Clinical Intervention Study Protocol

Resveratrol to Enhance Vitality and Vigor in Elders (REVIVE)

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Sponsor of IND (IDE): Stephen Anton

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PRÉCIS

The proposed study will utilize a prospective, parallel design to determine whether 90 days of resveratrol supplementation in older men and women (≥ 65 years) is associated with (i) increases in muscle mitochondrial respiration, (ii) increases in levels of PGC-1α, AMP-activated protein kinase (AMPK), and sirtuins (SIRT1 and SIRT3), and (iii) improvements in functional performance and metabolic parameters. To achieve these aims, 60 participants will be randomized to receive a placebo (n=20), 1000 mg/day of resveratrol (n=20), or 1500 mg/day of resveratrol (n=20) for a 90-day period. We will collect muscle specimens from the vastus lateralis at baseline and 90 days for biochemical analyses (see **Table 1** for planned data collection and procedures). In addition to the measures listed in **Table 1**, we will also monitor blood chemistries every 30 days at monthly clinic visits. To enhance retention and compliance, participants will receive biweekly phone calls throughout the 90-day treatment period. Following the completion of this trial, one in-person follow-up assessment will be conducted at approximately 30 days post-treatment to monitor blood chemistries and ascertain adverse events. In addition, approximately 10-days following muscle Biopsy procedure, a follow-up visit will take place to assure absence of biopsy complications and to monitor blood chemistries.

Eligible participants will be randomized to receive placebo (vegetable cellulose) or resveratrol product containing either 1000 mg resveratrol/day or 1500 mg resveratrol/day. In all conditions, participants will ingest three pills per day and will be instructed to consume one pill following each meal. Participant safety will be our highest priority, and we will monitor potential adverse events associated with resveratrol consumption by blood work analysis (complete metabolic panel and complete blood count), and assess reported adverse events using the National Cancer Institute (NCI) criteria during all study visits, including the 10 and 30-day follow-up visits.

Table 1. Timeline for tests, evaluations, and protocols to be conducted and performed

Assessment	Phone Pre-Screen	Screening Visit	Baseline Visit 1	Baseline Visit 2	10 days Post-Biopsy Visit	30-Day Visit	60-Day Visit	90-Day Visit 1	90-Day Visit 2	10 days Post-Biopsy Visit	Follow-up 30 Day
Telephone interview pre-screening	X										
Informed Consent		X									
Informed Consent for Data/Tissue Bank (optional)			X								
Medical history (inclusion/exclusion criteria)		X									
Medical History Update			X	X	X	X	X	X	X	X	X
Height (without shoes)		X									
Physical Exam		X									
Mini-Mental Status Exam (MMSE)		X									
Center for Epidemiologic Studies - Depression Scale (CES-D)		X									
Weight/Girth Measurement		X	X			X	X	X			
CMP & CBC w/Diff		X	X		X	X	X	X		X	X
Inflammation Markers in blood			X					X			
Vitals (blood pressure + heart rate)		X	X	X	X	X	X	X	X	X	X
Short Physical Performance Battery (SPPB)		X	X					X			
Physical Function (6 min walk test)			X					X			
Activity Monitor			X					X			
Biodex (knee extension and flexion)			X					X			
Muscle Biopsy				X					X		
Randomization				X							
Product Dispense				X		X	X				
Product Compliance						X	X	X			

Adverse Event Assessment Toxicity (NCI) criteria			**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**

Abbreviations: CBC, Complete Blood Count; CMP, Comprehensive Metabolic Panel.

Resveratrol to Enhance Vitality and Vigor in Elders (REVIVE)

Objectives

Primary Objective

Specific Aim 1. To examine the effects of resveratrol supplementation on mitochondrial function (State 3 and 4 respiration on permeabilized muscle fibers, cytochrome oxidase (COX), citrate synthase (CS) enzyme activities, and mitochondrial DNA content) in muscle samples of older adults.
Hypotheses. Compared to placebo, resveratrol supplementation will increase mitochondrial respiration (State 3=primary outcome), COX, CS enzymes activities, and mitochondrial DNA content in muscle specimens from the *vastus lateralis.*

Secondary Objectives

Specific Aim 2. To evaluate the effects of resveratrol supplementation on levels of PGC-1α, AMPK, and sirtuins (SIRT1 and SIRT3) in muscle samples of older adults.
Hypotheses. Compared to placebo, resveratrol treatment will increase PGC-1α (primary outcome), AMPK, SIRT1, and SIRT3 levels in muscle specimens obtained from the *vastus lateralis.*

Exploratory Aims. Explore the effects of resveratrol supplementation on measures of physical function, metabolic risk factors, and changes in physical activity in older adults.
Hypotheses. Compared to placebo, resveratrol supplementation will (1) increase walking speed, (2) improve physical performance, (3) increase resistance to muscle fatigue, (4) reduce established metabolic risk factors, (i.e. diastolic blood pressure and blood levels of insulin and glucose), and (5) increase levels of spontaneous physical activity.

Design and Outcomes

The proposed study will utilize a double-blind, placebo controlled, parallel design to determine whether 90 days of resveratrol supplementation in older men and women (≥ 65 years) is associated with (i) increases in muscle mitochondrial respiration, (ii) increases in levels of PGC-1α, AMP-activated protein kinase (AMPK), and sirtuins (SIRT1 and SIRT3), and (iii) improvements in functional performance and metabolic parameters.

Interventions and Duration

Eligible participants will be randomized to receive placebo (vegetable cellulose) or resveratrol (1000 mg/day or 1500 mg/day) for a 90-day period. Following the completion of this trial, two in-person follow-up assessments will be conducted at approximately 30 days post-treatment to monitor blood chemistries and ascertain adverse events. In addition, approximately 10-days following muscle Biopsy procedure, a follow-up visit will take place to assure absence of biopsy

complications and to monitor blood chemistries.

***S*ample Size and Population**

A total sample of 60 older adult participants (age ≥ 65 years) with a body mass index (BMI) between 20–39.9 kg/m^2 will participate in this trial.

