PROTOCOL TITLE: Improve PAD **PER**formance with **MET**formin: The PERMET Trial

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OBJECTIVES:
Our work and that of others has established that people with lower extremity peripheral artery disease (PAD) have greater functional impairment, faster functional decline, and increased rates of mobility loss compared to people without PAD (1-7). PAD-related ischemia-reperfusion during walking activity is associated with calf skeletal muscle pathophysiologic changes that include reduced mitochondrial activity and increased reactive oxygen species (ROS) (8-13). Calf skeletal muscle abnormalities in PAD are associated with functional impairment and mobility loss (14-17). However, few therapies improve functioning or prevent functional decline in people with PAD.

Metformin is an inexpensive, widely available, well-tolerated biguanide medication and the most commonly prescribed drug for Type 2 diabetes worldwide. Recent pre-clinical and preliminary human evidence suggest that metformin has previously unrecognized therapeutic properties (18-58). Therapeutic properties of metformin in pre-clinical models that may benefit people with PAD include: calf skeletal muscle increases in peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) (a major regulator of mitochondrial biogenesis), skeletal muscle increases in mitochondrial protein expression and activity, increases in capillary density in ischemic tissue, reductions in oxidative stress, increases in autophagy (clearance of cellular damage), and improved endothelial function (18-58). These therapeutic properties target pathophysiologic conditions present in PAD. Therefore, we hypothesize that metformin will improve lower extremity functioning in people with PAD, by facilitating favorable changes in calf skeletal muscle and by increasing calf skeletal muscle perfusion. No randomized controlled clinical trials have studied whether metformin improves lower extremity functioning in PAD. A definitive trial is needed.

PERMET is a placebo controlled double-blind randomized clinical trial to establish whether metformin (2,000 mgs daily) improves and/or prevents decline in walking performance in people with PAD. Participants will be 212 people with PAD who do not have diabetes mellitus, since metformin is a first-line therapy for Type 2 diabetes. Our primary outcome is change in six-minute walk at 6-month follow-up. Secondary outcomes are 6-month changes in treadmill walking performance, brachial artery flow-mediated dilation (FMD) (a measure of endothelial function), calf muscle biopsy biochemical measures, patient-reported walking performance (measured by the Walking Impairment Questionnaire (WIQ)), and quality of life (measured by the Short Form-36 Physical Functioning (SF-36 PF) score). Calf skeletal muscle outcomes

consist of changes in PGC-1α abundance, mitochondrial quantity and activity, capillary density, ROS-related tissue damage, and autophagy.

SPECIFIC AIMS

Primary Aim. Among 212 participants with PAD, we will determine whether those randomized to metformin have greater improvement or less decline in six-minute walk at 6-month follow-up, compared to those randomized to placebo. *We hypothesize that PAD participants randomized to metformin will achieve greater improvement or less decline in the six-minute walk at 6-month follow-up, compared to placebo.*

Secondary Aim #1. Among 212 participants with PAD, we will determine whether those randomized to metformin have greater improvement or less decline in maximal treadmill walking time, pain-free treadmill walking time, the WIQ distance score, the WIQ speed score, and the SF-36 PF score at 6-month follow-up, compared to those randomized to placebo. *We hypothesize that PAD participants randomized to metformin will have greater improvement or less decline in maximal treadmill walking time, pain-free treadmill walking time, the WIQ distance score, the WIQ speed score, and the SF-36 PF score at 6-month follow-up, compared to placebo.*

Secondary Aim #2. Among 212 participants with PAD, we will determine whether those randomized to metformin have greater improvement or less decline in brachial artery FMD, compared to those randomized to placebo. *We hypothesize that PAD participants randomized to metformin will achieve greater improvement or less decline in FMD at 6-month follow-up, compared to placebo.*

Secondary Aim #3. Among 106 participants with PAD, we will perform muscle biopsies to determine whether metformin promotes favorable changes in calf muscle measures of AMP activated protein kinase (AMPK), mitochondrial biogenesis (PGC-1α), mitochondrial mass (citrate synthase, mitochondrial to nuclear DNA ratio), mitochondrial activity (COX and SDH enzyme activity), calf muscle capillary density, autophagy (LC3, P62, PINK1, PARKIN), and oxidative stress damage (4-hydroxynonenal, protein carbonyls), compared to placebo. *We hypothesize that PAD participants randomized to metformin will have more favorable changes in each calf skeletal muscle measure at 6-month follow-up, compared to placebo.*

If our hypotheses are correct, the PERMET Trial results will have a major impact on preserving mobility and improving quality of life in the large and growing number of people with PAD.

BACKGROUND:

Lower extremity PAD is common and associated with mobility loss. PAD affects 8 million people in the U.S. and more than 200 million worldwide (59,60). Our work and that of others demonstrate that people with PAD have greater functional impairment, more rapid functional decline, and faster mobility loss than those without PAD (1-7). Chronic disability, such as that associated with PAD, accounts for more than half of the U.S. health burden (61). Yet therapeutic advances have not kept pace with the growing burden of disability from chronic disease (61).

Only two medications, pentoxifylline and cilostazol, are FDA approved for treating PAD-associated walking impairment. Of these, recent data show that benefits from pentoxifylline are comparable to placebo and benefits from cilostazol are modest (62-66). No new medications for PAD-related walking impairment have been FDA approved since 1999. New therapies are urgently needed to improve walking performance in patients with PAD.

Patients with PAD experience ischemia of calf skeletal muscle during walking activity, when metabolic demands exceed oxygen supply. Patients with PAD experience reperfusion of calf

skeletal muscle during rest, when blood supply increases to meet oxygen requirements. Re-perfusion of target tissue (i.e. calf muscle) after ischemia increases oxidative stress, generates reactive oxygen species (ROS), damages skeletal muscle fibers, and is associated with impaired mitochondrial activity (8-10,13,14). Consistent with this paradigm, calf muscle from PAD patients has a quantitative mitochondria dysfunction, with reduced ATP production (8,11-14,16). In two separate studies, poorer mitochondrial activity was associated with poorer walking performance in people with PAD (14,16).

