PROTOCOL TITLE: Resveratrol To Improve Outcomes in older pEople with PAD (The Restore Trial)

PROTOCOL TITLE:
RESveratrol To Improve Outcomes in older pEople with PAD (The RESTORE Trial)

PRINCIPAL INVESTIGATOR:
Mary McDermott, MD
Department of General Internal Medicine
312-503-6419
mdm608@northwestern.edu

VERSION NUMBER:
Version #5.

VERSION DATE:
11.13.15
## Table of Contents

1.0 Objectives ................................................................................................................ 3  
2.0 Background ............................................................................................................... 4  
3.0 Inclusion and Exclusion Criteria .............................................................................. 8  
4.0 Study-Wide Number of Subjects ........................................................................... 10  
5.0 Study-Wide Recruitment Methods ........................................................................ 10  
6.0 Multi-Site Research ............................................................................................... 10  
7.0 Study Timelines ..................................................................................................... 11  
8.0 Study Endpoints ..................................................................................................... 11  
9.0 Procedures Involved ............................................................................................. 12  
10.0 Data and Specimen Banking ............................................................................... 21  
11.0 Data and Specimen Management ....................................................................... 22  
12.0 Provisions to Monitor the Data to Ensure the Safety of Subjects ......................... 24  
13.0 Withdrawal of Subjects* ...................................................................................... 24  
14.0 Risks to Subjects* ............................................................................................... 25  
15.0 Potential Benefits to Subjects .............................................................................. 26  
16.0 Vulnerable Populations ....................................................................................... 27  
17.0 Community-Based Participatory Research ......................................................... 27  
18.0 Sharing of Results with Subjects ......................................................................... 27  
19.0 Setting ................................................................................................................... 27  
20.0 Resources Available ............................................................................................ 27  
21.0 Prior Approvals .................................................................................................. 28  
22.0 Recruitment Methods ......................................................................................... 28  
23.0 Local Number of Subjects .................................................................................. 30  
24.0 Confidentiality .................................................................................................... 31  
25.0 Provisions to Protect the Privacy Interests of Subjects ........................................ 31  
26.0 Compensation for Research-Related Injury ....................................................... 31  
27.0 Economic Burden to Subjects ........................................................................... 31  
28.0 Consent Process .................................................................................................. 31  
29.0 Process to Document Consent in Writing ........................................................... 32  
30.0 Drugs or Devices ................................................................................................. 32  
31.0 References .......................................................................................................... 26  

---

PROTOCOL TITLE: Resveratrol To Improve Outcomes in older pEople with PAD (The Restore Trial)
1.0 Objectives

1.1 Purpose/ Objectives.

**PRINCIPAL SPECIFIC AIM**
Among 66 participants with PAD age 65 and older, we will determine whether resveratrol 500 mgs daily for six months and whether resveratrol 125 mgs daily for six months, respectively, each improve six-minute walk performance at 6-month follow-up, compared to placebo.

**SECONDARY SPECIFIC AIMS**

*Secondary Aim #1*. Among 66 participants with PAD age 65 and older, we will determine whether resveratrol 500 mgs supplementation daily for six months and whether resveratrol 125 mgs daily for six months each improve pain-free and maximal treadmill walking time at 6-month follow-up, respectively, compared to placebo.

*Secondary Aim #2*. Among 66 participants with PAD age 65 and older, we will determine whether resveratrol 500 mgs supplementation daily for six months and whether resveratrol 125 mgs supplementation daily for six months, respectively, each increase calf muscle biopsy-measured levels of peroxisome proliferative activated receptor-γ co-activator 1α (PGC-1α), SIRT-1, and calf muscle mitochondrial function as measured by citrate synthase and COX activity.

*Secondary Aim #3*. Among 66 participants with PAD age 65 and older, we will determine whether resveratrol 500 mgs supplementation daily for six months and whether resveratrol 125 mgs supplementation daily for six months each improve brachial artery flow-mediated dilation at 6-month follow-up, compared to placebo.

**Exploratory Aim**. Among participants with PAD age 65 and older, we will evaluate whether resveratrol 500 mgs supplementation daily for six-months is associated with greater increases or less declines in six-minute walk performance, and in maximal and pain-free treadmill walking performance, compared to resveratrol 125 mgs supplementation daily for six-months. Among participants with PAD age 65 and older, we will evaluate whether resveratrol 500 mgs supplementation daily for six-months is associated with greater increases or less declines in brachial artery flow-mediated dilation and in calf muscle mitochondrial measures, compared to resveratrol 125 mgs supplementation daily for six-months. Among participants with PAD age 65 and older, we will evaluate whether resveratrol 500 mgs supplementation daily for six-months is associated with greater increases or less declines in the physical function and mental health domains of the SF-36, compared to resveratrol 125 mgs supplementation daily for six-months. Among participants with PAD age 65 and older, we will evaluate whether resveratrol 500 mgs supplementation daily for six-months is associated with greater increases or less declines in the physical function and mental health outcomes domains of the SF-36, compared to placebo. Among participants with
PAD age 65 and older, we will evaluate whether resveratrol 125 mgs supplementation daily for six-months is associated with greater increases or less declines in the physical function and mental health outcomes domains of the SF-36, compared to placebo.

1.2 Hypotheses.

We hypothesize that PAD participants randomized to resveratrol 500 mgs daily and that PAD participants randomized to resveratrol 125 mgs daily will each achieve greater increases or less decline in six-minute walk performance at 6-month follow-up, compared to those randomized to placebo.

We hypothesize that PAD participants randomized to resveratrol 500 mgs daily and that PAD participants randomized to resveratrol 125 mgs daily will each achieve a greater increase or less decline in pain-free and maximal treadmill walking time at 6-month follow-up, compared to those randomized to placebo.

We hypothesize that PAD participants randomized to resveratrol 500 mgs daily and that PAD participants randomized to resveratrol 125 mgs daily will each achieve greater increases in calf muscle levels of PGC-1α, SIRT-1, and will each achieve greater increases in citrate synthase and COX activity, compared to those randomized to placebo.

We hypothesize that older PAD participants who receive resveratrol therapy 500 mgs daily and that older PAD patients who receive resveratrol therapy 125 mgs daily will each have greater improvement or less decline in brachial artery flow-mediated dilation, compared to those randomized to placebo.

2.0 Background

2.1 Relevant experience.

Lower extremity peripheral artery disease (PAD) affects 10-15% of community dwelling men and women age 65 and older and 15 to 20% of community dwelling men and women age 75 and older (1-3). Our prior work demonstrates that older people with PAD have greater functional impairment and faster mobility loss over time than those without PAD (4-7). PAD-related functional limitations are associated with increased morbidity and mortality, increased health care costs, and loss of independence (8-13). Yet few medical therapies have been identified that improve functional performance or prevent mobility loss in older people with PAD.

Emerging evidence, including our pilot data, demonstrates that calf skeletal muscle mitochondrial dysfunction contributes to functional impairment in people with PAD (14-20). Resveratrol, a polyphenol and natural supplement, has pharmacological properties that target the specific mitochondrial impairments that
are associated with functional impairment in older people with PAD (21-24). Animal models and preliminary human evidence show that resveratrol protects against ischemia-reperfusion injury, reduces inflammation, and improves endothelial dysfunction (25-28). Ischemia-reperfusion injury, inflammation, and endothelial dysfunction are all present in people with PAD and contribute to adverse outcomes in PAD (29-34). In summary, resveratrol specifically targets several pathophysiologic impairments associated with walking difficulty and mobility loss in people with PAD.

2.2 Significance of the research.

A1. PAD is common and is associated with functional impairment and functional decline in older men and women. PAD affects 10-15% of community dwelling men and women age 65 and older (1-3). PAD will be increasingly prevalent as the U.S. population survives longer with chronic disease. Our prior work demonstrates that men and women with PAD have greater functional impairment and more rapid functional decline than those without PAD (4-8). The functional impairments documented in PAD are associated with loss of independence, increased mortality, and poor quality of life (9-13).

A2. Few medical therapies improve functional impairment in PAD. Only two medications, pentoxifylline and cilostazol, are FDA-approved for treating PAD-associated walking impairment. Of these, pentoxifylline is usually ineffective and benefits from cilostazol are modest (35-38). New therapies are urgently needed to improve walking performance and prevent mobility loss in patients with PAD.