1. STUDY OBJECTIVES

1.1 Primary Objective

Specific Aim 1. To examine the effects of resveratrol supplementation on mitochondrial function (State 3 and 4 respiration on permeabilized muscle fibers, COX, CS enzymes activities and mitochondrial DNA content) in muscle samples of older adults.
Hypotheses. Compared to placebo, resveratrol supplementation will produce statistically significant increases in mitochondrial respiration (State 3=primary outcome), COX, CS enzymes activities, and mitochondrial DNA content in muscle specimens from the *vastus lateralis.*

1.2 Secondary Objectives

Specific Aim 2. To evaluate the effects of resveratrol supplementation on levels of PGC-1α, AMPK, and sirtuins (SIRT1 and SIRT3) in muscle samples of older adults.
Hypotheses. Compared to placebo, resveratrol supplementation will increase PGC-1α (primary outcome), AMPK, SIRT1, and SIRT3 levels in muscle specimens obtained from the *vastus lateralis.*

Exploratory Aims. Explore the effects of resveratrol supplementation on measures of physical function, metabolic risk factors, and changes in physical activity in older adults.
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2. BACKGROUND AND RATIONALE

2.1 Background on Condition, Disease, or Other Primary Study Focus

General Overview. The life expectancy of older Americans continues to increase, with persons aged ≥70 years representing the fastest growing segment of the US population.[1] While prolongation of life remains an important public health goal, of even greater significance is that extended life should involve preservation of the capacity to live independently, and to function well.[2] Therefore, identification of proven interventions to maintain physical function and to prevent disability is a major public health challenge.[3] Mobility and activities of daily living represent tasks that are necessary for the maintenance of basic independent functioning,[4;5] and inability to perform these activities marks a serious decline in functional health, conferring increased risk of institutionalization and death.[6;7] Most older adults are sedentary.[8;9] Among this population, many are mobile and free of disability but have reductions in mobility marked by slower walking speed, which is a key predictor of functional decline and of increased risk of

mortality.[10] It is these individuals who represent the target population for the proposed intervention.[11-13]

Causes of Physical Disability in Older Persons. In many cases, physical disability is directly caused or aggravated by acute events (e.g. stroke or hip fracture) and chronic conditions (e.g. heart failure, coronary heart disease, diabetes, arthritis, or peripheral artery disease).[14;15] In contrast, a large and growing number of older adults experience progressive declines in physical function culminating in age-related physical disability with no clear connection to a single disease. A number of factors have been associated with the pathogenesis of functional decline and physical disability (in non-acute disease conditions), but the exact mechanisms contributing to this process remain largely undefined. A growing body of evidence strongly implicates the mitochondria as having a key role in the initial onset and progression of functional decline in many individuals.[16-19] Thus, the mitochondria may be an important target of interventions aimed to improve physical function in older persons.

Biological Mechanisms Contributing to Functional Decline and Physical Disability. In older persons, a large proportion of mobility disability follows a progressive course over several years.[20] Accumulating evidence from both preclinical studies and recent human clinical trials strongly suggests that mitochondria have a key role in the pathogenesis of age-related functional decline, which is marked by slower walking speed. For example, impairments in mitochondrial function are commonly observed in aged tissues, and are particularly prominent in highly oxidative tissues such as skeletal muscle.[21-23] Walking speed appears to be a robust indicator of functional status[24], a key predictor of functional decline, and also subsequent mobility disability, morbidity, and mortality in older adults.[10;25] Our preliminary data, including pilot data collected for this study, suggest that functional declines are indeed strongly associated with reduced mitochondrial respiration, decreases in oxidative mitochondrial enzyme activities and enzyme content, and a large decline in PGC-1α and SIRT3 (regulators of mitochondrial biogenesis or mitochondrial redox status) levels in skeletal muscle. These findings suggest that these biological changes may directly impact muscle function, and may therefore underlie the pathogenesis of physical disability. Based on these observations, we have developed a working model to explain this potential dynamic relationship between mitochondrial function and skeletal muscle, which is briefly described below.

Reasons for why there is a decrease in mitochondrial function with aging remains under debate, but free radicals (i.e. oxidants) have been implicated in observed mitochondrial damage. In line with this, the mitochondrial theory of aging proposes that cumulative damage caused by the production of oxidants can alter mitochondrial DNA (e.g. point mutations and increase deletions).[26] In turn, defects in mitochondrial DNA can lead to a decline in mitochondrial DNA abundance and a reduced number of genes encoding mitochondrial proteins that are essential for the proper assembly of the electron transport chain and oxidative phosphorylation.[27] Indeed, lower mitochondrial protein synthesis rates,[28] abnormalities in mitochondrial enzyme activity,[27;28] and lower oxidative capacity and ATP synthesis[27] have been observed in the skeletal muscle of older persons. In addition, PGC-1α, a powerful regulator of mitochondrial oxidative metabolism,[29-31] shows marked declines with aging.[32;33] Specifically, declines in PGC-1α have been associated with reductions in mitochondrial oxidative metabolism, which in turn has been associated with poor muscle quality and function.[17;18;34] Thus, the available evidence to date strongly implicates mitochondria as having a pivotal role in the pathogenesis of age-related functional decline.

2.2 Study Rationale

Strategies to reduce functional decline in older adults. Few therapies have been identified to improve functional performance in older adults. There are currently no FDA-approved medications for the treatment of functional impairment in older adults. Given the increasing number of older adults at risk for functional decline, new therapies are urgently needed to improve functional ability and prevent mobility disability, particularly among adults 65 years and older. Resveratrol, a polyphenol found in the skins of grapes and red wine, has received considerable attention for its pharmacological properties as a gene regulator affecting mitochondrial metabolism, possibly by activating AMP-activated protein kinase (AMPK) and sirtuins (SIRT1 and SIRT3).[35;36] These beneficial biological effects have been demonstrated across multiple species, such as mice and flies, and have been associated with improvements on a variety of functional tasks and health-span measures in humans. In several species, resveratrol has even been found to extend healthy lifespan.[37-39] These findings are not unexpected given that resveratrol has been found to activate a wide variety of targets, and produces broad, systemic effects that are very similar to caloric restriction, the only method to date found to increase healthy lifespan in multiple species.[40]

Based on the findings described above, resveratrol may have unique promise to improve age-related functional decline, because it appears to activate similar pathways as caloric restriction, without leading to muscle loss. Yet, very few studies have examined the effects of resveratrol in older adults, and critical questions regarding whether resveratrol works in humans, and by what mechanisms, have not been answered. Recently, in one of the first completed clinical trials examining the effects of resveratrol in adults taking 150 mg of resveratrol daily for 30 days, remarkable beneficial effects on multiple biological and clinical parameters linked to healthy aging were observed. Participants in this double-blind, cross-over study were obese (mean Body Mass Index (BMI)=31.5kg/m^2) but otherwise healthy, middle-aged men (mean age=52.5 years), who served as their own control group (N=11). Following the 30 day resveratrol supplementation period, marked improvements in mitochondrial function (i.e. ability to utilize substrates originating from fatty acids), increases in cell signaling proteins (AMPK, SIRT1, and PGC-1α) causing mitochondrial biogenesis, reductions in inflammatory cytokines (TNF-α), as well as clinically relevant reductions in parameters such as blood pressure, blood levels of glucose, and insulin, were observed.[41] Noteworthy, these changes were not present during the placebo condition. The findings of this study strongly suggest that resveratrol holds promise as a candidate drug for attenuating age-related declines in mitochondrial function, and therefore may also have the potential to attenuate the declines in physical function observed with aging.

