadmill exercise stress test will be performed. A blood sample will be obtained and height and weight will be measured. Participants will be asked to undergo an MRI but will remain eligible if they are unable or unwilling to participate in the MRI portion of the study. If the participant agrees to the optional muscle biopsy and if the participant is eligible for the biopsy, they will be asked to undergo a biopsy. Baseline testing will require testing at multiple visits performed over multiple days (see Table 1 below). Research procedures are described in more detail below.

Table 1: Order of Baseline Visit Measures[1]

	Baseline Visit 1	Baseline Visit 2	Baseline Visit 3
Consent	X		
ABI	X		
Six-minute walk	X		
Treadmill stress test		X	
MRI[2]		X	X
Muscle Biopsy			X

[1] *Additional measures such as the short physical performance battery (SPPB), questionnaires, blood collection, and height and weight will be performed in combination with either baseline visit 1 or 2. The MRI may be performed at visits 2 or 3. If the MRI is scheduled for baseline visit 2, it will be performed either before the treadmill stress test or at least 45 minutes after the treadmill stress test. If the MRI occurs the same day as the biopsy, the MRI will be performed first. In some instances, a participant may need an additional visit or visits to complete baseline visits if they are not able to perform the tests over three separate days.*

Table 1 is a general guide to the order of data collection. Please note that in some instances, the six-minute walk is performed at an additional visit that occurs before the treadmill stress test, but after visit #1. Typically three visits are required to complete baseline testing, but in some instances up to six baseline visits may be needed. The six-minute walk test is not performed on the same day as either treadmill testing or muscle biopsy. The treadmill test is not performed on the same day as the muscle biopsy.

Run-in. The run-in is designed to exclude people who may not be adherent to the study drug and to ensure the participant has adequate storage in their home for the study drug. To help ensure that randomized participants are adherent to study drug, we will ask all potential participants to complete a one-week run-in prior to randomization. For the run-in, participants will be taught to self-administer the study drug subcutaneously. Participants will receive a total of seven pre-filled placebo vials, syringes, and other supplies (e.g. alcohol swabs) for home administration as well as a sharps container for syringe disposal. The Northwestern Investigational Pharmacy will dispense the placebo vials for the study run-in. A study coordinator will deliver the vials and supplies to the participant's home and will assess their home freezer to ensure the participant can properly store the study drug. Alternatively, the study participant will pick up the vials and supplies. A temperature monitor will be placed in the participant's freezer to ensure the temperature is adequate for the study drug. Only those who self-administer at least five syringes in 7 days will be eligible for randomization. Participants will be asked to return following the run-in period and a study coordinator will collect and count the used syringes/empty vials and review the medication adherence log to ensure the participant is eligible for randomization. Information from the temperature monitor will be reviewed. If the freezer is not adequately cold (-15 degrees Celsius or colder), study drug post-randomization will be dispensed every 14 days. Potential participants who administer fewer than five

subcutaneous injections during the run-in, who want to attempt the run-in again, and who are expected to be able to successfully complete the run-in may have up to two additional attempts to complete the run-in.

Randomization. We will randomize participants to unacylated ghrelin or placebo using a computer-generated list of random assignments. Participants will be randomized using randomly selected block sizes from 4 and 8. Randomization will be stratified by baseline functional performance, measured by a six-minute walk distance ($\geq$ 1,100 feet vs. < 1,100 feet). Randomization assignment will be communicated directly by the data management team at Northwestern to the pharmacy dispensing study drug. Because the amount of unacylated ghrelin was limited, in order to complete the clinical trial, as of approximately December 2022 it was determined that approximately 40% of participants would be randomized to unacylated ghrelin and approximately 60% to placebo.

Intervention and placebo. Participants will be asked to self-administer the study drug or placebo subcutaneously once daily. Participants randomized to unacylated ghrelin will self-administer 20 ug/kg unacylated ghrelin daily. Unacylated ghrelin is diluted with bacteriostatic saline. Participants randomized to placebo will self-administer an identical-appearing solution of bacteriostatic saline daily. The study is double blinded. For participants who are dispensed drug after September 2023, it is necessary to unblind 1-2 coordinators (as NU pharmacy will dispense placebo and Mark Drugs will dispense study drug). The unblinded coordinators who pick up the supplies, deliver to participants, and collect empty vials/syringes will not conduct data collection visits at follow-up.

A study coordinator will obtain a monthly supply of vials from the dispensing pharmacy and deliver to the participant's home on dry ice. The coordinator will ensure the study drug is placed into the participant's freezer immediately upon delivery. Approximately 30 days of study drug will be administered at a time. A temperature monitor will be left in the participant's freezer throughout the study. If a participant's freezer is not adequately cold (-15 degrees Celsius or colder), study drug will be dispensed every 14 days and may be stored in the fridge. Drug that is dispensed every 14 days will either be delivered to the participant's home in a cooler pack or the participant will be asked to pick it up and will transport home in a cooler pack.