Pathophysiologic changes observed in skeletal muscle of people with PAD are similar to pathophysiologic changes observed in aging skeletal muscle (14-16,67-71). Reduced mitochondrial activity, increased oxidative stress, reduced peripheral skeletal muscle perfusion, and reduced skeletal muscle capillary density are observed both in people who have PAD and in elderly people who do not have PAD (8-10,13-16,67-71). Interventions that improve skeletal muscle health and functioning in PAD may be potentially therapeutic for elderly people with functional impairment and decline due to sarcopenia and muscle dysfunction.

Metformin, a biguanide medication, derived from the French lilac (Galega officinalis), is the most widely prescribed medication for Type 2 diabetes. First discovered in 1922, metformin has been available for prescription use in the United States since 1995. Metformin is inexpensive, widely accessible, and well-tolerated. Growing preclinical evidence shows that metformin has previously unrecognized therapeutic properties that include beneficial effects on skeletal muscle and direct, favorable effects on the vasculature, resulting in improved perfusion and blood flow (21-31,43,44,47-57). These therapeutic properties target pathophysiologic abnormalities present in PAD. Specific therapeutic properties of metformin that may improve walking ability in PAD include increased expression of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), a major regulator of mitochondrial biogenesis, increased mitochondrial activity, increased capillary density of ischemic calf muscle, reduced oxidative stress, improved endothelial function, and increased autophagy (19-33,37,42-54,72-74). Most of these favorable changes are mediated by AMP activated protein kinase (AMPK). Metformin increases levels of AMPK (23,24,30,51,75,76), including in ischemic tissue (54). We hypothesize that favorable effects of metformin on calf skeletal muscle and on calf muscle perfusion will improve walking performance and prevent mobility loss in PAD. Examples supporting our hypotheses are provided below.

Metformin increases abundance of PGC-1α, a major regulator of mitochondrial quantity and activity, and increases mitochondrial enzyme activity in skeletal muscle (30-31). For example, Suwa and colleagues fed metformin enriched chow to rats vs. regular chow (without metformin) to separate rats and examined the soleus and gastrocnemius muscles after 14 days. Phosphorylated Acetyl CoA Carboxylase levels in the soleus and gastrocnemius muscles were higher in rates fed metformin group, by 55% and 32%, respectively, compared to rats in the control group (30). PGC-1α levels in soleus Type 1 muscle fibers, soleus Type 2 muscle fibers, and gastrocnemius muscle increased by 35%, 49%, and by 79%, respectively, in rats who received metformin-enriched chow, compared to the control group. Levels of pyruvate kinase and hexose kinase were also higher in skeletal muscle of the metformin treated group, compared to control (30). These and other preclinical data showed that metformin increased skeletal muscle abundance of PGC-1α and improved mitochondrial protein activity, consistent with increased energy production (29-53). We hypothesize that metformin-induced increases in mitochondrial mass, mitochondrial biogenesis, and mitochondrial activity in calf skeletal muscle will improve skeletal muscle function and walking ability in people with PAD.

Metformin is well known to increase AMPK (23,24,30,51,75,76). AMPK activators, such as metformin, improve oxidative metabolism and promote mitochondrial regeneration, by increasing mitophagy and mitochondrial biogenesis, and by stimulating mitochondrial activity (45,46,75-78). Baltgalvis and colleagues studied whether the AMPK activator, R118, decreased skeletal muscle fatigability and improved exercise tolerance in a mouse model of PAD (78). The AMPK activator R118 significantly reduced muscle fatigability, increased citrate synthase activity in the plantaris muscle, and increased muscle perfusion immediately after exercise. Exercise capacity and total wheel running time also improved in the mouse models of PAD fed the AMPK activator R118, compared to those who did not receive R118 (78). These changes were accompanied by increased mitochondrial activity, improved skeletal muscle perfusion, and increased nitric oxide availability. Importantly, in this study, cilostazol, one of two drugs FDA approved for walking impairment in PAD, did not increase citrate synthase or increase running time to the same degree as the AMPK activator (78). In a Phase I trial, R118 was found to have excessive adverse events in humans (79) and therefore will not be pursued as a therapy for PAD. In separate studies, AICAR another agent that activates AMPK, improved skeletal muscle oxidative enzyme activity and increased PGC-1α in rodents, indicating improved mitochondrial efficiency and/or mitochondrial biogenesis (46,80). Together, these data support our hypothesis that metformin, a potent activator of AMPK, will improve skeletal muscle function and improve walking performance in people with PAD.

Pre-clinical evidence shows that metformin reduces ROS by direct and indirect mechanisms (29,48,49,52). For example, metformin reduces ROS by increasing mitochondrial biogenesis (levels of PGC-1α) (29) and by upregulating expression of antioxidants (29,48). Metformin has been shown to attenuate mitochondrial ROS production without reducing respiratory capacity (49,52). In human aortic endothelial cells, metformin blocked palmitic acid-induced increases in ROS in a dose dependent fashion (48). In summary, pre-clinical evidence shows that metformin reduces ROS and that this benefit occurs via multiple biologic pathways (29,48,49,52). PAD patients are an ideal population in which to study the effect of metformin on oxidative stress, because PAD patients have higher levels of oxidative stress than those without PAD (8,12,13). In the PERMET Trial, we will determine whether metformin reduces markers of ROS induced tissue damage in calf skeletal muscle.

Autophagy, the mechanism for disposing of damaged organelles, is particularly relevant to PAD, because of the calf muscle reactive oxygen species (ROS) generated from exposure to ischemia/reperfusion during daily walking activity (8-10). Decleves and colleagues studied the effects of metformin on ischemia reperfusion injury in rats, by removing one kidney in Wistar rats and ligating the renal artery supplying the remaining kidney (82). The ischemic kidney was subsequently re-perfused for 24 hours to induce ischemia reperfusion injury. In the kidney damaged by ischemia reperfusion, metformin increased the macroautophagy protein LC3 and normalized ischemia reperfusion induced changes in P62/SQSTM1 and PINK1 expression (82). The authors concluded that metformin promotes autophagy, protects cells, and limits damage to tissue that is exposed to ischemia reperfusion injury (82). Metformin also promotes mitophagy (58,82), a specific process by which damaged mitochondria are removed from tissue.