Working Model. Mitochondria provide an essential source of energy by producing large quantities of ATP through oxidative metabolism. Mitochondrial activity in skeletal muscle is a highly controlled process, under the influence of a variety of nuclear and mitochondrial factors that adapt to cellular perturbations by increasing the content of mitochondria through the process of mitochondrial biogenesis. Perhaps among the most well-known regulators of mitochondrial activity are peroxisome proliferator-activated receptor γ (PPARγ) and coactivator-1 (PGC-1α).[42-45] The importance of PGC-1α as a master regulator of mitochondrial activity and content has been demonstrated both in cell culture and in transgenic animal models. In particular, overexpression of PGC-1α in mice results in a fiber-type transition from white muscle, with predominantly glycolytic fibers, to muscle that appears red and have high oxidative capacity.[46] In humans, PGC-1α protein is strongly correlated with muscle oxidative capacity and mitochondrial enzyme markers such as

cytochrome c oxidase and citrate synthase (CS).[34;47;48] COX activity is also used as an indicator of mitochondrial content. On the other hand, CS, a rate-limiting Krebs cycle enzyme, is often used as a marker of oxidative metabolism and a broad index of mitochondrial enzymatic "volume." The renewal of mitochondria is vital for maintaining mitochondrial integrity. Further, diminished PGC-1α levels and organelle biogenesis have been implicated in the pathogenesis of several age-related disease conditions including neurodegeneration and type 2 diabetes.[49;50] Basic science research and animal studies have demonstrated that higher levels of AMPK, PGC-1α, SIRT1, and SIRT3 in skeletal muscle induce mitochondrial biogenesis, promote a switch in fuel consumption to fatty acids, and substantially improve skeletal muscle performance. These biological changes would be expected to translate to improvements in physical function among older adults.

Resveratrol appears to oppose the reductions in mitochondrial function associated with aging by affecting the expression of genes such as PGC-1α,[51] which support oxidative phosphorylation and mitochondrial biogenesis. Specifically, resveratrol activates SIRT1, which in turn increases PGC-1α activity by reducing its acetylation.[51] Similar to SIRT1, SIRT3 can also induce mitochondrial biogenesis by activating PGC-1α and AMPK in skeletal muscle but it is currently unknown if resveratrol can activate SIRT3 *in vivo*.[52] SIRT3 is localized to mitochondria and regulates levels of ATP and the activity of complex I of the electron transport chain.[53] Mice lacking SIRT3 display the hallmarks of fatty acid oxidation disorders, indicating that SIRT3 modulates mitochondrial fatty acid oxidation in mammals.[54] Moreover, the activation of AMPK increases fatty acid oxidation, an effect that involves the phosphorylation and inhibition of acetyl-CoA-carboxylase.

For the first time, we will investigate whether resveratrol supplementation activates AMPK, PGC-1α, and both SIRT1 (known to be induced by resveratrol) and SIRT3 in older adults. Despite favorable associations identified in basic science, and one single clinical study in obese, middle-aged men, the effects that resveratrol has on key biological targets, such as mitochondrial respiration, PGC-1α, AMPK, and Sirtuins (SIRT1 and SIRT3), which appear to be directly linked with physical functioning, has not yet been explored in older adults. The proposed study will fill this gap in the literature, as well as extend previous findings, by examining whether these predicted cellular adaptations are associated with improvements in clinically relevant measures of physical function in older adults.

Brief Description and Justification of Intervention Regimen(s)

Eligible participants will be randomized to receive placebo (vegetable cellulose) or resveratrol (1000 mg/day or 1500 mg/day) for a 90 day period. In all three conditions, participants will orally consume one capsule following each of their main meals (i.e. breakfast, lunch, and dinner) – three (3) times a day. The company *Resverage Organics* will provide the resveratrol product and placebo capsules.

Justification for Intervention*:* As noted in a recently published study,[55] many consumers are using resveratrol supplements for their potential health benefits with annual sales being close to $30 million in the United States alone, but few randomized clinical trials have yet justified its use. In the Timmers et al. study,[41] resveratrol supplementation (150 mg/day) for 30 days in middle-age, obese men (mean age=52.5 years) exerted favorable metabolic adaptations, including increased skeletal muscle **mitochondrial function** and **PGC-1 α** levels. Another recent study by Yoshino et

al. (2012),[55] however, found that supplementation with resveratrol at a dose of 75 mg/day for a 12-week period did not improve metabolic function in non-obese, women with normal glucose tolerance. Noteworthy, the findings of this study are in contrast to two other recently published clinical trials in which resveratrol supplementation at much higher doses (1000–2000 mg/day) improved metabolic outcomes in participants with impaired glucose tolerance,[56] and at much lower doses (10 mg/day) in participants with type 2 diabetes.[57] Thus, findings from studies to date suggest that resveratrol improves metabolic function among participants with impairments in glucose metabolism, but not normal glucose tolerance. It is possible that the selected dose in the Yoshino et al. study (75mg/day) was not high enough to exert beneficial effects on metabolic parameters. To our knowledge, the pilot study published by Crandall et al. (2012)[56] is the first to demonstrate improvements in glucose metabolism and insulin sensitivity in older adults (mean age=72 years) following resveratrol supplementation at doses of 1000 mg/day and 2000 mg/day. In addition to these recently published findings, our 12-week resveratrol pilot study demonstrated that resveratrol supplementation at a dose of 1000 mg/day, but not 300 mg/day, improved both cognitive and physical function, as well as markers of metabolic function (e.g. blood glucose) in healthy older adults (age $\geq$ 65 years). Based on the findings described above, particularly the findings of the Crandall et al. (2012) study, we have chosen to test two doses of resveratrol in the proposed trial: 1000mg/day and 1500 mg/day.

Potential Risks of the interventions. In our resveratrol pilot study, resveratrol supplementation of 300 mg/day and 1000 mg/day had minimal effects on blood chemistries, and there were no significant differences in the number of participants reporting adverse or toxic events (e.g. headaches) across the three conditions (all p values > 0.05). Noteworthy, the 1000 mg/day dose has been shown to be safe and well tolerated in previous safety trials in young and middle-aged adults.[58-60] Additionally, a recent repeated dosing study found that a dose of 200 mg of resveratrol at eight hour intervals for a three day period was well tolerated by older adults (age $\geq$ 65 years),[61] and similarly, 1000 mg/day of resveratrol supplementation was well tolerated by older adults in a four-week intervention.[56]

3. STUDY DESIGN

The proposed study will utilize a double-blind, placebo controlled, parallel design to determine whether 90 days of resveratrol supplementation in older men and women ($\geq$ 65 years) is associated with (i) increases in muscle mitochondrial respiration (state 3 and 4), (ii) increases in levels of PGC-1α, AMP-activated protein kinase (AMPK), and sirtuins (SIRT1 and SIRT3), and (iii) improvements in functional performance and metabolic parameters. To achieve these aims, 60 participants will be randomized to receive a placebo (n=20), 1000 mg/day of resveratrol (n=20), or 1500 mg/day of resveratrol (n=20) for a 90-day period. We will collect muscle specimens from the vastus lateralis at baseline and after 90 days of intervention, for biochemical analyses (See **Table 1** for planned data collection and procedures). In addition to the measures listed in **Table 1**, we will also monitor blood chemistries every 30 days at monthly clinic visits. To enhance retention, participants will receive biweekly phone calls throughout the 90-day treatment period. Following the completion of this trial, one in-person follow-up assessment will be conducted at approximately 30 days post-treatment, to monitor blood chemistries and ascertain adverse events. In addition, approximately 10-days following muscle Biopsy procedure, a follow-up visit will take place to assure absence of biopsy complications and to monitor blood chemistries.