Participants will be asked to keep a daily log to record each time they inject the study drug. This log will be reviewed at the monthly home visits. In addition, an adverse event questionnaire will be administered monthly. They will be asked to check the temperature of their freezer using the study-provided monitor.

The participants will be instructed to keep the study drug frozen in the participant's home. However, the study drug may be refrigerated for up to 14 days in the event that a participant does not have access to a freezer (for example, if the freezer temperature at run-in was not adequate or if the participant needs to transport the study drug in a cooler pack during travel).

Four-month follow-up testing. Participants will return after subcutaneously administering study drug for four months to complete final follow-up testing including muscle biopsy and MRI. Measures and tests that were conducted at baseline will be repeated and the final logs and syringes/vials will be collected. See Table 2 below for timing of follow-up measures.

Table 2: Order of Follow-Up Visit Measures

	Follow-Up Visit 1	Follow-Up Visit 2	Follow-Up Visit 3

Six-minute walk	X		
Treadmill stress test		X	
Muscle Biopsy			X

Note: Additional measures such as the ABI, SPPB, questionnaires, blood collection, and height and weight will be performed in combination with either baseline visit 1 or 2. The MRI may be performed at visits 1, 2, or 3. If the MRI occurs at visits 2 or 3, the MRI will be performed before the treadmill test or before muscle biopsy. In some instances, a participant may need an additional visit or visits to complete follow-up visits if they are not able to perform all tests during these three visits.

Some or all study measures may be repeated at baseline or follow-up for data quality (e.g. if a treadmill test must be stopped due to extremely high blood pressure before the patient completed the test is one potential example of why a measure may need to be repeated). In some cases, it may be necessary to take an additional, unscheduled blood pressure measurement. For instance, if a participant has high blood pressure during the ABI and investigators would like to double check their arm pressure measurement before performing the six-minute walk either at the same visit or at a subsequent visit. Determinations about blood pressure checks will be made on a case-by-case basis in consultation with Dr. McDermott or other qualified personnel.

In some cases, participants may be asked to take study drug for longer than four months. For example, if a participant has an illness that prevents them from returning at four months for follow-up testing or if they are out of town during their four-month follow-up window. In these instances, participants may be asked to continue taking the study drug for more than four months. Each individual situation, in which the participant misses study drug for an extended period of time due to unforeseen circumstances, will be decided on by the principal investigator with regard to whether study drug will be extended beyond four months after randomization. In other cases, participants may take the drug for less than four months, if some drug doses are missed and a new 30 batch would be required in order for a participant to complete the full four months of study drug. The principal investigator will determine the duration of therapy depending on individual participants situation.

Research procedures:
Ankle Brachial Index (ABI). After the participant rests supine for five minutes, the right brachial, dorsalis pedis (DP), posterior tibial (PT) and left DP, PT, and brachial artery pressures are measured using a hand-held Doppler probe. Pressures are measured twice. The ABI is calculated for each leg by dividing the average of the DP and PT pressures by the average brachial pressure. If an ankle brachial index result yields an equivocal result or suggests that a participant's lower extremity arteries are calcified, investigator discretion may be used to order a lower extremity vascular test with toe pressures at Northwestern Memorial Hospital to determine eligibility at baseline. The test will repeat blood pressures in the arms and legs and will include a toe pressure measurement, which is performed by placing a small blood pressure cuff around the great toe and attaching a plethysmography probe (circulatory sensor) on the pulpy part of the tip of the great toe.

Questionnaire Administration. Participants will be administered IRB-approved study

questionnaires by a trained and certified study coordinator. Health-related quality of life will be assessed with the Walking Impairment Questionnaire (WIQ) and the PROMIS mobility questionnaire. Patient report will be used to document comorbidities and physical activity.

Six-minute walk. In the six-minute walk, participants walk back and forth along a 100-ft hallway for six minutes after standardized instructions to complete as many laps as possible. Distance covered in six minutes is recorded.

Treadmill testing. The Gardner graded treadmill exercise test will be used to measure change in maximal treadmill walking time in response to the study intervention. Speed is maintained at 2.0 miles per hour (mph) and treadmill grade increases by 2.0% every two minutes. If patients cannot walk at 2.0 mph, a Modified Gardner will be used, where treadmill speed is started at 0.5 mph and increased by 0.5 mph every 2 minutes until the participant reaches 2.0 mph, after which the treadmill grade is increased every two minutes while the speed remains at 2.0 mph.