Preclinical evidence shows that metformin increases capillary growth in ischemic tissue (54,56,83). Metformin-induced increases in AMPK, for example, increase angiogenesis (84). Increases in endothelial nitric oxide synthase (eNOS) also promote blood vessel growth (85). Takahashi and colleagues demonstrated that metformin stimulates revascularization via an eNOS dependent pathway in an ischemic hind limb mouse model. In this mouse model of PAD, Takahashi and colleagues assigned mice to receive either oral metformin or oral saline (54). The metformin treated mice had greater capillary and arteriole density, measured by CD31

staining, and greater flow, measured by laser Doppler, in the ischemic hind limb as compared to the control group (54). However, eNOS deficient knock-out mice with hind limb ischemia did not experience improved hind limb flow with metformin, suggesting that the improved perfusion was related to metformin-induced increases in eNOS. These data support our hypothesis that metformin will increase capillary density and vascular flow in calf muscle in PAD.

Metformin is the only anti-diabetic drug that improves cardiovascular health (26-28,54 34-40,86-88). This benefit is associated with improved vascular tone and tissue perfusion. AMPK is well known to increase levels of eNOS in endothelial cells (89,90,91). By increasing AMPK, metformin increases levels of NOS in endothelial cells (29,54,57). eNOS catalyzes the production of nitric oxide (NO), a major cell signaling molecule that modulates vascular tone. NO acts directly on the endothelium to promote vasodilation and increase vascular flow. Because metformin induces increases in AMPK-stimulated eNOS, we hypothesize that metformin will improve endothelial function, vasodilate lower extremity vessels, and increase perfusion to calf skeletal muscle. We hypothesize that this action of metformin, which has been demonstrated in several animal models, including an animal model of PAD, will maximize flow to calf muscle (31,54,57,84,91). Consistent with our hypothesis, two small non-randomized and non-controlled studies of PAD patients showed that metformin was associated with increased lower extremity flow, measured by plethysmography (43,44). However, no randomized controlled studies of metformin in PAD have been conducted.

Pathophysiologic changes in calf skeletal muscle in people with PAD include reduced mitochondrial activity and increased levels of oxidative stress damage compared to people without PAD (8,11-14,16). Pathophysiologic changes in calf skeletal muscle have been linked to impaired walking ability in PAD (14-17). Metformin increases AMPK (75,76), which is well known to promote mitochondrial biogenesis and increase the quantity and activity of mitochondria (29,30,33,46,77,78). Metformin also reduces damage due to oxidative stress, increases capillary growth, and promotes autophagy (20,27,32,47,52-56). We hypothesize that favorable effects of metformin on calf skeletal muscle will improve walking performance in PAD. The activation of AMPK and eNOS by metformin are expected to improve systemic endothelial function and enhance calf perfusion in PAD.

A small uncontrolled trial supports our hypothesis that metformin will improve walking performance in PAD (44). Montanari and colleagues studied metformin (2.0 grams per day) in 11 men with symptomatic PAD who did not have diabetes (44). Participants were age 49 to 70. No women were included. There was no control group. At baseline and every month for six-months, participants were tested with lower extremity strain gauge plethysmography and a treadmill walking test. At six-month follow-up, post-ischemic lower extremity blood flow improved from 4.20+0.30 to 5.22+0.41 100 ml/min ($P<0.001$). Pain free walking time improved from 1.22 + 0.40 to 3.22+0.45 minutes ($P<0.001$). Maximal treadmill walking time improved from 2.67 + 0.62 to 5.68+0.63 ($P<0.001$) (44). A separate study by Sirtori and colleagues showed that metformin improved plethysmography-measured blood flow in 15 patients with PAD who were treated with 2,500 mgs of metformin daily for six months (43). Lower extremity arterial flow increased by 17.3% at 3-month follow-up and by 40.0% at 6-month follow-up (43). These improvements dissipated when participants were switched from metformin to placebo (38). However, neither study was randomized. The importance of improvement of blood flow, demonstrated by Sirtori et al, is unclear if walking performance is not simultaneously measured. A definitive randomized controlled trial is needed.

Innovation

No prior or ongoing randomized trials are studying metformin in people with PAD. The PERMET Trial will capitalize on recent evidence identifying previously unrecognized therapeutic properties of metformin and determine whether metformin can be re-purposed to improve walking performance in PAD. Metformin is an inexpensive, widely available, and well-tolerated drug for Type 2 diabetes. If our hypotheses are correct, results of this trial can be applied immediately in clinical practice.

No prior studies have assessed the effects of metformin on calf skeletal muscle characteristics in people with PAD. Using muscle biopsies at baseline and 6-month follow-up, we will determine whether metformin promotes favorable changes in calf muscle measures based on preclinical evidence of favorable effects of metformin on: AMPK, mitochondrial biogenesis, mitochondrial mass, mitochondrial activity, calf muscle capillary density, autophagy, and oxidative stress damage, compared to placebo. Preclinical evidence supports our hypothesis that metformin will improve each of these measures. Our plan to study a drug that increases mitochondrial quantity and activity and favorably alters other impaired aspects of calf skeletal muscle in PAD is an innovative approach to improving walking performance in PAD.

Our plan to simultaneously study the ability of metformin to increase walking performance and improve vascular function and calf skeletal muscle characteristics is innovative. PAD is associated with both calf skeletal muscle pathophysiologic impairments and impaired endothelial function. Our plan to simultaneously measure metformin-related changes in walking performance and metformin-related changes in calf skeletal muscle measures and vascular function (brachial artery FMD) allows us to determine whether improvement in each muscle measure and improvement in vascular function each corresponds to improvement in walking performance in PAD. This approach allows us to identify the most promising new biologic pathways for improving walking performance in people with PAD.