Importantly, previous data demonstrate that 30 days of supplementation is sufficient for detecting meaningful changes in muscle mitochondrial biomarkers and clinical parameters in obese middle-aged adults, as proposed in the current study;[41] however, we propose to extend the supplementation duration to 90 days and to recruit a mixed population of men and women with diverse functional status. The justification for this extension is two-fold: our selected participant sample is comprised of older adults, who have been found to have lower levels of mitochondrial function than younger adults, and The RIPE Trial, our pilot resveratrol study, showed increased mitochondrial activity, as well as improvements in cognitive function, following a 90-day intervention.

4. SELECTION AND ENROLLMENT OF PARTICIPANTS

4.1 Inclusion Criteria

Participants must meet all inclusion criteria listed below in order to participate in this study.

- Ability to understand study procedures and to comply with them for the entire length of the study;
- Age 65 years and older;
- Body Mass Index (BMI) range: 20–39.9 kg/m^2;
- Willingness to undergo all testing procedures.

4.2 Exclusion Criteria

All candidates meeting any of the exclusion criteria listed below at baseline will be disqualified from study participation.

- Non-English speaking individual.
- Failure to provide informed consent;
- Allergy/sensitivity to grapes or Japanese knotweed;
- Current dietary supplementation of grape seed extract or ginko biloba;
- Consumption of ≥ 8 oz. of red wine/dealcoholized red wine/red or purple grape juice more than once weekly;
- Consumption of any dietary supplements containing resveratrol, quercetin, or *P. cuspidatum* in the previous 90 days;
- Active treatment for cancer, stroke (< 6 months), peripheral vascular disease, coronary artery disease, myocardial infarction (< 6 months), congestive heart failure (stage III or IV), valvular heart disease, major psychiatric disease, severe anemia (blood levels of Hemoglobin < 8 g/dl), bleeding disorders or other blood disorders, liver or renal disease, diabetes, severe osteoarthritis, blindness or deafness, fracture in upper or lower extremity (< 6 months), upper or lower extremity amputation, or Parkinson's disease;

- Cognitive impairment (i.e. Mini Mental Status Exam ≤ 23);
- History of significant head injury that currently interferes with functioning;
- Severe physical limitations (i.e., < 4 on the Short Physical Performance Battery);
- Hard exercise (i.e. running, bicycling, etc.) ≥ 30 min on five or more days per week;
- Excessive alcohol use (> 2 drinks/day) or alcohol abuse (> 5 drinks/day for males, or > 4 drinks/day for females);
- History of substance abuse within the past six months;
- Mood disorder (i.e. Center for Epidemiological Studies – Depression (CES-D) ≥ 16);
- History of tobacco use within the past three years;
- Resting heart rate > 120 bpm at screening visit;
- Systolic blood pressure > 160 mm Hg at screening visit;
- Diastolic blood pressure > 90 mm Hg at screening visit;
- Fasting glucose ≥ 126 mg/dL at screening visit;
- Abnormalities in blood chemistry parameters, defined by blood chemistry marker outside of healthy range) as determined by study physician;
- Current use of
    - anabolic treatments (e.g. growth hormone or testosterone),
    - anticholinesterase inhibitor (e.g. Aricept),
    - hormone replacement (e.g. Estrogen),
    - anticoagulant therapies (note: aspirin -anti-platelet use (≤ 81mg/day) is permitted) or
    - use of anti-inflammatory medications more than 3 times per week
- Participation in another non-observational clinical trial, or has received an investigational product within 30 days prior to screening/enrollment;
- Refuse to refrain from CoQ10 or alpha-lipoic acid while enrolled in the study.

Temporary Exclusion Criteria

A person meeting any of the following temporary exclusion criteria at the time of screening would not be enrolled but may be re-screened at a later date. A period of 4 weeks is the minimum amount of time required before re-screening for the following conditions can be done.

- Recent bacterial/viral infection (< 2 weeks);
- Acute febrile illness in past 2 months;
- High blood pressure (i.e. ≥ 140/90 mm Hg but ≤ 160/90) at the screening visit;

- Major surgery or hip/knee replacement (< 6 months);
- Persons reporting use of exclusionary amount of alcohol or dietary supplements containing grape seed extract or ginko biloba.

4.3 Study Enrollment Procedures

Recruitment. We will use multiple resources for recruiting our participants that include those originating at the Department level, but also the UF Clinical Translational Science Institute (CTSI). We have a wealth of experience recruiting high and moderate functioning older adults, which will suit this study well. We have a subject pool of 43 individuals with low-to-high functioning physical characteristics (N=43) who participated in the pilot study described above, as well as a registry of over 2,240 participants, out of which 69.4% are over the age of 70. Thus, we have a good initial base of potential participants to be recruited for the present study. Additional participants, if needed, will be enrolled from the North/Central Florida region. The North/Central Florida region includes Gainesville (home to the University of Florida), its home county (Alachua), and the eight immediately surrounding counties (Bradford, Clay, Columbia, Gilchrist, Levy, Marion, Putnam, and Union). The total population of older adults falling within the target age range in these counties is 216,217. We recognize that fewer people may be interested in a study involving muscle biopsies due to the relatively invasive nature of the biopsy procedure. Given the current demographics of North/Central Florida, the fact that we have a large base of potential participants already identified, and that this is an intervention study, we do not anticipate difficulties with the enrollment of 60 subjects. Moreover, there were no untoward outcomes associated with completed muscle biopsies on participants in our previous pilot studies. Thus, we do not anticipate difficulty obtaining our proposed sample size of 60 participants.

In addition to the subject pool described above, we will use the UF Pepper Older Americans Independence Center (OAIC) Recruitment Core registry to recruit potential participants for this study. This registry was built in four major ways: (1) offering registry membership to all participants who do not qualify for a study (in which they have expressed interest), or who have completed participation in a study; (2) a direct mail campaign (10,000 pieces) sent to Alachua County residents aged 65 and older; (3) offering registry participation at community outreach events (e.g. the community presentation series detailed below); and (4) other miscellaneous contacts received by the OAIC and Institute on Aging staff from seniors interested in research.

The OAIC Recruitment Core has developed many assets for successful recruitment that include: (1) participant real-time tracking from initial contact to study enrollment; (2) web-facilitated tracking database, which tracks each (a) informal participant inquiry/contact, (b) screening contact, and (c) enrollment; (3) development and growth of the OAIC participant registry; (4) staff of up to four screeners/schedulers; (5) a "Speaker Series" at two locations (described below); (6) stable relationships with advertising personnel at all local radio, television, and print outlets; (7) established and disseminated new formal procedures/policies for access to core; and (8) developed templates for all study advertising materials (i.e. flyers, brochures, newspaper ads, radio ads, and classified ads).