Blood collection and long-term storage. At baseline and four-month follow-up study visits, participants may have approximately 50 ml of blood drawn for processing and long-term storage at -70 degrees Celsius. A comprehensive chemistry panel will be performed on blood at baseline. Stored blood will await later analyses for biomarkers and other emerging blood markers related to peripheral artery disease that may change in response to the intervention. Genetic testing may also be performed on stored DNA if the participant agrees to this optional study element on the consent document. Results of the genetic testing on the sample will be stored with other data collected. Samples will be labeled with the participant's study identification number and will not be stored with other health or identifying information. Information associated with the sample will be stored a secure database on password protected computers that are secured by Northwestern University firewalls. Access is limited to study staff. If the samples are shared with other researchers not part of the current study, the PI will grant permission to the other researchers to analyze the samples after receiving IRB approval. Samples will be identified with a study identification number and the other researchers will not have access to PHI. Results of testing on the blood samples for long-term storage will not be shared with the study participants. Results of the comprehensive chemistry panel will be shared with participants upon request or if a value is medically significant and requires follow-up.

In addition, at four-month follow-up, participants will have approximately 6 ml of blood (3 ml at two time points) drawn for ghrelin testing. Blood will be collected prior to a ghrelin injection and again approximately one hour later. Participants will be asked to fast for 8 hours prior to blood collection at follow-up. Blood will be sent to the University of Virginia for ghrelin testing.

Calf muscle/fat biopsies. Muscle biopsies will be performed by co-investigator Robert Sufit, MD, a board-certified neurologist with > 30 years of experience performing muscle biopsies. Muscle biopsies are obtained in the medial head of the gastrocnemius muscle in the leg with lowest ABI, at the point that is 67% of the distance between the medial malleolus and the medial aspect of the proximal tibia. Anesthesia is achieved with subcutaneous lidocaine. Subcutaneous and adipose tissue are dissected. Approximately 250 mgs of muscle is removed and immediately prepared for long term storage at -70 degrees Celsius. Muscle tissue is frozen directly in liquid nitrogen for protein analyses and also mounted in trigacanth gum on cork and snap frozen in liquid nitrogen cooled isopentane for histochemical analysis. At four-month follow-up, we will repeat the biopsy adjacent to the original biopsy, identifiable by the scar. Approximately 100 to 150 mgs of fat may be removed from the subcutaneous fat and approximately 100 to 150 mgs of fat may be removed from below the fascial line during the

muscle biopsy. In patients with more fat tissue, up to approximately 250 mgs of fat will be removed from the subcutaneous fat and from below the fascial line, respectively, at the discretion of the physician performing the muscle biopsy.

All calf muscle measures will be compared between baseline and four-month follow-up.

Calf muscle biopsy measures will be de-identified and sent to off-site laboratories of co-investigators and/or consultants for measures defined in the specific aims, using standard methods (63-67). Measures of senescence, including P16+ cells and senescence associated beta-galactosidase + cells, may be identified in fat tissue obtained during the muscle biopsy in the laboratory of Dr. Peterson and/or Dr. Tchkonia at Mayo Clinic.

Participants who give consent to the optional muscle biopsy will be asked to undergo biopsy if they are eligible. Possible reasons for not being eligible for muscle biopsy include the following. Participants taking warfarin or other anti-coagulant therapy (such as direct oral anticoagulant therapy) will not be eligible for muscle biopsy and therefore will not be eligible for the study, unless their physician indicates that it is safe to come off of their anticoagulation therapy for up to 7 days prior to the biopsy. Participants who are taking anti-platelet therapy will provide their prescribing physician's name. The physician will be contacted for permission to ask the participant to withhold their anti-platelet therapy for seven days prior to the biopsy. If the physician does not allow the participant to discontinue the anti-platelet therapy, the participant will not qualify for the biopsy unless Dr. Sufit and the participant agree to carry out the biopsy while the participant remains on anti-platelet therapy. Participants who are HIV+ will not be eligible for a muscle biopsy. HIV may result in muscle changes independently of PAD and could make interpretation of the findings difficult. Participants with a BMI $\geq$ 40 may not qualify for a muscle biopsy due to possible difficulty accessing muscle tissue.

Potential participants who decline muscle biopsy or are not eligible for muscle biopsy will still be able to participate in the trial if they meet other eligibility criteria.