Preliminary Data

Our prior work established that people with PAD have greater functional impairment and faster functional decline than people without PAD (1-6). Preliminary evidence from our observational studies shows that PAD participants with diabetes taking metformin have better functional performance, slower functional decline, less mobility loss, and greater improvement in brachial artery FMD than PAD participants with diabetes not taking metformin. We have completed 166 calf muscle biopsies in people with PAD and demonstrated our ability safely complete these biopsies. A definitive randomized controlled trial is now needed to test our hypotheses that metformin prevents functional decline in PAD participants without diabetes. Our preliminary work demonstrates the validity and feasibility of our methods.

INCLUSION AND EXCLUSION CRITERIA:

Inclusion criteria. All participants will have PAD. PAD will be defined as follows. First, an ankle brachial index (ABI) ≤0.90 at the baseline study visit is an inclusion criterion for PAD. Second, potential participants who have an ABI > 0.90 but ≤ 1.00 and experience a 20% or higher drop in ABI after heel-rise exercise will be eligible. Third, potential participants with an ABI > 0.90 who have vascular lab evidence of PAD or angiographic evidence of PAD who have ischemic symptoms during the six-minute walk and/or treadmill exercise stress test will be eligible. Fourth, potential participants with a history of lower extremity revascularization who do not meet the criterion above and have an ABI > 0.90 with a 20% or higher drop in ABI after heel-rise exercise will be eligible.

Exclusion criteria.

1. Above- or below-knee amputation.
2. Critical limb ischemia.

3. Wheelchair-bound or requiring a walker to ambulate.
4. Walking is limited by a symptom other than PAD.
5. Current foot ulcer on bottom of foot.
6. Diabetes mellitus defined as one or more of a) patient report of physician diagnosed diabetes mellitus, b) use of one or more diabetes medications, c) two baseline hemoglobinA1C values of ≥6.5, d) two fasting glucose values >126 mg/dl. [NOTE: the second fasting glucose and hemoglobin A1C values will be at the discretion of the principal investigator. For example, if the first glucose value is >300 or the first A1C value is >6.9, then investigators may decide not to repeat the value. Potential participants who report a prior history of diabetes mellitus, which is now resolved (for example after significant weight loss) will be eligible at the investigator's discretion, if they are not taking metformin.
7. Chronic kidney disease defined as GFR ≤45. [NOTE: if GFR is 40-44, investigator discretion will be used to determine if a repeat test may be performed. If the second GFR value is >45, the participant may be included.]
8. Chronic liver disease defined as two or more hepatic function tests ≥2.0 times the upper limit of normal. [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.]
9. Failure to successfully complete the 2-week study run-in, defined as unable to tolerate metformin and/or failing to take the medication daily for 10 or more days in the two-week period.
10. Planned lower extremity revascularization, orthopedic surgery, or other major surgery during the next six months.
11. Lower extremity revascularization, 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 or dementia. However, investigator discretion may be used to allow some people below this threshold to participate, if the investigator determines there is another reason for their lower score, including lack of sufficient familiarity with the English language or lack of sufficient education to achieve a score of 23 or higher. Note that the MMSE includes some spelling and English writing proficiency.
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 metformin or has taken metformin in past six months.
16. Increase in angina or angina at rest
17. Non-English speaking.
18. Visual impairment that limits walking ability.
19. 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.

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

STUDY-WIDE NUMBER OF PARTICIPANTS: NA

STUDY-WIDE RECRUITMENT METHODS: NA

MULTI-SITE RESEARCH:
To ensure recruitment targets are met, we will use additional sites, Ochsner Medical Center and Tulane University. Dr. Lydia Bazzano will serve as the site investigator for PERMET at Ochsner and Tulane. Participants will complete all study measures at Ochsner or Tulane's site. However, muscle biopsies and long-term storage of blood may not be offered at Oschner or Tulane. The Tulane IRB acknowledges Ochsner's IRB decision based on a reliance agreement between each site's IRB to facilitate single IRB review of multi-site research. The University of Florida (UF) will also be used as an additional site for recruitment and data collection. Dr. Scott Berceli will serve as the site investigator at UF. Participants will complete all study measures at UF; however, muscle biopsies and long-term storage of blood may not be offered at UF. The University of Chicago will also be used as an additional site for recruitment and data collection. Dr. Tammy Polonsky will serve as the site investigator at the University of Chicago. Participants may complete some study measures (e.g. brachial artery FMD and/or muscle biopsy) at Northwestern after signing a Northwestern consent form.

STUDY TIMELINES:
Each participant will participate in the study for six months after randomization. We plan to enroll 212 participants over a 48 month period.

STUDY ENDPOINTS:
Our primary outcome is change in six-minute walk at 6-month follow-up. Secondary outcomes are 6-month changes in treadmill walking performance, brachial artery flow-mediated dilation (FMD) (a measure of endothelial function), calf muscle biopsy biochemical measures, patient-reported walking performance (measured by the Walking Impairment Questionnaire (WIQ)), and quality of life (measured by the Short Form-36 Physical Functioning (SF-36 PF) score). Calf skeletal muscle outcomes consist of changes in PGC-1α abundance, mitochondrial quantity and activity, capillary density, ROS-related tissue damage, and autophagy.

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.

Baseline testing. Participants will provide informed consent at baseline. An ankle brachial index (ABI) will be performed. Questionnaires will be administered and physical functioning tests will be performed. A treadmill exercise stress test will be performed. A blood sample will be obtained and height and weight will be measured. A brachial-artery flow-mediated dilation (FMD) test will be performed. If the participant agrees to the optional muscle biopsy, they will be asked to undergo a biopsy and return approximately seven days afterward for an incision site check. Baseline testing will require testing at multiple visits performed over multiple days. Research procedures are described in more detail below.