A3. Impairments in calf skeletal muscle mitochondrial energy production and mitochondrial biogenesis contribute to functional impairment in PAD (14-20). Peroxisome proliferator-activated receptor gamma co-activator 1-alpha (PGC-1α) is a key promoter of mitochondrial function and mitochondrial biogenesis. Our preliminary data (see section C2) show that older patients with PAD have low calf muscle levels of PGC-1α and that lower calf muscle levels of PGC-1α are associated with poorer six-minute walk performance in people with PAD. Calf muscle mitochondria oxygen consumption is lower people with PAD, compared to those without PAD (15,20). Our preliminary data (section C2) show that calf muscle levels of citrate synthase, a measure of mitochondrial function, correlate with six-minute walk performance in people with PAD. In summary, mitochondrial function and biogenesis are impaired in PAD and our preliminary data show that these impairments are associated with walking impairment in people with PAD.

A4. Resveratrol is a natural supplement that increases calf muscle levels of PGC-1α and improves mitochondrial energy production in skeletal muscle. Extensive evidence from basic and animal research shows that resveratrol improves skeletal muscle oxidative metabolism, increases PGC-1α, and increases mitochondrial biogenesis (21-24). A recent randomized controlled trial of 11 obese men without PAD demonstrated that resveratrol improves thigh muscle mitochondrial function. In a randomized controlled cross-over trial of 11 obese...
men (mean age 52.5 years ± 2.1), Timmers and colleagues demonstrated that 150 mgs of resveratrol therapy for 30 days improved muscle mitochondrial function (Table 1) (39).

Table 1. Mitochondrial function after 30 days of placebo or resveratrol in obese men (N=11).

<table>
<thead>
<tr>
<th>Global Mitochondrial Function</th>
<th>Placebo</th>
<th>Resveratrol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitochondrial Respiration: MOGS = M, malate; O, octanoylcarnitine, G, glutamate and S, succinate ([pmol/mg protein]/ mtDNA)</td>
<td>66.77 ± 5.81</td>
<td>79.50 ± 6.22*</td>
</tr>
<tr>
<td>Muscle Citrate Synthase (µmol/g protein/min)</td>
<td>65.0 ± 6.0</td>
<td>84.0 ± 6.0*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Measures of Mitochondrial Biogenesis</th>
<th>Placebo</th>
<th>Resveratrol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle PGC-1α (PGC-1α/actin units)</td>
<td>0.95 ± 0.047</td>
<td>1.07 ± 0.069*</td>
</tr>
<tr>
<td>Muscle SIRT1 (SIRT1/tubulin actin units)</td>
<td>0.67 ± 0.047</td>
<td>0.85 ± 0.079*</td>
</tr>
<tr>
<td>Muscle AMPK (pAMPK/AMPK)</td>
<td>0.79 ± 0.019</td>
<td>0.96 ± 0.045*</td>
</tr>
</tbody>
</table>

If resveratrol improves mitochondrial function and biogenesis in older people with PAD, the resulting improvement in calf muscle energy production is expected to improve functional performance and reduce mobility loss in older people with PAD.

A5. Resveratrol improves brachial artery flow-mediated dilation (FMD).
Resveratrol improves endothelial dysfunction and slows progression of vascular remodeling in rats with cardiopulmonary disease (40). Two recent human studies demonstrated improved brachial artery FMD in humans treated with resveratrol (27,41). In a double-blind crossover control study of 28 obese (mean BMI=33.3 ± 0.6 kg/m²) but otherwise healthy participants, Wong and colleagues reported recently for the first time that chronic resveratrol therapy (75 mgs daily) was associated with a 23% increase in brachial artery FMD at six-week follow-up (P=0.02) (41). Separate study demonstrated a dose-dependent increase in brachial artery FMD in 19 mildly obese individuals with mild hypertension treated for one day with resveratrol. Brachial artery FMD values were 4.0%, 6.5%, 6.0%, and 7.2% following consumption of resveratrol doses of zero, 30 mgs, 90 mgs, and 270 mgs, respectively (27). The 19 participants were mean age 55.0 ± 2.0 with mean BMI= 28.7 ± 0.50. These studies demonstrate that resveratrol improves brachial artery FMD in obese men and women. However, no prior studies have assessed the effects of resveratrol on brachial artery FMD in participants with PAD.

A6. Theoretical Framework for the RESTORE Trial. Evidence in animals and from small cohorts of humans without PAD shows that resveratrol improves several key pathophysiologic abnormalities that are linked to functional impairment in older people with PAD (21-28,39-41). We hypothesize that by reversing these pathophysiologic impairments, resveratrol will improve functional impairment in older people with PAD.
**Figure 1. Theoretical Model for the pathway by which resveratrol improves walking impairment in PAD**

- **Impaired endothelial function**
- **Reduced calf muscle mitochondrial ATP production and reduced mitochondrial biogenesis**

**Resveratrol**

**Walking impairment and mobility loss over time**

**Control**

- Continued decline in
  - Endothelial function
  - Calf muscle ATP production
  - Calf muscle mitochondrial biogenesis

**Improved mobility**

**Improved mobility**

**A7. Importance of this Proposal.** Effective therapies are urgently needed to improve walking impairment in older people with PAD. The RESTORE Trial will provide preliminary evidence needed to determine whether a large definitive trial of resveratrol is warranted in older people with PAD. If effective, resveratrol will improve the health of millions of older people in the U.S. who are currently disabled by PAD.

2.3 **Innovation of the research**

**B1. Innovation of Primary Aim #1 and Secondary Aim #1:** The RESTORE Trial will test, for the first time, whether resveratrol therapy improves walking impairment in older people with PAD. Although emerging evidence from basic research and animal studies suggests that resveratrol improves many of the pathophysiologic impairments that have been linked to walking disability in older people with PAD (Figure 1) (21-28,39-41) resveratrol has never been studied previously in people with PAD.

**B2. Innovation of Secondary Aim #2:** The RESTORE Trial will be the first study to test whether a supplement that improves mitochondrial function and mitochondrial biogenesis in basic research studies is effective in older people with PAD. Emerging evidence from basic research and preliminary data from a small study of obese humans (39) demonstrate that resveratrol targets the calf muscle mitochondrial deficits that are present in people with PAD (14-20). Our proposed pilot trial will be the first to determine whether resveratrol improves these specific calf skeletal muscle mitochondrial abnormalities in older people with PAD and simultaneously improves walking endurance.

**B3. Innovation of Secondary Aim #3.** To our knowledge, the RESTORE Trial will be the first to test whether resveratrol therapy simultaneously improves endothelial function and functional performance in any population.
People with PAD have significantly impaired endothelial function as compared to individuals without PAD (29,42,43). Impaired endothelial function is associated with increased cardiovascular event rates and lower physical activity in people with PAD (33,34). No prior studies have ever assessed whether resveratrol improves brachial artery flow-mediated dilation in people with PAD.

**B4. An additional unanswered question** is whether resveratrol is well tolerated by older people with chronic medical conditions who are taking multiple medications. We will address this unanswered question over a six month period among older PAD participants in the RESTORE Trial.

### 3.0 Inclusion and Exclusion Criteria

#### 3.1 Screening for eligibility.

**Recruitment.** We plan to consent 375 subjects to enroll and randomize 66 participants into RESTORE. This estimate is based on the recruitment from our SILC trial. We expect to consent an additional 75 subjects, totaling 375, to enroll and randomize 66 into the trial.

We will randomize 66 eligible participants over 8 months. We anticipate a dropout rate of 10% and that 60 participants will complete the study. We will identify older PAD participants using methods we have used successfully in the past (44-46). We will use Northwestern’s Enterprise Data Warehouse to identify consecutive patients with PAD who will receive recruitment letters. We will mail recruitment postcards to community dwelling men and women age 65 and older in the Chicago area, inviting those with PAD or PAD symptoms to contact us.

#### 3.2 Criteria.

**Inclusion Criterion.** All participants will be age 65 and older. All will have an ABI < 0.90 or, if ABI ≥ 0.90, medical evidence of prior lower extremity revascularization at baseline. ABI < 0.90 is a well accepted standard for defining PAD (47-50).