MRI. We will use arterial spin labeling with cardiovascular magnetic resonance imaging to measure changes in calf skeletal muscle perfusion at 3 Tesla. Christopher Kramer MD, from University of Virginia, is an internationally recognized expert in this measurement and will assist investigators with interpreting results. Results will be read blinded to group assignment. The procedure is performed as follows: A thigh cuff is inflated in the leg with lowest ABI up to 250 mm Hg. After five minutes, the blood pressure cuff is rapidly deflated. Seven control-tagged image pairs are acquired over 60 seconds using PASL pulse sequence with single-shot echo-planar imaging readouts (field of view 200x200 mm, matrix 64x64, repetition time 4000 ms, echo time 32 ms, slice thickness 10 mm). Perfusion is measured and quantified on a Siemens Healthcare workstation by selectively drawing regions of interest of the hyperemic areas on the perfusion maps. Images for calf muscle fat and skeletal muscle quantity may also be obtained.

Participants will fill out an MRI safety form prior to being scheduled for an MRI to ensure it is safe for them to undergo MRI. Participants will be excluded from the MRI if they have an implant or other item (such as metal flakes or shrapnel in their body) that is not considered safe at 3 Tesla. Participants who are claustrophobic will not be encouraged to participate in MRI testing. Participants who are not eligible or willing to undergo MRI will still be eligible for the trial.

Other measures. Body mass index (BMI) will be assessed at baseline and follow-up by objectively measuring height and weight. A four-meter walk test will be administered at usual and fastest pace at the baseline and follow-up visits. Participants will be asked to perform the

usual paced four-meter walk at usual pace and the “fast paced” four meter walk at their fastest pace. Each of these short walks will be performed twice. Participants will be asked to complete a series of standing balance tests and five chair stands. Names and doses of participants’ medications will be systematically recorded at baseline and at follow-up.

DATA AND SPECIMEN BANKING
Muscle/fat specimens and blood specimens for long-term storage will be stored in a freezer belonging to Dr. McDermott's research program at Northwestern University, in the freezer farm in the basement of Olson Pavilion. Specimens will be stored for up to 70 years, after which they will be destroyed.

Specimens will be coded; meaning that a key will exist that can link the codes back to the direct subject identifiers. Each participant will be assigned a unique study ID number that can be traced back to the study participant. The muscle specimens and the blood samples that are stored will be labeled with this unique identifier and the date and time of the blood collection.

Only Dr. McDermott has control over release of study data or specimens. Any investigators seeking to analyze blood or muscle specimens must contact Dr. McDermott for permission. Each request, if it occurs, will be considered on a case-by-case basis.

SHARING RESULTS WITH PARTICIPANTS
Participants will receive results of their ankle brachial index (ABI) test and will be provided with a “result letter” at the end of their baseline visit. ABI and stress test results will be mailed to the participant’s physician upon request. They will not be provided with other study results routinely. However, participants will be notified of medically significant stress test, blood, or blood pressure results identified in the stress test or ABI.

STUDY TIMELINES
Each participant will participate in the study for approximately four months after randomization. We plan to enroll 30 participants over an approximate 13-month period.

INCLUSION AND EXCLUSION CRITERIA
Inclusion criteria. All participants will be age 55 and older. All participants will have PAD. PAD will be defined as either 1) an ankle-brachial index (ABI) $\leq$0.90 at the baseline study visit or 2) vascular lab evidence of PAD or angiographic evidence of PAD with ischemic leg symptoms during the six-minute walk and/or treadmill exercise stress test.

Exclusion criteria.
1. Above- or below-knee amputation.
2. Critical limb ischemia.
3. Wheelchair-bound or requiring a cane or walker to ambulate.
4. Walking is limited by a symptom other than PAD.
5. Current ulcer on bottom of foot. The participant may become eligible after the ulcer heals.
6. Significant liver or kidney impairment defined as two or more hepatic function enzymes >3.0 times the upper limit of normal and/or eGFR < 20. [NOTE: participants who meet this criterion may undergo a re-test of hepatic function tests to determine whether initially elevated hepatic enzymes represented a transient or spurious phenomenon.]
7. Unwilling or unable to self-administer study drug.
8. Failure to successfully complete the study run-in.
9. Planned lower extremity revascularization or other major surgery during the next four months.

10. Lower extremity revascularization, major orthopedic surgery, cardiovascular event, coronary revascularization, or other major surgery in the previous three months.
12. Major medical illness including renal disease requiring dialysis, lung disease requiring oxygen, Parkinson's disease, a life-threatening illness with life expectancy less than six months, or cancer requiring treatment in the previous two years. [NOTE: potential participants may still qualify if they have had treatment for an early stage cancer in the past two years and the prognosis is excellent. Participants who only use oxygen at night may still qualify.]
13. Mini-Mental Status Examination (MMSE) score <23
14. Participation in or completion of a clinical trial in the previous three months. [NOTE: after completing a stem cell or gene therapy intervention, participants will become eligible after the final study follow-up visit of the stem cell or gene therapy study so long as at least six months have passed since the final intervention administration. After completing a supplement or drug therapy (other than stem cell or gene therapy), participants will be eligible after the final study follow-up visit as long as at least three months have passed since the final intervention of the trial.]
15. Currently taking study drug(s) or has taken study drug(s) in past six months.
16. Increase in angina in last month or angina at rest.
17. Non-English speaking.
18. Visual impairment that limits walking ability.
19. Women who are pregnant or who are pre-menopausal will not be eligible.
20. Potential participants who recently participated in or are currently participating supervised treadmill exercise and those planning to begin a supervised treadmill exercise regimen will become eligible four months after their participation in the supervised treadmill exercise program has ended.
20. In addition to the above criteria, investigator discretion will be used to determine if the trial is unsafe or not a good fit for the potential participant.
21. The potential participant does not have adequate refrigeration for storing study drug.