Run-in. Participants will be given a 14-day supply of 500 mgs daily of metformin. The participant will return at the end of the 14 days to monitor adherence and will be instructed to contact study staff if they experience adverse symptoms.

Randomization. Participants will be randomized to either metformin or placebo using a SAS computer program. We will use block randomization with block sizes randomly selected from 4 to 6 to ensure balance. Randomization will be stratified on two factors: according to whether or not the participant has impaired fasting glucose and according to baseline six-minute walk performance. Impaired fasting glucose will be defined as a fasting glucose value of 101-125 mg/dl and/or an A1C value >5.6 and <6.5. For six minute walk performance, participants will be stratified according to whether their value is above or below the baseline median value for prior PAD trials conducted by the investigative team.

Intervention and placebo. Metformin will be supplied in 500 mg pills. Participants randomized to receive metformin will begin by taking 500 mgs twice daily (i.e. two pills for a total of 1,000 mgs daily) for two weeks. Participants tolerating 1,000 mgs daily will have their dose increased to 2,000 mgs daily (i.e. two pills twice per day for a total of 2,000 mgs daily) for the remainder of the study. If a participant is not able to tolerate their dose, they will be prescribed the highest dose they are able to tolerate. Participants randomized to placebo will receive identical-appearing pills and will have their dose increased from taking one pill twice daily (i.e. two pills daily) to two pills twice daily (i.e. four pills daily) on the same schedule as those randomized to metformin in order to maintain blinding. Participants will be asked to keep a daily log tracking their pill adherence.

Three-month follow-up testing. Participants will be asked to return to the medical center after three months to monitor adherence (comparing the pill log to the number of pills remaining in the bottle) and to receive a new three-month supply of study pills. Participants will also be telephoned every month to ask whether they have been hospitalized, assess their adherence to study medication, and ask whether they have undergone a procedure involving iodinated contrast dye.

Participants may be asked to return for additional visits at the discretion of the investigator to monitor adherence or for safety during the study period. For example, participants will be asked to return for a repeat GFR test if they undergo a procedure with their physician requiring intravenous contrast dye.

Six-month follow-up testing. Participants will return after taking the study pills for six months for final follow-up testing. All measures and tests that were conducted at baseline will be repeated.

In some cases, participants may be asked to take study pills for longer than six months. For example, if a participant has an illness that prevents them from returning at six months for follow-up testing or if they are out of town during their six-month follow-up testing window. In these instances, participants will be asked to continue taking their study pills for more than six months. Participants may be asked to take up to an additional three months of study pills.

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 Dr. Lloyd-Jones or other qualified personnel.

COVID-19 Questionnaire. Currently enrolled and past participants may be called to see if they are willing to complete a questionnaire related to the COVID-19 pandemic. The questionnaire will be completed over the telephone and will help investigators determine how the pandemic is affecting older adults with PAD and how physical activity levels are affected during this time.

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 a participant qualifies for a heel-rise ABI based on their initial ABI, they will be asked to perform 50 heel-rises at a rate of one per second followed by additional measurements of the right and left brachial, DP, and PT pressures. 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.

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 (1-5,119). Distance covered in six minutes is recorded. Change in six-minute walk performance between baseline and 6-month follow-up is our primary outcome.

Treadmill testing. The Gardner graded treadmill exercise test is the standard, accepted treadmill protocol for measuring change in maximal treadmill walking time in response to interventions in PAD participants (92,93,99,104-106). 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, treadmill speed is started at 0.50 mph and increased by 0.50 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 (92,93,99,104-106).

Brachial Artery FMD. Brachial artery imaging will be performed by our Registered Cardiac Sonographer, with more than 15 years' experience measuring brachial artery FMD (92,107,108). With the participant supine, a blood pressure cuff over the upper arm is inflated for five minutes at a specified supra-systolic pressure according to protocol. The brachial artery is imaged (B-mode and Doppler) 5 to 9 cm above the antecubital fossa using a linear array vascular ultrasound transducer (Siemens Medical Solutions, Sequoia Model #256, frequency 8 MHz). FMD is calculated as the percent change in brachial artery diameter 60 seconds after cuff release. Changes in FMD will be read by Dr. James Stein's University of Wisconsin Atherosclerosis Imaging Research Program Core Laboratory by a single reader blinded to participant characteristics and group assignment. Measurement reproducibility in Dr. Stein's laboratory has a median FMD difference of 0.02% (inter-quartile range: -0.03–0.04).

Blood collection and long-term storage. At baseline and six-month follow-up study visits, participants may have approximately 50 mls of blood drawn for processing and long-term

storage at -70 degrees Celsius. Blood will be tested for glucose, hemoglobinA1C, and kidney and liver function at baseline. If the participant had glucose, hemoglobinA1C, or kidney or liver functioning tested with their own physician within the previous six months prior to the study baseline testing date, the results from the prior test may be used for eligibility, in place of the study test. If the fasting glucose level is >126 or if the glomerular filtration rate (GFR) is 40-44 or if the A1C is 6.5-6.9, blood may be drawn for repeat testing. GFR will be calculated using the CKD-EPI formula. Approximately 10% of participants selected by chance will have an additional set of 45 mls of blood drawn for quality assurance, for a total of 95 mL at each visit. A subset of participants will be invited to have an extra 9 ml tube of blood obtained to measure senescent cells (i.e. P16+ cells). For participants who agree to the optional element, blood will be obtained in blood tubes supplied by Sapere Bio, labeled with study ID number, and sent to Sapere Bio (400 Park Offices Dr, Research Triangle Park, NC 27709) for measurement of senescent cells. Sapere Bio will either destroy or return to Northwestern the remaining blood after measuring senescent cells. 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 will not be shared with the study participants.