The Recruitment Core also supports participant retention by: (1) weekly real-time review of missed visits; (2) retention planning for all missed visits (including phone calls, mailings, and home visits

as needed); and (3) repeated contact with hard to reach participants until a stable final disposition is reached (i.e. scheduled visit, partial or telephone visit, withdrawal). In general, withdrawal is a last resort, and other supports (i.e. home visits and provision of transportation) are offered to minimize loss of participants. More on the transportation option is described below.

A community-based Speaker Series is conducted monthly at two different locations in Gainesville. One is presented at Oak Hammock, a retirement community affiliated with the University of Florida, as part of the "Institute for Learning in Retirement." The other is presented at the Alachua Country Senior Recreation Center, as part of a local educational cooperative entitled "Primetime for Seniors." These Speaker Series offer monthly opportunities for researchers to present their research and to recruit participants (where relevant).

Transportation can be a major barrier for participation in research for older persons. To overcome this barrier, participants will be offered transportation services by the OAIC. The OAIC has access to a vehicle that can provide any participant with transportation to and from the clinic and transportation will be available for all participants enrolled in the proposed study.

The Investigators will actively contribute to all efforts targeted towards ensuring the success of study recruitment, especially in terms of providing adequate representation of women and ethnic/racial minority participants. This includes weekly monitoring of the overall pace of recruitment relative to the study goals, both the overall goals and goals for the recruitment of women and ethnic/racial minorities. Proactive efforts will be required to ensure successful recruitment. In response to this challenge, the Investigators will employ a multi-stage approach.

We will primarily utilize the UF Pepper OAIC Recruitment Core. The Recruitment Core of the OAIC will coordinate overall recruitment (e.g. mailings and phone screenings), and will track and report on progress in recruiting women and ethnic/racial minorities. Study materials and screening will be developed to facilitate overall recruitment, and more importantly, to enhance the recruitment of ethnic/racial minorities.

The investigators will employ a number of recruitment strategies such as cultivating the coverage of the study in the free media, community presentations, requesting referrals from health professionals who treat at-risk older adults, direct mail, targeted mass mailing, community advertising, health fair participations, and soliciting volunteers among participants from previous studies. A sample of ≥ 65 year-old residents within each catchment area will be selected from USAData, which has provided commercially available consumer lists for adults in our age range for the past five years. Their lists are culled from credit card owner lists, voter registrations, tax records, driver registration, and other sources. Staff from the UF Pepper OAIC recruitment committee will first contact sampled participants by mailing the study brochure. The mailed brochure has both a call back number and a postage paid reply card, allowing interested participants to self-identify themselves. During an initial phone call, participants will be pre-screened for eligibility and then scheduled for an in-person clinic visit, to consent individuals to participate in the study and to determine if they meet the eligibility criteria. At this clinic visit, a thorough medical screening will take place to ensure the participants are generally healthy and suitable to participate in the proposed study. The direct mail methodology proved to be very effective in our previous studies, and the investigators anticipate further success. Typically, we have a 1-2% response rate to community mailings. We will also

employ a variety of other strategies including newspaper advertisements and community presentations, as well as other strategies previously outlined.

Since July 2007, the OAIC has maintained a participant registry. This is an IRB approved database containing contact information and minimal demographics (i.e. age, race, sex, and ethnicity) for persons who consent to be contacted with recruitment opportunities. The registry contains no information regarding health or functional status since these are labile phenomena and need to be rescreened by each individual study at time of recruitment. Registrants are recruited at the time they consent to participate in individual studies; they are also recruited if they fail to qualify for an OAIC-supported study. Registrants have also been identified through a direct-mail campaign (10,000 pieces sent to elders in the catchment area, resulting in approximately 300 phone calls) and community presentations. Unsolicited inquiries from older adults seeking to participate in research are also directed to the registry. Each registrant receives a consent form (which is reviewed with a member of the RC4 staff prior to signing), a contact record (including name, address, phone, and email), and a demographic questionnaire. The contact information is entered and stored separated from the demographic information. At the present time, the registry contains 2,240 individuals (684 women, 1,556 men).

To aid with registry retention, we strive to maintain personal relationships with participants (e.g. mailing cards, letters, newsletters, etc.), both as a total center, and as individual, studies. When specific studies are recruiting, registrants are contacted in one of three ways: (1) the quarterly newsletter, entitled *THRIVE*, highlights specific studies and includes short summaries for individual studies; (2) direct mail of flyers to eligible registrants; and (3) follow-up phone calls to eligible registrants.

Interested individuals will be encouraged to call the research office to learn more about the study and to answer questions in an initial telephone pre-screening form. If the caller is found to not be eligible to participate in this study based on the inclusion and exclusion criteria listed above, then the reason the participant was not eligible will be recorded by the telephone screener. If eligibility criteria change, we will call back previously excluded participants and inform them of these changes should they still be interested in participating in the trial. If the individual is found to be potentially eligible to participate in this study, based on their responses to the telephone screen, then he or she will be invited to attend an in-person screening visit.

At this visit, the potential participant will be asked to give their informed consent and will then be screened for cardiovascular and other major diseases by means of a health review. The health review consists of a medication inventory, medical and hospital admission history, a review of the telephone screen, vital signs, including blood pressure, radial pulse and anthropometric measures as height without shoes, weight, and waist circumference. Participant's height and body weight will be assessed at this visit to determine if their BMI falls within the acceptable range for this study (BMI = 20.0–39.9 kg/m^2). At this visit, participants will also be asked to provide a fasting blood sample that will be utilized to evaluate clinical laboratory parameters and ensure participants are suitable to participate in the trial. The Mini Mental Status Exam-2 (MMSE-2) will be administered to screen for potential cognitive impairments (MMSE score ≤ 23). Center for Epidemiological Studies – Depression (CES-D) scores will help in detecting mood disturbances (CES-D Score ≥ 16) that might interfere with study participation and safety. Participants will also complete the Short

Physical Performance Battery (SPPB) to ensure that their physical function is not marked by severe limitations (SPPB Score < 4). If eligibility criteria change, we will call back previously excluded participants and inform them of these changes should they still be interested in participating in the trial.

If all study entry criteria are met at the in-person screening visit, participants will be scheduled to return to the clinic for two baseline visits prior to randomization. During the first baseline visit, study staff will initially assess body mass and vital signs (e.g. pulse, blood pressure). Participants will then be asked to provide a fasting blood sample that will be utilized to evaluate clinical laboratory parameters. Following collection of the blood sample, participants will complete the following physical function tests: (1) the Short Physical Performance Battery (SPPB), (2) the Six Minute Walk Test, where participants will be asked to walk as far and fast as possible for 6-min on a 40 m track, and (3) isokinetic peak torque of the knee extensors and flexors of the dominant limb, unless contraindicated, will be assessed by dynamometer (Biodex Medical Systems, New York, NY) will be performed at this visit. Finally, the study coordinator will provide participants with a physical activity monitor. Participants will be asked to wear this monitor for a typical seven day period to objectively evaluate baseline physical activity habits. Also, during the Baseline Visit 1, study participants will be asked if they agree that left-overs of their tissue (muscle, blood) already collected for this study can be stored in the Tissue/Data Bank for future research. The Tissue/Data Bank has been approved by the UF IRB (IRB201300725, PI – Dr Leeuwenburgh, Department of Aging and Geriatric Research, University of Florida). The bank study has a separate consent form that needs to be signed by those who agree to participate in the bank.