Vulnerable populations (fetuses, pregnant women, children, prisoners, and institutionalized persons) and adults unable to consent will not be included in the study.

Note: Potential participants who are not able to undergo MRI or who decline the MRI will still be eligible for the trial.

PARTICIPANT POPULATION(S)

Accrual Number:	Category/Group: (Adults/Children Special/Vulnerable Populations)	Consented: Maximum Number to be Consented or Reviewed/Collected/Screened	Enrolled: Number to Complete the Study or Needed to Address the Research Question
Local	Adults 55 and older	1000	30
Study-wide	N/A		
Total:		1000	30

RECRUITMENT METHODS

Participants will be identified from among individuals with PAD who have participated previously in research conducted by Dr. McDermott and/or who have expressed an interest in participating in future studies conducted by Dr. McDermott. Participants who we screen for ongoing studies who may have PAD but are ineligible for that study and interested participating in a study may be screened for this study.

We will also obtain lists of consecutive patients with a diagnosis of lower extremity peripheral arterial disease, individuals at high risk for peripheral artery disease, and individuals seen in a non-invasive vascular laboratory from Northwestern's Enterprise Data Warehouse (EDW). EDW lists will be provided by an individual who has permission to obtain the lists from the EDW.

A research assistant will contact referring physicians of potential participants identified from the vascular laboratory via fax, phone, page, or electronic message (EPIC or e-mail), to ask for permission to contact their patient about the study. If a reply is not received within three weeks, up to five letters are mailed from Dr. McDermott about the research study. In the recruitment letters, recipients are asked to call us if they are interested in participation or if they do not want to be contacted further. Potential participants who do not call us within three weeks of the first mailed recruitment letter may be telephoned by study staff and invited to participate.

Letters may be mailed to potential participants from the University of Chicago site using lists obtained from the medical center. Potential participants who do not call within two weeks of the receipt of the letter may be telephoned and up to five letters may be mailed.

In addition, we may use newspaper, television, and radio advertising to identify potential participants for this study. We may also use brochures, flyers, or posters that we will post in relevant office practices and public areas. We may use advertising on public transportation (i.e. Chicago Transit Authority) or online advertisements.

We will obtain a list of patients who live in the Chicago area from a mass mailing company. Using this, we will send postcards to those individuals on the list. The postcards will instruct people to call a study number if they are interested.

Recruitment materials for methods referenced above will be submitted to the IRB for approval prior to their use.

We may also obtain IRB approval at the Jesse Brown VA Medical Center to recruit patients from these medical centers. If IRB approval is obtained from U of C and JBVAMC, letters will be mailed by an approved staff member to potential patients with known PAD. Participants recruited through these methods at JBVAMC will sign a VA consent document and will undergo some study testing on-site at the VA. Study tests include questionnaires and functional performance measures.

COMPENSATION FOR PARTICIPATION IN RESEARCH ACTIVITIES

Participants will receive $50 per month when study drug is delivered and remaining syringes/vials are picked up at 1, 2, and 3-months (for a total of $150) and $50 for completing four month follow-up testing. Participants who receive study drug every 14 days will receive $25.00 when study drug is delivered. Those receiving drug every 28 days (per month) will receive $50 when the drug is delivered. If the participant undergoes the optional muscle biopsy portion of the study, they will receive $100 per muscle biopsy.

Participants will be given assistance and/or reimbursement for expenses related to travel such as parking, bus/train fare, taxi fare or rideshare service (i.e. Uber/Lyft), and mileage, if requested. A receipt will be required for reimbursement greater than $40. Participants will be provided up to $90 per visit for travel reimbursement. If they require the use of our taxi or rideshare service, we will estimate the fare on taxifarefinder.com or on the rideshare website. A one-way fare estimate must be less than or equal to $45 (i.e. round trip of $90) in order for the study to provide taxi or rideshare service. In some instances, a participant's travel estimate may be within the $90 limit for their first visit, but may unexpectedly increase at a later visit due to price fluctuations with Uber/Lyft. In these instances, the study will continue to provide travel to participants and pay the increased travel fare. In addition, if after randomization, a participant becomes unable to attend study visits and requires transportation such as a shared ride service in order to continue participation, then the travel service will be provided, using investigator discretion, so that the randomized participant can continue in the trial. In these cases, the amount of travel using our taxi or Uber/Lyft service may exceed $90.