Calf muscle biopsies. Muscle biopsies will be performed by co-investigator Robert Sufit, MD, a board-certified neurologist with > 30 years of experience performing muscle biopsies. Dr. Sufit completed all biopsies for our pilot study. 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. This site represents greatest calf muscle diameter in >95% of individuals (109). Anesthesia is achieved with subcutaneous lidocaine. Subcutaneous and adipose tissue are dissected. Approximately 250 to 300 mgs of muscle tissue is removed. 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. 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. Participants return for an incision site check one week later. At 6-month follow-up, we will repeat the biopsy, making the follow-up incision adjacent to the baseline incision. In the open biopsy, muscle tissue is directly visualized, providing major advantages over blind needle biopsy.

Measures of muscle protein and mitochondrial activity will be performed by Dr. Leeuwenburgh's laboratory at University of Florida. Methods are well standardized and validated with excellent test re-test reliability in the University of Florida laboratory (110). Muscle specimens obtained in Chicago will be immediately frozen and stored at -70° Celsius. Specimens will be shipped on dry ice to the University of Florida for testing. Protein expression of PGC-1α, AMPK (and its

phosphorylation state), measures of autophagy (LC3, P62, Parkin, PINK) and oxidative damage (4HNE and protein carbonyls) in whole tissue homogenate will be performed using Standard Western Blot techniques as described previously (111). mtDNA content will be measured using quantitative real time PCR (qRT-PCR) following standard protocols and as described previously (112). Succinate dehydrogenase (SDH) and citrate synthase activity will be measured via standardized assay kits according to the manufacturer's instructions (110). COX activity will be determined spectrophotometrically at 30°C as the maximal rate of oxidation of fully reduced Cytochrome C, measured by the change in absorbance at 550 nm. (110). In exploratory analyses, eNOS and mTOR (and its phosphorylation state) will be measured in Dr. Leeuwenburgh's laboratory using standard methods. Dr. Peterson will receive specimens at University of Kentucky for measurement of capillary density and SDH content activity on cryostat sections. Fluorescently-labeled lectin-positive endothelial cells will be expressed per muscle fiber using automated image analysis (113). SDH activity per muscle fiber will be quantitfied using standard histochemical techniques.

Other measures related to skeletal muscle quality and function may also be performed at Northwestern or at other institutions (including University of Florida and University of Kentucky) as designated by the Principal Investigator.

Participants will be asked to return approximately seven days following the calf muscle biopsy for an incision site check.

Medications. Participants and their physicians will be asked not to change their medications during the study if possible. We will systematically record names and doses of medications and inquire about medication changes at follow-up. If we find differences in the use of specific medications between study groups (such as statins or cilostazol), we will adjust for these differences.

Other measures. Body mass index (BMI) will be assessed at baseline and follow-up by objectively measuring height and weight. Weight will also be measured at monthly adherence visits. Patient report will be used to document comorbidities. 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 chair stands.

DATA AND SPECIMEN BANKING:
Muscle 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. Dr. McDermott will obtain

IRB approval prior to releasing any blood or muscle specimens for analysis, other than those tests specifically named in this application.

DATA AND SPECIMEN MANAGEMENT:

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 calculations. In the power calculations for our primary and secondary aims, we anticipate that 192 participants of 212 randomized (i.e. 91%) will complete the 6-month trial. This 91% 6-month follow-up rate is a conservative estimate based on our previously completed randomized controlled trials of PAD participants (92,93). For our primary specific aim, we will determine whether metformin improves six-minute walk performance at 6-month follow-up, compared to placebo. In our GOALS trial of home-based exercise (93), the estimated standard deviation (SD) of change in 6-min walk at 6-month follow-up was 69 meters in both the intervention and control groups. A total of 192 participants completing the PERMET Trial provides 80% power to detect a minimum difference of 0.41 SD, or a 28 meter change in the six-minute walk test between the metformin vs. placebo groups, using a two-sided two-sample t-test with a significance level of 0.05. Prior studies have defined clinically meaningful changes in the six-minute walk as 30 meters (small meaningful change) and 50 meters (large meaningful change) (100-102). Thus, power should be adequate to detect a clinically meaningful difference in six-minute walk change between the two groups.

For Secondary Aim 1, 192 participants completing 6-month follow-up testing provide 80% power to detect a minimum difference of 0.41 SD in change of maximal treadmill walking distance, the WIQ, and the SF-36 PF score at 6-month follow-up, respectively, between the metformin and placebo groups. From our GOALS trial of exercise in PAD participants (93), the observed differences in change in maximal treadmill walking distance, the WIQ distance, and the WIQ speed scores at 6-month follow-up between the intervention and control groups were 0.50 SD, 0.46 SD, and 0.44 SD, respectively. From our SILC trial of exercise in PAD participants (92), the observed differences in change in SF-36 PF score at 6-month follow-up between the intervention and control groups was 0.61 SD. Thus, we should have sufficient power to detect similar effect sizes in the PERMET Trial. For secondary aim #2, 192 participants completing 6-month follow-up provides 80% power to detect a minimum difference of 0.41 SD in change of FMD at 6-month follow-up between the metformin and placebo groups. In our SILC randomized trial of exercise in PAD participants (92), the observed difference in FMD was 0.49 SD. Thus, we should have sufficient power to detect similar effect sizes for brachial artery FMD. For secondary aim #3, we will use calf skeletal muscle biopsies to determine whether metformin significantly increases calf skeletal muscle levels of AMPK, PGC-1α, citrate synthase, mitochondrial to nuclear DNA ratio, activity of COX and SDH enzymes, capillary density, measures of autophagy, 4-hydroxynonenal, and protein carbonyls. A total of 96 participants undergoing muscle biopsy and completing 6-month follow-up testing provides 80% power to detect a minimum difference of 0.58 SD in change of each muscle measure at 6-month follow-up between the metformin and placebo groups. The observed improvements in calf skeletal muscle levels of PGC-1α and AMPK were 2.03 SD and 4.92 SD in a previous study of resveratrol in older men and women (114). In the study by Suwa et al in which rats were treated with metformin for 14 days, PGC-1 alpha and Citrate Synthase levels increased in red and white gastrocnemius muscle by more than 1.60 SD and 1.10 SD, respectively (30). Based on these data, and anticipating that metformin is at least as efficacious as the dietary supplement resveratrol, our power should be adequate.