Following this first baseline visit, participants will be invited to attend a second baseline visit to complete a muscle biopsy. Following completion of the Baseline Biopsy Visit, participants who satisfy all inclusion/exclusion criteria will be randomly assigned to receive resveratrol (1000 mg/day or 1500 mg/day) or placebo (vegetable cellulose). Participants will be provided with up to a 40-day supply of study drug capsules at the Baseline Biopsy Visit and will be asked to return to the clinic in approximately 30 days (± 7 days) for their 30-Day Visit.

Missed visits. Specific procedures (discussed below) will involve documentation of any participants who appear to be "at risk" of attrition. We will implement a weekly retention conference, during which coordinators present information about each participant who has missed a visit during the previous week, has been difficult to schedule, or for whom other information suggests retention difficulties exist. For our current trials, we include members of both the intervention and assessment team at these meetings, but all information is presented in a blinded format. This facilitates communication regarding past contacts and attempted contacts with specific participants. Following group discussion about each participant, an action plan (e.g., send letter, follow-up phone call, or home visit) will be developed and the outcome of the selected course of action will be reviewed during the following week's meeting.

The following procedures will be implemented to carefully document and monitor missed assessment visits:

- Preparing for the next visit at the end of each current visit by making the appointment and giving instructions for the next visit
- Sending out pre-visit reminders and making reminder phone calls before all visits
- Charting and monitor local clinic attendance, so that clinic staff would be immediately alerted to a missed visit; missed visits are reviewed at weekly staff meetings
- Immediately contacting participants (usually by telephone) when they miss a visit.
- For participants who are unreachable via telephone, planning a schedule of repeated phone calls at different times of the day to reach them
- Asking participants to identify "someone who does not live with you, but who would always know how to reach you" as a second contact. This proxy/secondary contact should help us can maintain contact with all participants.
- For those participants who do not respond to repeated phone calls, sending personal "check" in cards, asking them to call.

For the Muscle Biopsy Visit in particular, we will employ strategies to ensure that participant discomfort is as low as possible and that this procedure goes smoothly. Although there are some risks associated with this procedure, including discomfort during the muscle biopsy procedure, scarring from the muscle biopsy skin incision, risk of bleeding, and risk of infection, we will do all we can to minimize these risks and thereby increase the likelihood the participant will return for a second muscle biopsy visit. First, as in previous studies conducted at our center, the muscle biopsy will be performed under sterile conditions using sterile technique. Second, local anesthesia will be used with topical lidocaine to minimize discomfort during the procedure. Third, Dr. Sandesara, who will be the primary physician performing these biopsies, has extensive experience with this procedure and has performed muscle biopsies in multiple studies conducted over the past five years.

We will also provide compensation to participants for completing study visits, as described below. Participants will complete two Baseline Assessment visits, two visits during the study (day 30 and day 60), two Post-Treatment Assessment study visits, and two follow-up visits at 10 days and 30 days, following completion of the Post-Treatment muscle biopsy visit at clinic. To ensure health and safety after biopsy visit, participants will be contacted by telephone 24 hours after procedure, 3 – 5 days after procedure, and will attend an in-person visit 10 days after the procedure. Participants will receive $75 in compensation for completion of the first Baseline Assessment visit,

and $150 in compensation for completion of the second Baseline Assessment Visit (i.e., muscle biopsy visit). Participants will receive $75 in compensation for completion of the first Post-Treatment Assessment visit, and $150 in compensation for completion of the second Post-Treatment Assessment visit (i.e., muscle biopsy visit). Participants will also receive $50 in compensation for completion of the day 10 visit, day 30 visit, and day 60 visit, as well as $50 for completion of each of the two follow-up visits at 10 days and 30 days following completion of the second muscle biopsy visit.

Managing participants using prohibited medication/interventions after enrollment

If the participant reports any change in medications, including use of prohibited medications, engagement in any prohibited interventions during the study, this information will be recorded in the participant's file (i.e. source documents and case report forms). The Principal Investigator together with the study physician will need to determine whether the participant should continue in the study.

5. STUDY INTERVENTIONS

5.1 Interventions, Administration, and Duration

Supplementation Regimen. Eligible participants will be randomized to receive capsules containing either placebo (vegetable cellulose) or resveratrol (1000 mg/day or 1500 mg/day) from our UF clinic. The company Resverage Organics will provide the resveratrol product and placebo in the finished dosage form. The UF Health Investigational Drug Service Pharmacy will be provided bulk supply of open label investigational product as follows:

- Resveratrol Product: ReserveAge capsule contains Organic French Red Grape & Vine [Vitis vinifera (Full Spectrum Polyphenol Profile)], Muscadine USDA Certified Organic Red Grape & Seed [Vitis rotundifolia (Grape Pomace)], Trans-ReserveAge [Organic French Red Grape & Vine (Vitis vinifera) and wild natural Japanese Knotweed (Polygonum cuspidatum)], Standardized to a minimum of 50% purity, containing no less than 250mg of the Trans-ReserveAge Isomer. The active compound of interest in ReserveAge is *trans*-Resveratrol. *trans*-Resveratrol (3,5,4'-trihydoxystilbene) is a polyphenol that occurs naturally in grapes, peanuts, and a number of other plants. It belongs to the stilbene family of phytoalexins.
- Placebo: vegetable cellulose.

The product will remain in its original sealed containers until receipt of a medication order for a randomized subject. Upon receipt of the investigational medication request, the IDS Pharmacist will review the treatment assignment for the subject (confirming subject number, name, and treatment assignment). The pharmacist will then prepare up to a 40-day supply of the designated treatment for the subject using Memory Pacs. Each subject will receive a total of 6 One-Week Memory Pac cards containing up to a 40 day supply of study drug. Instructions will be to take one capsule following each meal three times daily.

A dispensing log will be used to document appropriate Memory Pacs layout for each subject medication order. Subjects will be asked to return any unused Resveratrol/Placebo Memory Pacs at the follow-up visit. The study coordinator will count the number of capsules returned for the

morning, noon, and evening doses and record this in the case report form along with a compliance assessment. All Resveratrol/Placebo Memory Pacs will then be returned to the Investigational Drug Service Pharmacy for documentation of return counts and final destruction as per institutional policy.

Participants in all conditions will receive up to a 40-day supply of a resveratrol product or placebo, or a combination thereof for the 1000 mg/day condition. The reason participants will be provided with up to a 40 day supply is to ensure that they have a sufficient supply of study product before their day 30 visit, which will be scheduled at 30±7days from their second Baseline visit. Participants will also be given a up to 40 day supply at their day 30 and day 60 visits. Participants in all conditions will be instructed to consume three capsules per day, one capsule following each of their main meals (i.e. breakfast, lunch, and dinner).