WITHDRAWAL OF PARTICIPANTS

We anticipate that participant withdrawal from the research without their consent will be infrequent. However, a potential example is if a participant develops symptoms during the study and the principal investigator feels that the symptoms could make the study unsafe for the participant to continue. In this circumstance, the participant would be advised to follow-up with his or her physician. If the participant refuses to follow-up with their physician, it may be necessary for the participant to be withdrawn without their consent.

Some participants may decide they no longer want to participate in the study intervention. These participants will still be asked to return for follow-up testing.

Participants may withdraw from the research at any time. If they decide to leave the research, they should contact the principal investigator, Dr. Mary McDermott. If they stop being in the research, already collected data may not be removed from the study database.

RISKS TO PARTICIPANTS

Risks associated with unacylated ghrelin. Unacylated ghrelin has been administered intravenously to more than 70 humans without any serious adverse effects (25, 29, 34-39). Two participants in one study experienced mild facial swelling after receiving unacylated ghrelin intravenously (39). Symptoms resolved within hours (39). Dizziness was reported in one study in which intravenous acylated and unacylated ghrelin were administered simultaneously over the short term (37). However, this adverse effect was not observed when unacylated ghrelin was administered alone (37). A participant who received 10 ug/kg in the pilot study presented 24 hours later in atrial flutter. However, this participant had a prior history of atrial fibrillation. Participants will be administered questionnaires monthly to obtain data regarding any potential side effects and will be asked to call us immediately with any new untoward side effects. Safety will also be monitored by our DSMB.

Risks associated with the muscle/fat biopsy. The muscle/fat biopsy is associated with several potential risks. These include discomfort during the muscle biopsy procedure and for up to 1-2 days afterward, scarring from the muscle biopsy skin incision, bleeding (including a hematoma), and infection. Adverse effects of Lidocaine administered prior to the biopsy include pain at the injection site, allergic reaction (including swelling of the tongue or throat, wheezing, difficulty breathing, or death), emotional excitement, and a temporarily lowered heart rate and blood pressure. However, these adverse effects of lidocaine are rare. Side effects of the tape

used to bandage the incision site include a local allergic reaction. In addition, potential participants who are asked to hold their anti-platelet therapy during the week leading up to the biopsy procedure may experience a cardiovascular event related to the temporary discontinuation of the anti-platelet therapy. First, to minimize risk related to biopsy, all participants undergoing muscle/fat biopsy will receive a written handout regarding signs to watch for that may indicate wound infection. They will also be verbally instructed in this. Each participant will be instructed to call Dr. McDermott immediately if any signs of infection occur. Second, permission from the participant's physician will be required before participants are asked to discontinue anti-platelet therapy. Participants will be telephoned approximately seven days after the muscle biopsy. Participants who report any complaints about their biopsy site (such as significant pain or redness) will be scheduled for an evaluation of their biopsy site by a study physician.

Risks associated with the six-minute walk test, treadmill stress test, four-meter walks, balance, and chair stands. The physical functioning tests may be associated with muscle fatigue or soreness. These symptoms typically resolve with rest. These tests may be associated with the risk of falling, irregular heartbeat, heart attack, or coronary ischemia or dyspnea due to heart failure or lung disease. Rarely, falling may result in a fracture. However, the research assistant who will collect these data has been trained to prevent falling. The risk of a fracture secondary to a fall during the testing is less than 1 in 8,000. If a participant experiences chest pain, research assistants are trained to page Dr. McDermott immediately. If the chest discomfort does not immediately resolve with rest, participants are escorted to Northwestern's Emergency Department, which is located in the same building as the location of the tests. Dr. McDermott facilitates follow-up as appropriate, by contacting participants' physicians, for those who experience new chest discomfort during testing, for example. In our experience, the risk of chest discomfort is approximately 1 in 750. Symptoms or results from the treadmill stress test may lead to hospitalization or recommendations for procedures to improve blood flow to the heart.

Risks associated with ABI measurement. The ankle brachial index measurement consists of measuring systolic blood pressure in each extremity using a hand-held Doppler. The ABI is non-invasive, safe and does not have any known lasting side effects. During the ankle-brachial index test, participants may experience discomfort from the inflated blood pressure cuff. However, this discomfort resolves immediately when the cuff is released.