Statistical Analyses. Analyses will be performed according to the intention to treat principle. Data will be analyzed according to each participant's originally assigned group, irrespective of whether the participant adheres to his/her group assignment. For our primary specific aim, we will perform analysis of covariance (ANCOVA) based on multivariable linear regression analysis with the 6-month change in six-minute walk as the dependent variable to compare two groups adjusting for baseline six-minute walk and study site to estimate and test the mean treatment effect (115). The unadjusted two-sample t-test will also be conducted, but the primary analysis for the primary outcome is the ANCOVA based on the multivariable linear regression analysis.

For the secondary aims, we will conduct similar analyses as for the Primary Aim, except that the response (dependent) variables will be 6-month changes in maximal treadmill walking time, pain-free treadmill walking time, the WIQ distance score, WIQ speed score, and the SF-36 PF score for secondary aim #1, brachial artery FMD for secondary aim #2, and calf skeletal muscle measures for secondary aim #3, respectively, and the covariates adjusted for will consist of each respective outcome at baseline and study site. In addition to testing the treatment effect on each outcome separately, we will adaptively combine the statistical tests on related outcomes such as maximal treadmill walking time, pain-free treadmill walking time, the WIQ scores (distance and speed scores), and the SF-36 PF score and construct a statistical test for the overall treatment effect under the assumption that metformin has a synchronized effect on all pertinent outcomes. Specifically, we will construct a linear combination of the individual test statistics with optimal weights based on correlations as well as hypothesized alternatives. The null distribution of the linear combination can be generated via permutation method. This test will improve statistical power for detecting effects of the intervention on a set of correlated outcomes (115-116).

In a sensitivity analysis for the primary outcome, we will repeat the comparison adjusting for additional baseline covariates such as age, sex, race, ABI, BMI, smoking status and comorbidities. If there is evidence that the normality assumption for the distribution of change in six-minute walk is severely violated, we will either apply the appropriate transformation to the six-minute walk distance before conducting ANCOVA or perform an appropriate robust regression analysis instead of the linear regression.

In an exploratory analysis, we will determine the degree to which improvements in the calf muscle measures and brachial artery FMD are associated with improved 6-minute walk distance and other outcomes. We will first calculate the individual Pearson correlation coefficients between the 6-month change in six-minute walk and 6-month changes in brachial artery FMD and calf skeletal muscle measures, respectively, among participants receiving metformin. We will also estimate the adjusted partial correlation coefficient in a multivariate regression, which includes all aforementioned measures as independent variables. In addition, we will examine whether 6-month changes in brachial artery FMD and calf skeletal muscle measures may mediate the treatment effect of metformin on 6-month changes in six-minute walk and maximal treadmill walking time, respectively. To this end, we will first use a linear regression model to estimate the treatment effect of metformin on 6-month change in six-minute walk (as described above). We will then add 6-month changes in brachial artery FMD and each calf skeletal muscle measure as independent variables to the linear regression model. Each variable will initially be added individually and then all variables will be added together. We will compare the estimated treatment effects of metformin between these models. The statistical significance of the attenuation will be assessed using the nonparametric bootstrap method to account for the correlation between the estimated coefficients in the two regression analyses. If the treatment

effect of metformin on change in six-minute walk is significantly attenuated after adjusting for these potential mediators, this finding will suggest that the treatment effect of metformin on six-minute walk might be achieved by the improvement of these potential mediators by metformin. These analyses will be repeated for treadmill walking performance.

PROVISIONS TO MONITOR THE DATA TO ENSURE THE SAFETY OF PARTICIPANTS:
A Data and Safety Monitoring Board (DSMB) will monitor safety throughout the study. The DSMB will meet at least 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. The biostatisticians will work with the DSMB to perform interim analyses. Adverse events will be monitored continuously throughout the study and will be reported to the DSMB in a timely manner according to pre-specified requirements. Analyses for each DSMB meeting will be completed according to the requests of the DSMB. Adverse event rates and interim study results will be reviewed and discussed by the DSMB at the DSMB meetings. At least four categories of adverse events will be defined: a) death; b) cardiovascular events (myocardial infarction, stroke, and coronary arrhythmias); c) hospitalizations; and d) injury or illness causing chronic disability. We will report all serious adverse events to the DSMB in a timely fashion.

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.

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 metformin. Metformin is FDA approved for improving glycemic control in patients with Type 2 Diabetes. Side effects are infrequent (44,95,96). In several randomized clinical trials completed to date, approximately five percent of individuals randomized to metformin discontinued the medication due to side effects (97-98). The most common side effects are gastrointestinal and include diarrhea, nausea or vomiting, flatulence, asthenia, indigestion, abdominal discomfort, and headache. Rarely, in the setting of significant chronic kidney disease or hepatic disease, metformin can cause lactic acidosis. However, recent evidence shows that the incidence of lactic acidosis is extremely low, even among people with chronic kidney disease (94-95, 117-120). Therefore, potential participants with a glomerular filtration rate (GFR) $\leq$ 45 ml/min/1.73m2 and those with chronic liver disease (i.e. two or more liver function tests $\geq$ 2.0 times the upper limit of normal) will not be eligible. Current clinical practice guidelines support starting metformin at a GFR > 45 ml/min/1.73m2 (95). We will measure a renal and hepatic profile at baseline to identify people who have a GFR $\leq$ 45 or liver function test results that are $\geq$ 2.0 times normal. These individuals will not be eligible for the PERMET Trial. Participants will be provided with a list of metformin side effects and will be provided with Dr. McDermott's home and cellular telephone, in case the participant develops side effects or has questions. If a participant undergoes a diagnostic or therapeutic procedure requiring iodinated intravenous contrast dye with their own physician, participants are instructed to discontinue the study pills the day of a scheduled procedure with contrast dye. The

participant will not resume the study pills until a repeat GFR test shows GFR ≥45. GFR testing may be repeated as needed until a GFR ≥45 is achieved.