**Exclusion Criteria** and justification for each criterion: 1. Potential participants with below or above-knee amputation or critical limb ischemia, those who are wheel-chair bound and those who require a cane or walker to ambulate during baseline measures will be excluded because they have end-stage PAD and/or extreme walking impairment that may not respond to resveratrol; 2. Potential participants whose walking is limited by a symptom other than PAD will be excluded. Our study is designed specifically for individuals whose walking is limited by PAD. 3. Potential participants with lower extremity revascularization in the past three months, major orthopedic surgery in the past six months, a cardiovascular event, or coronary artery bypass grafting during the previous 3 months and those contemplating revascularization or major elective surgery during the next six months will be excluded because these events may affect outcome measures, independently of the intervention; 4. Potential participants with major medical illnesses including renal disease requiring dialysis or lung
disease requiring oxygen will be excluded because these may prevent full participation in the trial. 5. Potential participants with a Mini-Mental Status Examination (MMSE) score < 23 (51) or psychiatric illness will be excluded because these conditions may prevent full study participation or ability to provide accurate responses to questionnaires; 6. Potential participants enrolled in another clinical trial will be excluded because other interventions may alter outcome measures, independently of the RESTORE trial. 7. Potential participants with significant renal or liver dysfunction at baseline will be excluded because resveratrol has been shown to worsen renal and liver function when used at extremely high doses in animals (see section D2). 8. Potential participants who do not successfully complete the study run-in period will be excluded. 9. Potential participants with an extreme baseline six-minute walk value, specifically a baseline six-minute walk value < 500 feet or > 1,600 feet will be excluded. 10. Potential participants who were treated for cancer during the past two years will be excluded, unless their prognosis during the year after randomization is excellent (i.e. those treated for early stage breast or prostate cancer or non-melanoma skin cancer for example). 11. Potential participants with severe hearing impairment, other communication difficulties (e.g. non-English speaking), or who are legally blind will be excluded. 12. Potential participants with a diagnosis of Parkinson’s disease will be excluded. 13. Potential participants who are unable to return to the medical center at the required visit frequency will be excluded because they cannot fully participate in the study. 14. Potential participants thought to be poorly suited to the study intervention can be excluded at the discretion of the investigator. 15. Potential participants taking any supplement containing resveratrol will be excluded because this may affect their outcomes. 16. Potential participants who are allergic to resveratrol will not be eligible for safety reasons.

**Run-in period.** To assess likelihood of medication adherence at baseline, participants will be asked to complete a two week run-in at baseline, during which they will be provided with study medication (placebo) and asked to take one pill daily for two weeks. They will be asked to bring in all of their medications (including study medication) at the end of the run-in period. They will be asked to keep a medication diary during the two-week period. Potential participants who are not adherent at least 80% of the days during the two-week period, based on a medication count and their medication diary will be excluded.

**Inclusion of Women/Minorities.** PAD is common among older women and among African-Americans (1). We will ensure that our study population includes at least approximately 50% women and at least approximately 33% African-American (see enrollment table). We do not anticipate difficulty achieving these recruitment targets, since our recently completed SILC trial, a randomized controlled clinical trial of supervised exercise in participants with PAD included 52% women and 40% minorities (44). Similarly, our more recently completed GOALS trial included 50% women and 54% minorities (45). Our group has
substantial experience successfully recruiting and enrolling women and minorities for our observational and clinical trial research in older participants with PAD.

Methods to ensure adequate enrollment of women and minorities. To ensure that we achieve a study population that includes approximately 50% women and at least 33% minorities, we will make study participation as simple and enjoyable as possible for women and minority participants. Please note that our recently published SILC and GOALS clinical trials of exercise in people with PAD achieved exceptionally high rates of participation by women and minorities (44,45). To achieve these high participation rates from women and minority populations, we provide door-to-door transportation to study visits. We have also advertised our research opportunities on radio or in newspapers for which the audience includes a high proportion of minority populations. If we still have difficulty recruiting significant proportions of minorities, we will enlist minority leaders in the community to assist with recruitment and we will make a substantial effort to hire an African-American research assistant to assist us with recruitment. However, based on our track record, we anticipate no difficulty achieving targeted enrollment of 50% women and at least 33% minorities.

Alternatives should the above methods be insufficient. If the above methods are not sufficient, then we will expand our recruitment of participants to hospitals in which the majority of patients are minorities. For example, colleague and vascular surgeon Dr. Walter McCarthy is director of the non-invasive vascular laboratory at Cook County Hospital in Chicago. Similarly, co-investigator Dr. Melina Kibbe is a vascular surgeon at the VA Chicago Medical Center. If necessary, we will work with Drs. McCarthy and Kibbe to identify additional participants from Cook County Hospital and VA Chicago, where many patients are members of under-represented minority populations.

Inclusion of Children. PAD does not affect children. Therefore, children will not be included in the RESTORE Trial.

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

4.0 Study-Wide Number of Subjects
NA

5.0 Study-Wide Recruitment Methods
NA

6.0 Multi-Site Research
NA
7.0 Study Timelines

**STUDY OVERVIEW.** We will randomize 66 participants age 65 and older with PAD to resveratrol vs. placebo. We anticipate a 10% drop-out rate and that 60 participants will complete the study.

### Timeline for the RESTORE Trial

<table>
<thead>
<tr>
<th>Months</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
<th>12</th>
<th>14</th>
<th>16</th>
<th>18</th>
<th>22</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Start-up</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recruitment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline and follow-up testing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intervention</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Data analyses and manuscript writing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

8.0 Study Endpoints

8.1 *Primary and secondary study endpoints.*

Primary and secondary outcome measures that will be collected for this study are shown in Table 3 and include the six-minute walk test, treadmill walking performance, calf muscle biopsy measures, and brachial artery flow-mediated dilation. We will also administer questionnaires to participants at baseline and at each follow-up visit to assess their medical history, including presence of comorbidities, medication use, smoking status, and other health characteristics. Data collection will not make use of existing records or data. The prevalence of comorbid disease will be measured using patient report, based on previous study (44,45). The timeline for recruitment, data collection, and administration of the intervention are shown below.

### Table 3 shows data collection planned.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Three Month Follow-up</th>
<th>Six-month follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Six-minute walk</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Treadmill walking</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>performance</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calf muscle biopsy</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brachial artery flow-mediated dilation</td>
<td>X</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Ankle brachial index</td>
<td>X</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 3. Data collection planned among all participants randomized in the RESTORE Trial**

**Primary Outcome:** Our primary outcome is change in six-minute walk test distance between baseline and 6-month follow-up. The six-minute walk is a well
validated measure of walking endurance that is responsive to therapeutic interventions in older people with PAD (44,45). Our work demonstrates that in PAD participants, performance on the six-minute walk test is more closely correlated with physical activity during daily life than performance on a treadmill test (56). Our work also shows that the six-minute walk predicts subsequent mortality and mobility loss and that greater declines in six-minute walk are associated with greater mortality and mobility loss (8,10,57). In the six-minute walk, participants walk back and forth along a 100 foot corridor for six minutes (44,45,56,58). Distance covered in six minutes is recorded. The intra-class correlation coefficient for the test-retest reliability of the six-minute walk test among 156 PAD participants in our SILC exercise trial was 0.90 (p<0.001) when two six-minute walks were completed one to two weeks apart (45).

9.0 Procedures Involved

9.1 Study design.

We now propose a pilot study: a double-blind, randomized controlled clinical trial to test our hypothesis that resveratrol significantly improves calf skeletal muscle oxidative metabolism, increases skeletal muscle mitochondrial biogenesis, and improves systemic endothelial function, thereby improving lower extremity functioning in older people with PAD. If our findings support our hypotheses (below), results will be used to design a large, definitive randomized controlled trial of resveratrol therapy to improve lower extremity functioning and prevent mobility loss in older people with PAD. In the currently proposed pilot study, we will randomize 66 participants with PAD age 65 and older to resveratrol therapy or placebo. Of the 66 participants randomized, 22 will be randomized to 500 mgs of resveratrol, 22 will be randomized to 125 mgs of resveratrol, and 22 will be randomized to placebo. All participants will receive their study drug (resveratrol or placebo) daily for six months.

Randomization. Participants will be randomized to resveratrol vs. control using a SAS computer program. Randomization in block sizes of 4 and 6 will be used to ensure balance between the two groups. Randomization will be stratified by baseline six-minute walk performance.