Risks associated with questionnaire administration. Participation includes a risk of loss of confidentiality regarding personal health information or emotional distress. However, all research staff have undergone formal human subjects training. They are trained to protect the privacy of research subject participants.

Risks associated with drawing blood. The potential risks of drawing blood include a bruise at the site of vein puncture, inflammation of the vein, and infection. Participants undergoing a blood draw may experience lightheadedness, dizziness, or fainting.

Risks associated with MRI testing. The MRI scanner makes loud banging noises while doing a measurement. Participants will be provided with earplugs or earphones to protect against the noise. Participants will fill out an MRI screening form prior to participating in the MRI portion of the study to ensure they are safe for MRI testing. MRI testing can be difficult for people with claustrophobia. Participants who are claustrophobic will not be encouraged to participate in MRI testing. Participants may experience leg discomfort while the cuff is inflated.

In addition to these risks, this research may cause harm in ways that are unknown. These may be a minor inconvenience or may be so severe as to cause death.

POTENTIAL BENEFITS TO PARTICIPANTS
Participants who are randomized to receive unacylated ghrelin could experience improved functional performance or less decline in functional performance, if our hypotheses are correct.

DATA MANAGEMENT AND CONFIDENTIALITY
Data is recorded using preprogrammed instruments and an electronic case report form using secure, HIPAA-compliant REDCap database software on servers maintained by Northwestern's Clinical and Translational Sciences Institute. We have substantial experience with REDCap.

Power Estimation. The GIFT Trial will obtain preliminary data to determine whether there is sufficient 'signal' from unacylated ghrelin to perform a definitive randomized trial of unacylated ghrelin in older patients with PAD. In addition, the GIFT Trial will allow us to obtain an estimate of effect size and standard deviation of change for each outcome. Power calculations take into account a 5% drop-out rate at 4-month follow-up, based on our prior randomized trials in PAD participants (60-62). Power calculations are based on a one-sided two-sample t-test with a significance level of 0.10. The level of statistical significance and power were selected because the trial is a pilot study designed to collect preliminary data. Thirty participants completing the trial provides 70% power to detect a difference of 0.69 standard deviations (SD) in change in six-minute walk distance (primary outcome) and in maximal treadmill walking time and calf muscle perfusion (secondary outcomes) at 4-month follow-up between the unacylated ghrelin and the placebo group. Using the SD from our completed SILC trial, 0.69 SD represents a 35.6 meter difference for the six-minute walk. Prior studies have defined clinically meaningful changes in the six-minute walk as 20 to 30 meters (small meaningful change) and 50 meters (large meaningful change) (68,69). The observed difference in change in maximal treadmill walking time was 1.06 SD in our SILC trial (60). The effect size for change in calf muscle perfusion will be comparable or larger than for change in six-minute walk distance.

Statistical Analyses. Analyses will be performed according to the intention to treat principle. Prior to analyses, the distributions of the variables (changes) will be examined and necessary transformation will be performed. We will use two sample t-tests to compare changes in each outcome at 4-month follow-up between the unacylated ghrelin and the placebo groups. The balance of participant baseline characteristics (such as age, sex, race, ABI, and smoking status) will be compared between the two groups using t-tests or chi-square tests. If there is indication of major imbalance in these factors, analysis of covariance will be used to adjust for potential confounders. Since it is a pilot study, no adjustment for multiple testing will be made, but we will report the treatment effect on all endpoints listed in the specific aims regardless of their significance level. Based on our prior trials, we anticipate a loss to follow-up of $\leq$ 5% (60-62). If the loss to follow-up rate is higher than 10%, we will conduct multiple imputation as a sensitivity analysis. In response to a request from the DSMB on January 11, 2022, investigators will conduct a sensitivity analyses in which analyses are repeated after excluding participants who were eligible based on the toe brachial index measurement.

Update 12/7/2022: As of December 7, 2022, 21 of 30 participants in the GIFT II randomized clinical trial were randomized. However, the pharmacy advised investigators that there was only sufficient unacylated ghrelin remaining for approximately two to three of the nine people who had not yet been randomized. The investigative team noted that maintaining the original sample size of 30 participants, despite the fact that only two of the final nine participants to be randomized would receive unacylated ghrelin, would improve statistical power, compared to

reducing the originally planned sample size because of the limited amount of remaining unacylated ghrelin. Therefore, the investigative team decided to maintain the sample size of 30 participants. Rather than changing the original randomization plan, which would have required substantial time and effort, in November of 2022, investigators decided to maintain the original randomization scheme, but randomly select a subset of those assigned to unacylated ghrelin to be re-assigned to placebo to reduce the sample size of the treatment group. The effect of this modification on study power was very small. This 'second' randomization will be carried out by the study statisticians, Drs. Lu Tian and Lihui Zhao. The investigative team will send all randomization assignments by the data management team to Drs. Tian and Zhao. Those randomized to placebo will remain in the placebo group. Those randomized to unacylated ghrelin will be randomly re-assigned by Drs. Tian and Zhao to placebo. The change in assignment will be communicated back to the data management team and the new assignment will be recorded in the database. Except for the data management team and the study statisticians, all other investigators and all participants will remain blinded to group assignment. The final statistical analysis will be based on the updated treatment assignment.