Participants will also be asked to record taking their study product on a daily basis in a medication diary. Participants will also be asked to record any adverse events they may have experienced on this diary, as well as any concomitant interventions that they participated in since the previous study visit. If the participant reports any change in medications, including prohibited medications, during the study, this information will be recorded in the participant's file. Additionally, if the participant reports engagement in any prohibited interventions listed below, this information will also be recorded in the participant's file.

5.2 Handling of Study Interventions

Study products, both active and placebo capsules, will be shipped directly from the manufacturer to the University of Florida's Investigational Drug Service (IDS) Pharmacy.

The products will be packaged in 200 ml amber glass or white HDPE bottles. The placebo (i.e. vegetable cellulose) will be similar to the active resveratrol product in terms of color, taste, size of capsule, and other aspects of appearance. The study coordinator or other members of the study team will not be able to distinguish the active product from the placebo. Thus, a double-blind design will be utilized.

Once acquired, the study product will be stored within the temperature-controlled drug room at the University of Florida's IDS. The product should be stored in cool, dry place according to instructions (50 - 85˚F and ≤ 65% RH). The drug room of the IDS is locked and can only be accessed by designated personnel. Reference specimens are stored under specific conditions in regards to temperature and moisture controls.

At the time of randomization a signed order is received at the pharmacy, the pharmacist while following the study specific pharmacy plan will first verify the treatment arm to which the participant was randomized and re-package the appropriate amount of study agent/placebo capsules into Memory Cards (similar to blister packs). The pharmacist will dispense the appropriate number of Memory Cards for up to 40 daily doses to the study coordinator or designee. The study coordinator or designee will be responsible for dispensing the Memory Cards to the participant and instructing the participant to take daily doses (1 capsule following each meal – 3 times a day) until their next study visit, reminding the participant to return unused Memory Cards. Any unused study products will be destroyed by the company Steriocycle, on a bi-annual basis. Records of the study product destruction will be filed with the study's documents.

5.3 Concomitant Interventions

Concomitant Interventions are not allowed during this trial.

5.3.1 Allowed Interventions

No additional interventions are allowed during this trial.

5.3.2 Required Interventions

There are no required interventions to participate in this trial.

5.3.3 Prohibited Interventions

- Active treatment for cancer, stroke, peripheral vascular disease, coronary artery disease, myocardial infarction, congestive heart failure (stage III or IV), valvular heart disease, major psychiatric disease, severe anemia, liver or renal disease, diabetes, severe osteoarthritis, blindness or deafness, fracture in upper or lower extremity, upper or lower extremity amputation, or Parkinson's disease;
- Physical activity (i.e., running, bicycling, etc.) ≥ 120 min/week;
- Use of anabolic medications (e.g. growth hormones or testosterone), anticholinesterase inhibitor (e.g. Aricept), or anticoagulant therapies (aspirin use is permitted);
- Dietary supplementation of grape seed extract or ginko biloba;
- Consumption of any dietary supplements containing resveratrol, quercetin, or *P. cuspidatum;*
- Use of other Investigational products;
- Dietary supplementation with CoQ10 or alpha-lipoic acid.

5.4 Adherence Assessment

For this study, adherence will be defined as 90% or greater compliance with prescribed regimen, based on pill counts (number of pills consumed/number of days since previous visit). Study drug intake compliance will be assessed by means of capsule count by a study team member at 30, 60, and 90-day visits. In order to encourage high levels of adherence, a member of the study team will call each participant on a biweekly basis to encourage continued adherence.

The gold standard for clinical trials is by intent-to-treat. An analysis of the prognostic significance of actual dose consumed over the active period has value, but a comparison with placebo is not recommended. This would be a secondary analysis and would allow for determination of whether higher consumption while on active medication resulted in greater changes to outcome variables. For this study, a completers analysis will be performed on the target population of older adults (age ≥ 65 years).

6. STUDY PROCEDURES

A Schedule of Evaluations is provided in section 6.1 below.

6.1 Schedule of Evaluations

Assessment	Phone Pre-Screen	Screening Visit	Baseline Visit 1	Baseline Visit 2	10 days Post-Biopsy Visit	30-Day Visit	60-Day Visit	90-Day Visit 1	90-Day Visit 2	10 days Post-Biopsy Visit	Follow-up 30 Day
Telephone interview pre-screening	X										
Informed Consent		X									
Informed Consent for Data/Tissue Bank (optional)			X								
Medical history (inclusion/exclusion criteria)		X									
Medical History Update			X	X	X	X	X	X	X	X	X
Height (without shoes)		X									
Physical Exam		X									
Mini-Mental Status Exam (MMSE)		X									
Center for Epidemiologic Studies - Depression Scale (CES-D)		X									
Weight/Girth Measurement		X	X			X	X	X			
CMP & CBC w/Diff		X	X	X	X	X	X	X	X	X	X
Inflammation Markers in blood			X					X			
Vitals (blood pressure + heart rate)		X	X	X	X	X	X	X	X	X	X
Short Physical Performance Battery (SPPB)		X	X					X			
Physical Function (6 min walk test)			X					X			
Activity Monitor			X					X			

Biodex (knee extension and flexion)			**X**					**X**			
Muscle Biopsy				**X**					**X**		
Randomization				**X**							
Product Dispense				**X**		**X**	**X**				
Product Compliance						**X**	**X**	**X**			
Adverse Event Assessment Toxicity (NCI) criteria			**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**

6.2 Description of Evaluations

Telephone interview pre-screen: Potential participants will undergo an initial telephone pre-screening to assess inclusion and exclusion criteria, which will include a brief medication and health history.

Informed consent: Potential participants will first provide informed consent to participate in this study. The study coordinator will explain to prospective participants the purpose, methods, and extent of the study. Potential participants will be asked to read the informed consent form and to ask questions. The research assistants will review all key aspects of the study verbally, and will then ask several questions to ascertain whether potential participants have understood the information. Two copies of the consent form are to be made, one for the participant and one for the investigators to be stored in the participant's individual file.

Informed consent to participate in the Tissue/Data Bank, PI – Dr Leeuwenburgh: Study participants will be asked if they agree that left-overs from the blood and muscle samples can be stored in the bank and used for future research. The research assistants will review all key aspects of the study verbally, and will then ask several questions to ascertain whether potential participants have understood the information. Two copies of the consent form are to be made, one for the participant and one for the investigators to be stored in the participant's individual file.

Medical history (inclusion/exclusion criteria): The information to be collected includes basic demographic and lifestyle factors including living situation, ethnicity and language, personal medical history, smoking history, alcohol use, known allergies, medication history, and level of education.

Medical history update: The information to be collected includes any changes in health status and/or medications.

Physical Exam: The physical exam consists of a medication inventory, medical and hospital admission history, a review of the telephone screen, a Mini Mental Status Exam (MMSE), and a physical exam including height, blood pressure, radial pulse, weight, waist circumference, and hip

circumference. Blood pressure and heart rate will be measured twice in the seated position following 5 minutes of rest using an oscillometric device. Height will be measured without shoes standing against a wall using a calibrated stadiometer. Body Mass Index will be calculated as the quotient of the participant's weight in kilograms by the square of their height in meters. In addition, participants will meet with a health care provider (study physician, nurse practitioner, or physician's assistant) for a brief physical exam of overall health and wellness.