Monitoring adherence. Participants will return each month to the medical center for monitoring adherence using pill counts and diaries. Participants will be asked to bring all of their medications to the medical center for review and pill counts will be performed while participants are administered a questionnaire to assess adverse events.

Potential Problems and Solutions. First, missing data may occur if participants are lost to follow-up. We will make every effort to ensure that participants return for the 6-month follow-up. These methods include use of proxies to help us locate participants we are unable to reach and mailing appointment reminders when
participants are not responsive to our telephone contacts. We will encourage participants who do not adhere to their assigned groups to return for six-month follow-up. We will provide transportation and monetary incentives. Thus, we anticipate that the proportion of participants without outcome data at six-month follow-up will be small. In addition, if dropout occurs completely at random, then the aforementioned analyses based on available data provide valid statistical inferences. When dropout is not completely at random, we will perform several sensitivity analyses. Specifically, we will employ the multiple imputation approach to account for missing data at 6-month follow-up under the assumption of missing at random. We will perform additional sensitivity analyses to guard against the possibility of missing not at random using pattern-mixture models and shared-parameter models (74). Second, we recognize that not all participants may want to return for a second muscle biopsy at six-month follow-up. However, to address this potential problem, we telephoned 19 participants who have undergone muscle biopsies in our pilot study (note- our resources allowed us to analyze data for just the first 17). Of these 19 participants, 18 (94%) indicated that they would be willing to return for a second muscle biopsy. Thus, loss to follow-up should be small.

9.2 Describe all research procedures.

Please see sections 7.0, 8.1, 8.2, and 9.1 above.

Six-minute walk. Our primary outcome is change in six-minute walk test distance between baseline and 6-month follow-up. The six-minute walk is a well validated measure of walking endurance that is responsive to therapeutic interventions in older people with PAD (44,45). Our work demonstrates that in PAD participants, performance on the six-minute walk test is more closely correlated with physical activity during daily life than performance on a treadmill test (56). Our work also shows that the six-minute walk predicts subsequent mortality and mobility loss and that greater declines in six-minute walk are associated with greater mortality and mobility loss (8,10,57). In the six-minute walk, participants walk back and forth along a 100 foot corridor for six minutes (44,45,56,58). Distance covered in six minutes is recorded. The intra-class correlation coefficient for the test-retest reliability of the six-minute walk test among 156 PAD participants in our SILC exercise trial was 0.90 (p<0.001) when two six-minute walks were completed one to two weeks apart (45).

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 (44,45,59-61). Speed is maintained at 2.0 miles per hour (mph) and treadmill grade increases by 2.0% every two minutes (44,45,59-61). 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.
**Brachial Artery Flow-Mediated Dilation (FMD).** Brachial artery imaging will be performed by a Registered Diagnostic Cardiac Sonographer (33,44,62). 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. Measurement reproducibility in Dr. Stein’s laboratory has a median FMD difference of 0.02% (inter-quartile range: -0.03–0.04).

**Muscle biopsy procedure.** Muscle biopsies will be performed by Robert Sufit MD, a board-certified neurologist with > 30 years experience performing muscle biopsies. Dr. Sufit completed all muscle biopsies for our pilot study (see Section C2). The muscle biopsy will be obtained in the medial head of the gastrocnemius muscle, at the measured 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 until muscle is identified. Approximately 250 mgs of muscle tissue is removed and immediately prepared for freezing at -70 degrees Celsius. The fascia, wound, and skin are closed with absorbable suture, subcuticular sutures, and steri-strips, respectively. Participants return for a wound check one week after the procedure. At six-month follow-up, we will repeat the measurement adjacent to the original biopsy, identifiable by the scar. In our open biopsy method, in contrast with a blind needle biopsy, muscle tissue is visualized, ensuring an optimal sample of muscle.

**Mitochondrial measures.** Our mitochondrial measures of PGC-1α, SIRT-1, citrate synthase (CS), and COX activity will be measured by the University of Florida mitochondrial laboratory, led by co-investigator Dr. Christiaan Leeuwenburgh. Dr. Leeuwenburgh’s laboratory performed all of the muscle measures completed in our pilot muscle biopsy study (see section C2). Proposed methods are well standardized, validated methods that have excellent test re-test reliability in the University of Florida laboratory (63). Muscle specimens obtained in Chicago are immediately frozen and stored at -70 degrees Celsius. Specimens are shipped on dry ice in batches to the University of Florida for testing. Whole muscle tissue homogenates are prepared using established methods, separated by gel electrophoresis on polyacrylamide gradient gels and transferred to a nitrocellulose membrane (64). Membranes are blocked using commercially available blocking buffer and incubated overnight with primary antibodies to PGC-1α and SIRT1 (Santa Cruz Biotechnology Inc). COX and citrate synthase enzyme activity is measured in whole muscle homogenates using established
methods (65). Other measures related to skeletal muscle quality and function may also be performed.

**ABI.** We have extensive experience measuring the ABI (5-7,44,45). Blood pressure cuffs are placed around each ankle and over each brachial artery. 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.

We will measure walking velocity at usual and fastest pace over four meters at baseline and at three-month and six-month follow-up visits. We will measure the short physical performance battery at baseline, three-month, and six-month follow-up visits. The short physical performance battery includes walking velocity over four meters, the time required to stand five times from a seated position as fast as possible, and a standing balance test.

**Other measures.** As in our previous clinical trials, patient report will be used to document comorbidities. Patient reported comorbid disease is highly correlated with medical record documented comorbidities (66-70). Patient-report is an accurate measure of cigarette smoking in PAD patients (71). At baseline and six-month follow-up, we will administer cognitive testing to study participants.

### 9.3 Adequacy of protection against risks and methods to minimize potential risks.

**Overview of protection against risks.** Prior to beginning data collection and study interventions, all study coordinators undergo training and are certified by Dr. McDermott using a detailed checklist for each data collection element. Research coordinators are certified in each element of the study visit including obtaining informed consent, administering questionnaires, protecting confidentiality of collected data, performing the six-minute walk, and measuring the ABI. Dr. McDermott re-certifies coordinators every six months to ensure continued adherence to protocol. Those not adhering to all aspects of the protocol undergo additional training followed by re-certification.

All research staff members have completed human subjects training required by Northwestern’s institutional review board (IRB). This training includes education about the importance of maintaining confidentiality of personal health information. Dr. McDermott or a co-investigator is available to answer questions that arise during the informed consent process as needed.

Participants are asked to sign a study consent form prior to data collection. The research coordinator reviews study procedures, including risks and benefits associated with study participation. The research coordinator answers participants’ questions. Dr. McDermott and other study investigators are available to answer participants’ questions. Both the participant and the individual administering the consent form will sign the consent form. Dr.
McDermott’s pager, direct telephone line, and home telephone number are provided to participants.

**Risks to the subjects.** As described above, participants will be randomized to one of the following three groups: a) resveratrol 500 mgs daily; b) resveratrol 125 mgs daily; or c) placebo. Study participation will last for six months. Those randomized to placebo will receive an identical-appearing placebo pill. Based on our prior work (44,45), we expect that 90% of participants will complete the study. Based on our previous work involving older participants with PAD, we anticipate that the average age will be approximately 76 years, that at least 33% of participants will be minorities, and that approximately 50% will be women. For example, in our recently completed SILC randomized controlled clinical trial of exercise in patients with PAD participants included 52% women and 45% African-Americans (44). In general, older patients with PAD have a high prevalence of comorbid diseases, particularly coronary artery disease, cerebrovascular disease, diabetes mellitus, and pulmonary disease. Thus the patient population is likely to be of generally poorer health than that of older men and women without PAD in the general population.

**Risks associated with Resveratrol.** As described in section C8 above, resveratrol has been administered in doses up to 5,000 without significant toxicity. Doses of resveratrol as high as 4000 to 5000 mgs daily have been administered to humans without serious adverse events (54,55). In a study that administered resveratrol 2000 mgs twice daily for eight days, only mild diarrhea/loose stool was reported, which resolved with discontinuation of the drug (54). We will minimize risk by carefully monitoring participants for adverse events related to resveratrol. As described below, participants will be asked to return monthly to the medical center for assessment of potential side effects and adverse events. A chemistry panel will be measured at baseline and at three months follow-up and at five months follow-up during the study. Our medical safety officer, Dr. Lloyd-Jones, will monitor safety and all adverse events will be reported to the DSMB as described below.