Risks associated with the muscle biopsy. The muscle biopsy is associated with several potential risks. These include discomfort during the muscle biopsy procedure and for 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. In addition, potential participants who are asked to hold their anti-platelet therapy during the week leading up to the muscle biopsy procedure may experience a cardiovascular event related to the temporary discontinuation of the anti-platelet therapy. First, to minimize risk related to muscle biopsy, all participants undergoing muscle biopsy will receive a written hand-out 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. A seven day follow-up incision site check will be performed by Dr. McDermott or Dr. Sufit. Second, permission from the participant's physician will be required before participants are asked to discontinue anti-platelet therapy.

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 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 and FMD. 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 and FMD measurement, 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. 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.

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 metformin could experience improved functional performance or less decline in functional performance, if our hypotheses are correct.

VULNERABLE POPULATIONS: NA

COMMUNITY-BASED PARTICIPATORY RESEARCH: NA

SHARING OF 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. They will be asked whether they would like ABI and stress test results mailed to their physician. They will not be provided with other study results routinely. However, participants will be notified of abnormal stress test, blood, or blood pressure results.

SETTING:
Baseline and follow-up data collection will take place at Northwestern Memorial Hospital in the Galter Pavilion (675 N. St. Clair) on floors 11 or 18 or in the Feinberg Pavilion (251 E. Huron) on the 15th floor, or at 750 N. Lake Shore Drive.

RESOURCES AVAILABLE:
Study coordinators who collect data are Bachelor's level or Master's level graduates who are Northwestern University employees. New study staff members are required to read study manuals and observe 1-2 study visits. Staff members are trained by senior, experienced staff in data collection and certification is performed by Dr. McDermott. A checklist is used for certification. Study staff receive feedback from Dr. McDermott on their certification performance. Staff members who are not fully ready to begin data collection are re-trained and then re-attempt certification with Dr. McDermott until data collection procedures are satisfactory as determined by Dr. McDermott. Staff members undergo re-certification, using detailed checklists, every six months. Dr. McDermott performs most of these re-certifications. Some of these re-certifications are performed by a project manager. Study staff members are directly supervised by a project manager and Ms. Kathryn Domanchuk, a Senior Clinical Research Associate, with more than 10 years' experience managing NIH-funded clinical trials.

PRIOR APPROVALS: NA

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.

In addition, PAD participants may be identified from among consecutive patients diagnosed with PAD in the non-invasive vascular laboratory at Northwestern Medical Group (NMG). Dr. Mark Eskandari is medical director of the non-invasive vascular laboratory at NMG and will assist with identifying potential participants from the non-invasive vascular laboratory. As director of the

vascular laboratory at NMG, Dr. Eskandari formally reads many of the non-invasive vascular laboratory tests. He maintains all non-invasive vascular test results in his vascular laboratory. As director of the vascular laboratory, Dr. Eskandari could conceivably contact the patients whose test results are maintained in his laboratory. However, Dr. Eskandari prefers that the contact of potential participants in studies come from the physicians referring him for testing. Lists of patients who have undergone lower extremity arterial testing in the non-invasive vascular laboratory are generated monthly and e-mailed from NMG to Dr. McDermott. 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. We have substantial experience with our recruitment methods for our previous or ongoing studies.

We also propose to obtain lists of consecutive patients with a diagnosis of lower extremity peripheral arterial disease and individuals at high risk for peripheral artery disease from Northwestern's Enterprise Data Warehouse (EDW). EDW lists will be provided by an individual who is employed by the Division of General Internal Medicine who has received training and permission to obtain the lists from the EDW.

Similar methods will be used as those described above, in which the patient's physician will be contacted via fax, telephone, page, or electronic message (EPIC or email) 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.

In addition, we will use newspaper, television, and radio advertising to identify potential participants for this study. We will also use brochures, flyers, or posters that we will post in relevant office practices and public areas. We will use advertising on public transportation 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.

We may also use CAPriCORN for recruitment. CAPriCORN is a PCORI-funded network of institutions in the Chicago area. The purpose of CAPriCORN is to assist investigators with recruitment for clinical trials. CAPriCORN has its own IRB (Chicago Area Institutional Review board - CHAIRB) and uses ICD-9 codes and the electronic health record to identify potential participants who have PAD. These patients with PAD will be mailed a recruitment letter that describes the trial and invites the patient to participate.

We will mail letters to Jesse Brown VA Medical Center patients with known PAD. A research coordinator with WOC status at JBVAMC will send letters to JBVAMC patients with PAD and make follow-up calls from JBVAMC. Participants recruited through these methods 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.

We may use Electronic Recruitment Through the NMHC Medical Record Committee to contact NM patients via MyChart. Patient lists will be obtained through the EDW for MyChart recruitment.

PAYMENT:
Participants will receive $25 for completing six-month follow-up testing.

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 or shared ride service (i.e. Uber/Lyft) fare, and mileage, if requested. A receipt will be required for taxi fare reimbursement. Participants will be provided up to $90 per visit for travel reimbursement. If they require the use of our taxi or shared ride service (i.e. Uber/Lyft service), we will estimate the fare on www.taxifarefinder.com or on the shared ride service 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 Uber/Lyft 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.

NUMBER OF LOCAL PARTICIPANTS:
We will identify and randomize 212 eligible participants. It will be necessary to consent many more than 212 participants to account for screen failures.

CONFIDENTIALITY: NA

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: NA

CONSENT PROCESS:
The "SOP: Informed Consent Process for Research (HRP-090)" will be followed. 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.

PROCESS TO DOCUMENT CONSENT IN WRITING:
The "SOP: Written Documentation of Consent (HRP-091)" will be followed.

DRUGS OR DEVICES:
Northwestern Memorial Hospital's Investigational Pharmacy will receive randomization assignments from the study's data management team and will prepare identical-appearing metformin or placebo according to the randomization assignment. A research study coordinator will pick up the study medication from the investigational pharmacy to give to the study participant.

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