Updated February 3, 2025. Because the baseline characteristics of participants show a slight imbalance between the two study groups by sex, investigators revised the statistical analysis plan to indicate that the analyses will be adjusted for sex and for the baseline value for each outcome measure.

PROVISIONS TO MONITOR THE DATA TO ENSURE THE SAFETY OF PARTICIPANTS

An independent Data and Safety Monitoring Board (DSMB) will monitor safety throughout the study. The DSMB will meet approximately every six months during the study. The DSMB will review and approve the protocol prior to beginning data collection. They will decide on stopping criteria for the study.

Hospitalizations will be reported to the DSMB within seven days of investigators learning of the event, if the hospitalizations are unexpected and may be related to study participation. All deaths will be reported to the DSMB within 24 hours. All new injuries or illness causing serious, chronic disability that are unexpected and that may be related to study participation will be reported to the DSMB within seven days of investigators learning of the event. At the DSMB meetings, held approximately every six months, a report of all hospitalizations by study group will be presented to the DSMB.

PROVISIONS TO PROTECT THE PRIVACY INTERESTS OF PARTICIPANTS

Research staff undergo training (human subjects training) in the protection of participant confidentiality and privacy. Research staff have access to medical records only for the purpose of conducting research that is approved by the IRB. Research procedures will be conducted in an enclosed space by a trained and certified research assistant. Dr. McDermott certifies research assistants in data collection to help ensure that participants are treated with the highest level of professionalism.

COMPENSATION FOR RESEARCH-RELATED INJURY

If the participant needs medical care because of taking part in this research study, they should contact the investigator and medical care will be made available. This care will be billed to the participant, their insurance, or other third party. Northwestern University has no program to pay for medical care for research-related injury.

ECONOMIC BURDEN TO PARTICIPANTS

Describe any costs that participants may be responsible for because of participation in the research. All of the study procedures and measures are paid for by the investigative team.

CONSENT PROCESS

Written informed consent will be obtained from the participant prior to study procedures. Participants will be consented by a research assistant who has been trained and certified by Dr. McDermott in obtaining informed consent. A research assistant will explain the study to potential participants by telephone prior to their first study visit. When a potential participant arrives to the medical center for study participation, the research assistant will explain the full details of the research study. The informed consent process will take place at the initial baseline study visit in a private area on Northwestern's medical campus. Potential participants will be provided plenty of time to read the consent form. The research assistant will answer questions and Dr. McDermott or another study investigator at Northwestern is also available to answer any questions that participants may have about the research. If the participant would like more time to discuss the research study with their physician or family member before signing the consent document, they will be allowed to do so and the study visit will be rescheduled for a later date. Potential participants who do not speak English, subjects who are not yet adults, cognitively impaired adults, and adults unable to provide written consent will not be eligible for study participation.

PROTECTED HEALTH INFORMATION (PHI AND HIPAA)

HIPAA Authorization will be obtained from all participants as part of the informed consent process.

QUALIFICATIONS TO CONDUCT RESEARCH AND RESOURCES AVAILABLE

The multidisciplinary investigative team for this randomized trial includes internationally recognized and leading experts in PAD, skeletal muscle, functional performance, and randomized trial design and statistical analyses. Members of the investigative team have been working together for up to 20 years on NIH, NIA, and PCORI-funded studies to identify clinical characteristics associated with functional decline in PAD and more recently to identify novel therapies to improve functional performance and prevent functional decline in people with PAD.

The investigative team is internationally recognized for its experience and expertise successfully completing randomized clinical trials. Since 2009, the investigative team, led by Dr. McDermott, has completed enrollment for six randomized trials of participants with PAD that were funded by NIH, NIA, or PCORI.

Health interviewers will be trained by a senior coordinator and certified by Dr. McDermott in each data collection element, using a detailed checklist. Health interviewers are re-certified approximately every six months. When deficiencies are identified, interviewers undergo additional training and re-assessment.

Baseline and follow-up data collection will take place at Northwestern Memorial Hospital in the Division of General Internal Medicine space located in the Galter Pavilion (675 N. St. Clair) or at 750 N. Lake Shore. Treadmill stress testing will be performed by Northwestern Medicine on the 8th floor of the Feinberg Pavilion (251 E. Huron).

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