**Risks associated with the muscle biopsy.** The muscle biopsy is associated with several potential risks. These include discomfort during the muscle biopsy procedure, scarring from the muscle biopsy skin incision, bleeding, and infection. Potential participants who are asked to hold or reduce their anti-platelet therapy or anti-coagulation therapy during the week leading up to the muscle biopsy procedure may experience a cardiovascular event related to the temporary reduction or discontinuation of the anti-platelet or anti-coagulant 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 of wound infection after the muscle biopsy. They will also be verbally instructed in this. The participant will be instructed to call Dr. McDermott immediately if any signs of infection occur. In addition, permission from the participant’s primary care physician will be required before switching participants on anti-coagulant therapy.
to low-dose aspirin therapy for the seven days prior to the procedure. If the potential participant’s primary care physician does not provide permission for switching to low-dose aspirin for patients taking anti-coagulant therapy, the potential participant will not undergo muscle biopsy. However, based on our muscle biopsy pilot study, we anticipate that the number of these exclusions will be very small. For patients taking anti-platelet therapy but not anti-coagulant therapy, we will contact the patient’s physician and ask for permission for the patient to stop taking anti-platelet therapy for seven days leading up to the biopsy. If the patient’s primary care physician does not provide permission for the patient to stop the anti-platelet therapy, then the biopsy will be performed while the patient is taking anti-platelet therapy.

Six-minute walk test. The six-minute walk test may be associated with the risk of falling or coronary ischemia or dyspnea due to heart failure or lung disease. Rarely, falling during the six-minute walk test 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 six-minute walk test is less than 1 in 5,000.

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. However, all research staff has undergone formal human subjects training. They are trained to protect the privacy of research subject participants.

Adequacy of protection against risks and methods to minimize potential risks. Overview of protection against risks. Prior to beginning data collection, all study coordinators undergo training and are certified by Dr. McDermott using a detailed checklist for each data collection element. Research coordinators are certified in each element of the study visit including obtaining informed consent, administering questionnaires, protecting confidentiality of collected data, performing the six-minute walk, and performing the ankle brachial index. Dr. McDermott re-certifies coordinators every six months to ensure continued adherence to protocol. Those not adhering to all aspects of the protocol undergo additional training followed by re-certification.

All research staff members have completed human subjects training required by Northwestern’s institutional review board (IRB). This training includes education about the importance of maintaining confidentiality of personal health information. The study principal investigator or a co-investigator is available to answer questions that arise during the informed consent process as needed. Participants are asked to sign a study consent form prior to data collection. The research coordinator reviews study procedures, including risks and benefits.
associated with study participation. The research coordinator answers participants’ questions. Dr. McDermott and other study investigators are available to answer participants’ questions. Both the participant and the individual administering the consent form will sign the consent form. Dr. McDermott’s pager, direct telephone line, and home telephone number are provided to participants.

Minimizing risks related to resveratrol. Participants will be asked to return to the medical center once monthly. At these visits, participants will be administered questionnaires to obtain data regarding any potential side effects such as change in bowel movements or diarrhea (53). Because of theoretical concerns regarding liver and kidney toxicity, we will obtain a chemistry measurement at baseline, three-month follow-up, and at five month follow-up. Participants with significant kidney or liver disease at baseline will be excluded (i.e. GFR < 30 mls/minute or liver enzyme values ≥ 2.0 times normal). In addition, the principal investigator, Dr. McDermott, will review results of the blood tests. If the blood test results indicate a significant decline in renal or liver function after randomization, Dr. McDermott will send these results to the medical safety officer, Dr. Lloyd-Jones, who will reduce the resveratrol dose or eliminate the resveratrol. However, given the lack of toxicity observed to date in humans prescribed higher doses than 500 mgs daily of resveratrol, we expect that toxicity will be minimal. Safety will also be monitored by our DSMB.

Minimizing risks related to muscle biopsy. The muscle biopsy procedure will be performed by Dr. Robert Sufit who has more than 30 years of experience performing these muscle biopsies, primarily as part of his clinical practice as a Board-Certified Neurologist. As in our pilot study, completed in preparation for this proposal, the muscle biopsy procedures will be performed under sterile conditions using sterile technique. Local anesthesia will be obtained using topical lidocaine. All participants will be provided with written and verbal instructions about wound care and will be advised to contact Dr. McDermott immediately if any signs of wound infection occur.

Many PAD participants take anti-platelet therapy to prevent cardiac and cerebrovascular events. If potential participants are taking anti-platelet therapy, they will be asked to either hold their anti-platelet therapy (if they are taking 81 mgs of aspirin or less per day) or switch to a low dose (81 mgs) of aspirin therapy (if they are taking clopidogrel or an equivalent medication or > 81 mgs of daily aspirin at study entry) during the seven days leading up to the muscle biopsy procedure. Participants who are taking warfarin or other anti-coagulant therapy will be asked to switch to low dose aspirin therapy (i.e. 81 mgs daily) for the seven days prior to the muscle biopsy procedure. However, permission from the participant’s primary care physician will be required before switching participants on anti-coagulant therapy to low-dose aspirin therapy for the seven days prior to the procedure. If the potential participant’s primary care physician does not provide permission for switching to low-dose aspirin for patients taking anti-coagulant therapy, the potential participant will not undergo muscle biopsy. However, based on our pilot study, we anticipate that the number of these exclusions will be very small.
Minimizing risk related to baseline and follow-up testing. All study coordinators undergo baseline training and are certified by Dr. McDermott before beginning data collection. Training and certification involves ensuring that coordinators are trained in methods to help minimize falls. Dr. McDermott re-certifies coordinators every six months to ensure continued adherence to study protocol. Those who are not adhering to protocol undergo additional training followed by re-certification.

Minimizing risk related to loss of confidentiality. The following methods will be employed to maintain confidentiality of participants. First, study recruitment letters will be mailed, using IRB-approved methods, only after receiving written permission from the participant’s physician. The personal physician of each study participant will have the option of not allowing investigators to contact the potential participant. Lists of potentially eligible participants will be obtained by individuals who normally have access to these lists as part of their daily work requirements. Recruitment letters for potential participants identified from hospital and outpatient lists are prepared by research staff members whose job is to assist study investigators with recruitment. These research staff members have completed training in the ethical conduct of human subject research, including maintaining participant confidentiality. Recruitment letters to potential participants identified from medical center lists are mailed in sealed envelopes and addressed to the potential participant. All potential participants who receive mailed information about the study after the approval from their physician will have the opportunity to call a voice-mail system to ask NOT to be further contacted about this study. Secondly, only study investigators and key research staff will have access to the study database. Third, participants will be assigned a unique study identifier. Individual names will ultimately be removed from the study database and only the unique study identifier will be used to distinguish participants in the database. Fourth, collected data will be maintained in locked computer files and file cabinets to which only study investigators have access. Collected data will be used only for research purposes. Any published data will not contain any individual identifiers.

Data and Safety Monitoring Board (DSMB). We have identified three individuals: Dr. Mark Creager, Professor of Medicine at Harvard Medical School, Dr. Michael Conte, Professor of Surgery and Division Chief of Vascular Surgery at the University of California at San Francisco, and Dr. Donald Hedeker, Professor of Statistics, University of Illinois at Chicago who have agreed to serve on the DSMB for the RESTORE Trial. Dr. Creager is an internationally recognized expert in vascular medicine and is a board-certified cardiologist. Dr. Conte is an internationally recognized expert in vascular surgery and vascular medicine and is board certified in vascular surgery. Dr. Hedeker is an internationally recognized statistician with expertise and experience with clinical trials and also missing data. The DSMB will meet at least every six months during the study. The DSMB will meet to review and approve the protocol prior to beginning data collection. They will decide on specific stopping criteria for the study. The biostatisticians and data manager will work closely with the DSMB to perform interim analyses. Adverse events will be monitored continuously
throughout the study and will be reported to the DSMB and IRB in a timely manner according to pre-specified requirements. Analyses will be done according to the requests of the DSMB. In addition, for each major adverse event, the group assignment of the patient will be provided. In this way the DSMB can determine whether the event is intervention related. 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) gastrointestinal outcomes (diarrhea, loose stool, abdominal discomfort); d) adverse changes in kidney and liver function. We will report all hospitalizations to the DSMB in a timely fashion. We will use a designated data collection form to record these events and they will be reported immediately to the Institutional Review Board and DSMB. As noted above, however, to date resveratrol has been found to have low toxicity in humans (53-55).

- **Drugs and devices used in the research.**

**Resveratrol supplementation.** Resveratrol Organics will supply resveratrol and placebo for the study. *Justification for dose and duration of resveratrol therapy.* Doses of resveratrol in previous clinical trials in humans have ranged from 75 mgs to 5000 mgs per day without demonstrating significant toxicity (52-56). Most of these studies have been performed for cancer prevention. However, as shown in Table 1, Timmers et al demonstrated that resveratrol improved calf muscle mitochondrial function at a dose of 150 mgs daily for 30 days (39). Wong and colleagues observed increases in brachial artery FMD at doses as low as 30 mgs, though the greatest benefit was observed at a dose of 250 mgs daily (see section A5) (27). A pilot study by co-investigators Drs. Anton and Leeuwenburgh in 32 overweight older adults without PAD demonstrated greater improvement in mitochondrial oxygen extraction at a dose of 1000 mgs per day than at a dose of 300 mgs per day (personal communication). Additional studies show that doses of 500 and 1000 mgs each reduce cell proliferation in human colon cancer (52) and that doses that improve insulin sensitivity in rats translate to human doses of 10 to 973 mgs daily (53). Doses of resveratrol as high as 4000 to 5000 mgs daily have been administered to humans without serious adverse events (54,55). In a study that administered resveratrol 2000 mgs twice daily for eight days, only mild diarrhea/loose stool was reported, which resolved with drug discontinuation (54). A recent study of rhesus monkeys who were fed a diet high in fat and sucrose for two years, a resveratrol dose of 40 mgs twice daily for 12 months prevented the high-fat diet induced increases in a) arterial wall inflammatory infiltration; b) arterial wall calcification and c) increase in pulse-wave velocity (75). However, subsequent increase in the resveratrol dose to 240 mgs bid for an additional year was associated with an increase in pulse-wave velocity during the additional year. Although the explanation for progression in pulse wave velocity during this second year is unclear, one potential explanation is that the higher dose of resveratrol during the second year was not as effective as the lower dose. Because improvements in mitochondrial function, brachial artery
FMD, and lower extremity functioning in humans have been observed at doses of 150 mgs, 250 mgs, and 1,000 mgs respectively, we will test a dose of 500 mgs of resveratrol daily. Because of uncertainty about the optimal dose to improve outcomes in PAD participants, and because of some recent evidence that lower resveratrol doses may be more beneficial than higher resveratrol doses (75), we will also test a dose of 125 mgs daily. Our decision to administer resveratrol for six months is based on our prior work demonstrating that without therapy, PAD participants decline in six-minute walk performance at six-month follow-up (44,45). Six months of resveratrol provides greater exposure of target tissues (i.e. calf muscle and the endothelium) to resveratrol, allowing greater potential for improved functional performance.

- Source records that will be used.

We will use standardized questionnaires to collect data about each study participant. Please see attached data collection forms. We may use medical records to determine whether the patient was diagnosed with PAD prior to study enrollment.

9.4 Data collected.
Please see section 9.2 above.

10.0 Data and Specimen Banking

10.1 Storage of specimens.

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.

10.2 Data to be stored or associated with each specimen.

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 blood samples that are maintained in long-term storage will be labeled with this unique identifier and the date and time of the blood collection.

10.3 Procedures to release data or specimens.

Only Dr. McDermott has control over release of study data or specimens. Any investigators seeking to analyze blood specimens must contact Dr. McDermott for permission. Each request, if it occurs, will be considered on a case-by-case basis.
PROTOCOL TITLE: Resveratrol To Improve Outcomes in older people with PAD (The Restore Trial)

Dr. McDermott will obtain IRB approval prior to releasing any blood specimens for analysis, other than those tests specifically named in this application.

11.0 Data and Specimen Management

11.1 Data analysis plan.

Data Management. We will use methods in place for our previous and ongoing PAD studies to customize a data management system for the currently proposed Study. Data from baseline and follow-up visits will be acquired on paper and processed using the Teleform system by Cardiff Software. We have successfully used the Teleform system for over 6,000 patient visits in previous and ongoing studies.

Data Safety Monitoring Board (DSMB). Our Human Subjects section provides details about the DSMB.

Power Estimates. Our power calculations take into account an anticipated 10% drop-out rate at 6-month follow-up, based on data from our GOALS and SILC randomized trials in PAD participants (44,45). For our Primary Specific Aim, we will compare changes in six-minute walk performance at 6-month follow-up between the resveratrol 500 mgs daily group and the placebo group and between the resveratrol 125 mgs daily group and the placebo group. Using the estimated standard deviation (SD) from our GOALS trial, a total of 20 participants in each group provides 70% power to detect a difference of 0.61 SD, which represents approximately 31.5 meters change in six-minute walk performance between each resveratrol group (n=20, respectively) and the placebo group (n=20), 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 RESTORE is a pilot study intended to collect preliminary data. Prior studies have defined clinically meaningful changes in the six-minute walk as 30 and 50 meters (72,73). Thus, under these assumptions, we will be able to detect meaningful differences.

Similarly, 20 participants in each group will provide 70% power to detect a difference of 0.61 SD in 6-month change of each outcome proposed in our Secondary Specific Aims between the resveratrol group and the placebo group, based on a one-sided two-sample t-test with a significance level of 0.10. For our Secondary Aim #1, we will compare changes in pain-free and maximal treadmill walking time at 6-month follow-up between each resveratrol group and the placebo group, respectively. In our SILC trial, the observed difference between the intervention and control groups was 0.61 SD in changes of maximal treadmill walking time. For our Secondary Aim #2, we will compare changes in calf muscle levels of PGC-1α, calf muscle levels of SIRT-1, and COX and citrate synthase enzyme activity at 6-month follow-up between each resveratrol group and the placebo group. From the study by Timmers et al. (39), the observed differences in calf muscle levels of PGC-1α, SIRT-1, muscle citrate synthase and mitochondrial oxidative function were 2.03 SD, 2.77 SD, 3.17 SD, and 2.12 SD,
respectively. For our Secondary Aim #3, we will compare changes in brachial artery flow-mediated dilation at 6-month follow-up between each resveratrol group and the placebo group. From the trial by Wong et al (41), the observed differences in brachial artery flow-mediated dilation was more than 2.0 SD. Thus, 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 assigned group. Prior to the analyses, the distributions of the variables (changes) will be examined and necessary transformation will be performed. The balance of participant characteristics (such as age, sex, race, ABI, and smoking status) will be compared between the three groups using ANOVA or chi-square tests. If there is any indication of major imbalance in these factors, proper adjustment will be taken into account in the data analyses. For our Primary Specific Aim, we will use a two sample two-tailed t-test to compare changes in six-minute walk performance at 6-month follow-up between the resveratrol 500 mg daily group and the placebo group and between the resveratrol 125 mg daily group and the placebo group, respectively. We will perform analysis of covariance (ANCOVA) with the change in six-minute walking distance as the response variable to evaluate differences in change in six-minute walking distance between the resveratrol 500 mgs daily group and the placebo group and between the resveratrol 125 mgs daily group and the placebo group, adjusting for potentially imbalanced baseline confounders such as age, sex and baseline six-minute walk performance. Again, the assumptions of normality and equal variance will be checked. When there is evidence that the normality assumption for the distribution of measures of interest is violated, we will either apply appropriate transformation to the original measures before conducting t-tests or perform Wilcoxon rank-sum test for the comparison. For our Secondary Specific Aims, we will repeat the analyses for our primary specific aim for each outcome, separately.

11.2 Power analysis.

A power analysis is not applicable, given the exploratory nature of this research.

11.3 Steps to secure data to maintain confidentiality during storage, use, and transmission.

First, all research assistants must complete training in protection of subject privacy and prevention of disclosure of identifying information.

Second, all data collection forms are maintained in a secure office space.

Third, our study databases are maintained in locked computer files or on secure hard-drives that are password protected; to which only authorized staff have
access. Dr. McDermott or a study manager must provide permission for programmers and research assistants to access study databases.

Fourth, a study identification number will be assigned to each participant. This identification number will be used to label blood specimens, for example. In addition, most pages of our data collection forms will have only the study identification number listed (and not the participant's name, for example).

11.4 Quality Control. As in our prior studies, health interviewers will be trained by a senior coordinator and certified by Dr. McDermott in each component of data collection, using a detailed checklist developed for RESTORE Trial. Health interviewers are rigorously evaluated for adherence to protocol, prior to beginning data collection and every six months after initial certification. The ABI will be measured an additional time in a randomly selected approximately 10% subset of study participants. In this randomly selected 10% subset, additional blood will be collected for storage and additional muscle sample will be collected and stored at -70 degrees Celsius so that measures can be run in duplicate for quality control purposes.

Blinding for data collection. The health interviewer collecting outcome data will be blinded to the study group assignment. Participants are instructed not to reveal their group assignment. If a participant reveals their group assignment, another certified health interviewer is paged to continue the visit.

12.0 Provisions to Monitor the Data to Ensure the Safety of Subjects

Serious adverse events will be reported to the DSMB within seven days of each serious adverse event. Adverse event data will be reported to the DSMB every six months during the study, and/or as requested by the DSMB. The DSMB will have the ability to stop the study at any time if there are concerns about safety.

13.0 Withdrawal of Subjects*

Procedures that will be followed when subjects withdraw from the research, including partial withdrawal from procedures with continued data collection.

Subjects may withdraw from the research at any time. If they decide to leave the research, they should contact the investigator, Dr. Mary McDermott.

If they stop being in the research, already collected data may not be removed from the study database. They will be asked whether the investigator can collect data from their routine medical care. If the subject agrees, this data will be handled the same as research data.
14.0 Risks to Subjects*

Potential Risks.

Risks associated with Resveratrol. As described in section C8 above, resveratrol has been administered in doses up to 5,000 without significant toxicity. Doses of resveratrol as high as 4000 to 5000 mgs daily have been administered to humans without serious adverse events (54,55). In a study that administered resveratrol 2000 mgs twice daily for eight days, only mild diarrhea/loose stool was reported, which resolved with discontinuation of the drug (54). We will minimize risk by carefully monitoring participants for adverse events related to resveratrol. As described below, participants will be asked to return monthly to the medical center for assessment of potential side effects and adverse events. A chemistry panel will be measured at baseline and at three months follow-up and at five months follow-up during the study. Our medical safety officer, Dr. Lloyd-Jones, will monitor safety and all adverse events will be reported to the DSMB as described below.

Risks associated with the muscle biopsy. The muscle biopsy is associated with several potential risks. These include discomfort during the muscle biopsy procedure, scarring from the muscle biopsy skin incision, bleeding, and infection. Potential participants who are asked to hold or reduce their anti-platelet therapy or anti-coagulation therapy during the week leading up to the muscle biopsy procedure may experience a cardiovascular event related to the temporary reduction or discontinuation of the anti-platelet or anti-coagulant 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 of wound infection after the muscle biopsy. They will also be verbally instructed in this. The participant will be instructed to call Dr. McDermott immediately if any signs of infection occur. In addition, permission from the participant’s primary care physician will be required before switching participants on anti-coagulant therapy to low-dose aspirin therapy for the seven days prior to the procedure. If the potential participant’s primary care physician does not provide permission for switching to low-dose aspirin for patients taking anti-coagulant therapy, the potential participant will not undergo muscle biopsy. However, based on our muscle biopsy pilot study, we anticipate that the number of these exclusions will be very small.

Six-minute walk test. The six-minute walk test may be associated with the risk of falling or coronary ischemia or dyspnea due to heart failure or lung disease. Rarely, falling during the six-minute walk test 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 six-minute walk test is less than 1 in 5,000.
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. However, all research staff has undergone formal human subjects training. They are trained to protect the privacy of research subject participants.

15.0 Potential Benefits to Subjects

15.1 The potential benefits that individual subjects may experience from taking part in the research.

Potential benefits of the proposed research. Lower extremity peripheral artery disease (PAD) affects 10-15% of men and women age 65 and older in the United States (U.S.) (1-3). PAD will be increasingly prevalent as the U.S. population survives longer with chronic disease. We have demonstrated that men and women with PAD have greater functional impairment and more rapid functional decline than those without PAD (4-10). The functional impairment experienced by people with PAD is associated with loss of independence, increased hospitalization rates and poor quality of life (11-13). Yet few therapies exist for improving functional performance and preventing mobility loss in people with PAD.

Only two medications, pentoxifylline and cilostazol, are FDA-approved for treating PAD-associated walking impairment. Of these, pentoxifylline is usually ineffective and benefits from cilostazol are modest (35-38). New therapies are urgently needed to improve walking performance and prevent mobility loss in patients with PAD. Resveratrol holds promise as a potential therapeutic agent for people with PAD, because it has pharmacologic properties that target several key pathophysiologic contributors to functional impairment and functional decline in people with PAD.

Importance of knowledge to be gained. PAD is common in older men and women. The number of individuals with PAD is expected to increase as the U.S. population lives to older ages with chronic disease. Our prior work and that of others shows that patients with PAD have greater functional impairment, increased rates of functional decline, and increased mobility loss compared to persons without PAD (4-10). Older patients with functional impairment are less likely to remain independent in the community, have higher rates of hospitalization, and have poorer quality of life than those without functional impairment (11-13). Yet few therapies have been identified that improve lower extremity functioning or prevent functional decline or mobility loss in persons with PAD. If our hypotheses are correct, results will be used to design a large,
definitive randomized controlled trial of resveratrol therapy to improve lower extremity functioning and prevent mobility loss in the large and growing number of older people who are disabled by PAD.

15.2 Indicate if there is no direct benefit. Do not include benefits to society or others.

There are no direct benefits.

16.0 Vulnerable Populations

NA

17.0 Community-Based Participatory Research

NA

18.0 Sharing of Results with Subjects

Participants will receive results of their ankle brachial index (ABI) test results immediately after this testing is completed. They will be provided with a “result letter” at the end of their baseline visit. Results of the baseline treadmill stress test will be mailed to the participant’s designated physician. They will not be provided with other study results, because these results are not clinically relevant at this time.

19.0 Setting

The research will be conducted at Northwestern Memorial Hospital. Please see section 22.0 for more details.

20.0 Resources Available

20.1 Qualifications of staff.

Investigative Team’s Prior Work Supporting the RESTORE Proposal. Our multidisciplinary investigative team includes internationally recognized experts in PAD (Drs. Criqui, Kibbe, McDermott), functional decline (Drs. Ferrucci, Guralnik), skeletal muscle pathophysiology (Drs. Ferrucci, Leeuwenburgh, Sufit), resveratrol (Drs. Anton, Leeuwenburgh), and statistics and clinical trial design (Drs. Liu, Tian, Zhao). We have successfully completed three NIH-funded randomized controlled trials of exercise or behavioral interventions in participants with PAD: (R01-HL073351, sample size=156, R01-HL073912, sample size=392, and R01-HL088589, sample size=194) (44-46). Our prior clinical trials of participants with PAD included measurement of six-minute walk, treadmill walking performance, and brachial artery FMD in participants with PAD (44-45). In addition, we completed a muscle biopsy pilot study in preparation for this trial. In summary, we have substantial experience conducting randomized controlled trials of PAD participants.

Our experience successfully performing muscle biopsies in participants with PAD. Over a seven-month period, 490 participants with and without PAD
provided written informed consent to participate in our muscle biopsy pilot study. Of the 490 participants, most had PAD. Our available resources allowed us to obtain muscle biopsies in 17 participants, including 11 with PAD. Biopsies were performed by co-investigator Dr. Robert Sufit. There were no adverse events associated with completed muscle biopsies. The mean ABI value was $0.74 \pm 0.26$ among participants with PAD and $1.17 \pm 0.12$ among those without PAD. Citrate synthase, a measure of mitochondrial oxidative metabolism and mitochondrial quantity, was correlated with the ankle brachial index (ABI), a measure of the presence and severity of PAD (correlation=0.347). Among PAD participants, citrate synthase was correlated with the six-minute walk (correlation=0.410). Lower PGC-1α levels were associated with poorer six-minute walk performance among participants with PAD (Table 2).

Table 2. Pilot Study Results: Associations of PGC-1α levels with the ABI and six-minute walk

<table>
<thead>
<tr>
<th>Tertile 1 (lowest PGC-1α level)</th>
<th>Tertile 2 PGC-1α</th>
<th>Tertile 3 (highest PGC-1α level)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-minute walk (N=11 PAD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>709 feet ±169</td>
<td>1,323 feet ± 388</td>
<td>1,521 feet ± 182</td>
</tr>
</tbody>
</table>

Our muscle biopsy pilot study demonstrates the feasibility of our muscle biopsy methods. We have experience successfully conducting large randomized controlled trials of participants with PAD. We have experience with all outcomes proposed in RESTORE. We are an ideal investigative team to conduct the RESTORE Trial.

Collaborating sites.

Dr. Christiaan Leeuwenburgh is an internationally recognized expert in skeletal muscle biology and mitochondrial function. Dr. Leeuwenburgh’s laboratory will measure oxidative stress proteins in samples obtained in the RESTORE Trial. Dr. Leeuwenburgh’s laboratory performed all of the measures from our muscle biopsy pilot study. Drs. Michael H. Criqui (University of California at San Diego), Jack M. Guralnik (University of Maryland), and Luigi Ferrucci (National Institute on Aging) have worked with Dr. McDermott on PAD studies of functional impairment for over eleven years and bring expertise in functional assessment, PAD, and clinical trials to the study team.

21.0 Prior Approvals

NA

22.0 Recruitment Methods

22.1 When, where, and how potential subjects will be recruited.

PAD 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.

In addition, some PAD participants may be identified from among consecutive patients diagnosed with PAD in the non-invasive vascular laboratory of Northwestern Memorial Hospital (NMH). Dr. Mark Eskandari is medical director of the non-invasive vascular laboratory at NMH and will assist with identifying potential participants from the non-invasive vascular laboratory. As director of the vascular laboratory at NMH, 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 by Dr. David Leibovitz from Northwestern Memorial Hospital to Dr. McDermott using an encrypted file. Dr. Leibovitz is the Director of Clinical Information Systems at Northwestern Memorial Hospital and he is Chief Medical Information Officer at Northwestern Medical Faculty Foundation. A research assistant, working on behalf of Dr. Eskandari, will contact referring physicians of potential participants identified from the vascular laboratory via fax, phone, page, or e-mail, to ask for permission to contact their patient about the study. Once physician permission is obtained, up to four letters are mailed from Dr. McDermott on behalf of the patient's physician about the research study. We have substantial experience with our proposed recruitment methods, which are IRB approved for our previous or ongoing studies.

We also propose to obtain lists of consecutive patients with a diagnosis of lower extremity peripheral arterial disease who are seen in our vascular surgery practice, cardiology practice, and general internal medicine practice in the Northwestern Medical Faculty Foundation (NMFF). Co-investigator Dr. Mark Eskandari is a member of the vascular surgery practice at NMFF. Co-investigator Dr. Lloyd-Jones is a member of the cardiology division at NMFF. Dr. McDermott is a member of the general internal medicine practice at NMFF. We also propose to obtain lists of consecutive patients with a diagnosis of lower extremity peripheral arterial disease who are patients in our vascular surgery, cardiology, geriatrics, and general internal medicine practices at NMFF. Again, similar methods will be used as those described above, in which the patient’s physician will be contacted to obtain permission to send a recruitment letter to their potentially eligible patient from Dr. McDermott on behalf of the patient's physician. We will contact the physicians up to three times, if we do not hear back after the first request, to ask if they are willing to have us contact their patients.

Up to four recruitment letters will be mailed, three weeks apart. We may also contact by telephone (after three weeks) those who do not respond to the first
mailing within three weeks. Lists of patients at NMFF will be obtained using the EDW system. These lists will be obtained 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.

In the recruitment letters, recipients are asked to call our voice mail line if they are interested in participation or if they do not want to be contacted. 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.

22.2 *Source of subjects.* Please see details regarding “source of subjects” in section 22.0 and 22.1.

22.3 *Methods that will be used to identify potential subjects.* Please see details regarding methods used to identify potential subjects in sections 22.0 and 22.1.

22.4 *Amount, timing, and method of any payments to subjects.*

Participants will be reimbursed for your transportation expenses and parking costs. Reimbursements for travel may require a receipt and you will be reimbursed at the time of the visit, up to $75 per visit. Total reimbursement will not exceed $75 per visit. If they require the use of our taxi service, we will estimate your fare on [www.taxifarefinder.com](http://www.taxifarefinder.com). A one-way fare estimate must be below $37.50 (i.e. round trip of $75) in order for the study to provide taxi service to and from study visits.

We will pay $100 in cash at the time of their muscle biopsy at the baseline visit. We will also pay $100 in cash at the time of their muscle biopsy at the six-month follow-up visit. Thus, the total they can obtain for study participation is $200. Participants will only be paid for having a muscle biopsy. If they withdraw from the study, they may still retain payment for muscle biopsies that they have completed at the time of study withdrawal. Participants will be paid in cash at the time of the visit. If they do not have a muscle biopsy, they will not receive payment for study participation.

23.0 **Local Number of Subjects**

**Recruitment.** We will randomize 66 eligible participants over 8 months. We anticipate a drop-out rate of 10% and that 60 participants will complete the study. We will identify older PAD participants using methods we have used successfully in the past (44-46). We will use Northwestern’s Enterprise Data Warehouse to identify consecutive patients with PAD who will receive recruitment letters. We will mail recruitment postcards to community dwelling men and women age 65 and older in the Chicago area, inviting those with PAD or PAD symptoms to contact us.
24.0 Confidentiality

NA

25.0 Provisions to Protect the Privacy Interests of Subjects

25.1 Steps that will be taken to protect subjects’ privacy interests and to make the subjects feel at ease with the research situation.

Questionnaires will be administered in an enclosed space by a trained and certified research assistant. Dr. McDermott personally certifies study participants in data collection to help ensure that participants are treated with the highest level of professionalism. The study drug injection and phlebotomy will also take place in an examination room with the door closed to ensure optimal privacy.

25.2 Indicate how the research team is permitted to access any sources of information about the subjects.

All 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.

26.0 Compensation for Research-Related Injury

26.1 If the research involves more than Minimal Risk to subjects, describe the available compensation in the event of research-related injury.

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

27.0 Economic Burden to Subjects

NA

28.0 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. Prior to attending their first study visit, a research assistant will explain the study to potential participants by telephone. When a potential participant arrives to the medical center for study participation, the research assistant will explain the full details of the research study, including risks and benefits. The informed consent process will take place first at initial study
visit at Northwestern. Data will be collected on the 11th floor of Galter in a private area. The research assistant will be collecting the signed consent form.

Potential participants will be provided plenty of time to read the consent form. The research assistant will answer study questions. However, if the participant would like more time to discuss the research study with their physician or family member, they will be allowed to do so. In this case, the study visit will not proceed. Dr. McDermott or another study investigator at Northwestern is also available to answer any questions that participants may have about the research.

Non-English Speaking Subjects

Potential participants who do not speak English will not be eligible for study participation.

Subjects who are not yet adults (infants, children, teenagers)
Children will not be involved in this research.

Cognitively Impaired Adults

Participants who are cognitively impaired will not be eligible.

Adults Unable to Consent

Written consent will be required from all study participants. Participants who cannot provide informed consent are not eligible for participation.

29.0 Process to Document Consent in Writing

The “SOP: Written Documentation of Consent (HRP-091): will be followed.

30.0 Drugs or Devices

30.1 Plans to store, handle, and administer drugs or devices.

Resverage Organics will supply resveratrol and placebo for the study. Resveratrol is a natural compound- it is not under FDA purview.
31.0 References


PROTOCOL TITLE: Resveratrol To Improve Outcomes in older pEople with PAD (The Restore Trial)


30. Brevetti G, Piscione F, Cirillo P, Galasso G, Schiano V, Barbato E, Scopacasa F, Chiaro, M. In concomitant coronary and peripheral arterial disease, inflammation of
the affected limbs predicts coronary artery endothelial dysfunction. *Atherosclerosis* 2008;201:440-446. PMID: 18358480.


PROTOCOL TITLE: Resveratrol To Improve Outcomes in older pEople with PAD (The Restore Trial)