Mini-Mental Status Exam (MMSE): The MMSE is a widely used and well-validated brief screening tool that is used to screen for cognitive impairment. It measures orientation to time and place, immediate recall, short-term verbal memory, calculation, language and construct ability.

Center for Epidemiologic Studies - Depression Scale (CES-D): The CES-D is a brief questionnaire that measures depressive feelings and behaviors during the past week. It is a self-administered screening measure developed to identify current depressive symptomatology related to major or clinical depression in adults.

Weight/Girth Measurement: Weight will be measured following the removal of excess clothing and with calibrated scales. Girth measurements will include measurements at the abdomen and hip area. The waist measurement is taken at the visually narrowest waist level of the participant, or if this is not apparent, at the mid-point between their lowest rib and the top of their hip bone. Waist circumference will be measured according to the NHANES III Protocol (http://www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=obesity.box.236). The hip girth measurement is taken over minimal clothing, at the level of the greatest protrusion of the clients gluteal (buttock) muscles. The participant stands erect with their weight evenly distributed on both feet and legs slightly parted, making sure that they do not tense their gluteal muscles.

CMP & CBC with Differentials: Fasting blood samples will be taken (analyzed by CLIA certified Laboratories) for a Comprehensive Metabolic Panel (CMP) and Complete Blood Count (CBC) with Differential.

Inflammation Markers: Blood samples will be taken to measure levels of inflammatory markers.

Short Physical Performance Battery (SPPB): The SPPB is designed to assess lower-extremity function by measuring three timed subtests: five timed repetitive chair stands, standing balance (i.e. hold tandem and semi-tandem foot position for 10 seconds), and gait speed during a 4 m walk at one's usual pace. Each subtest is scored from 0 to 4, with 0 indicating inability to complete test, and 4 indicating maximal performance. A summary score from 0 to 12 is calculated for the SPPB, where higher scores indicate better physical function.

Physical Function (6 min walk test): The 6 Minute Walk test measures the amount of distance the participant can complete on a standard walking course in six minutes without running or overexerting themselves.

Activity Monitor: Participants will also be instructed in the use of an activity monitor that they will be asked to wear for 24 hours for seven days and then return at their next visit. The monitor estimates energy expenditure by combining data from a tri-axial accelerometer with galvanic skin response, skin temperature, and heat flux.

Biodex (knee extension and flexion): This test involves using an isokinetic dynamometer set at 90 degrees per second. Participants will perform 50 maximal knee extension and flexion concentric repetitions, which will be administered by trained and certified research assistants.

Muscle Biopsy: Skeletal muscle samples will be obtained under local anesthesia from the *vastus lateralis* muscle using a percutaneous needle biopsy technique as previously described by our group[62] (see **Appendix 1** for detailed description of procedure). The skin will be closed using steri-strips.

Randomization: The randomization will be performed using the CTSI's standard methodology for double blind randomization.

Product Dispense: Up to a ~~A~~ 40-day supply of study product (i.e. placebo [vegetable cellulose] or resveratrol [1000 mg/day or 1500 mg/day]) will be provided in person to the participant at the conclusion of their baseline biopsy, 30-day, and 60-day clinic visit.

Product Compliance: All medication containers, whether empty or containing unused tablets, must be collected to allow medication compliance to be determined (via a pill count) and to allow for proper destruction of the medication. Compliance will be measured by the number of pills returned at the visit compared to the number expected with 100% compliance. The research assistant will discuss the participant's adherence to medication since the previous clinic visit.

Adverse Event (AE) Assessment Toxicity (NCI) Criteria*:* At the designated intervals for event collection, participants will be asked the following question: "Since your previous visit, were you diagnosed with any new disease or condition, or experienced significant worsening of the diseases you were previously diagnosed with?" AEs will be obtained by self-report and will not require supporting documentation. AEs will be reported quarterly to the Independent Safety Board (oversight committee) and the NCCIH for each blinded treatment group.

6.2.1 Screening Evaluation

Consenting Procedure. In compliance with the Health Insurance Portability and Accountability Act (HIPAA) and the Standards for Privacy of Individually Identifiable Health Information of the Department of Health and Human Services, we will request a waiver of consent for phone pre-screening from the University of Florida's IRB. Further screening information will be obtained following informed consent and permission.

According to the NIH and the Office of Human Research Protection (OHRP) guidelines the informed consent currently contains the following elements:

1. A statement that the study involves research
2. An explanation of the purposes of the research
3. The expected duration of the subject's participation
4. A description of the procedures to be followed
5. Identification of any procedures which are experimental
6. A description of any reasonably foreseeable risks or discomforts to the subject
7. A description of any benefits to the subject or to others that may reasonably be expected from the research
8. A disclosure of appropriate alternative procedures or courses of treatment, if any, that might be

advantageous to the subject
9. A disclosure of audio taping of cognitive test administrations to allow for ongoing review of the technician's skills related administration of the battery
10. A statement describing the extent to which confidentiality of records identifying the subject will be maintained
11. An explanation of whether any compensation and any medical treatments are available if a research-related injury occurs and, if so, what these consist of, or where further information may be obtained
12. An explanation of whom to contact for answers to pertinent questions about the research and research subjects' rights, and whom to contact in the event of a research-related injury to the subject
13. A statement that participation is voluntary, refusal to participate will involve no penalty or loss of benefits to which the subject is otherwise entitled, and the subject may discontinue participation at any time without penalty or loss of benefits, to which the subject is otherwise entitled
14. Anticipated circumstances under which subject participation may be terminated by the investigator without regard to the subject's consent
15. Any additional costs to the subject that may result from participation in the research
16. The consequences of a subject's decision to withdraw from the research and procedures for orderly termination of participation by the subject
17. A statement that significant new findings developed during the course of the research, which may relate to the subject's willingness to continue participation, will be provided to the subject
18. The approximate number of subjects involved in the study
19. Specifically for the biological samples repository, the consent will describe the operation of the repository, the conditions under which data and specimens will be released to recipient-investigators, the specific types of research to be conducted, the procedures for protecting the privacy of subjects and maintaining the confidentiality of data (i.e. recipient-investigators will not be provided access to the identities of donor subjects).

Participants who are eligible and who agree to go through the screening process will be invited to a screening visit. Written informed consent will be obtained after explanation to potential participants about all procedures and time commitments. The study coordinator will explain to prospective participants the purpose, methods, and extent of the study. Potential participants will be asked to read the informed consent form and encouraged to ask questions. The form will be written in simple, easy-to-understand language. We require the research assistant to review all key aspects of the study verbally. They will then question potential participants to ascertain whether they have understood the information. Dr. Anton and other study investigators are available to answer participants' questions as well. Both the participant and the individual administering the consent form will sign the consent form. Dr. Anton's direct telephone line, and home telephone number are provided to participants.

Potential participants who are illiterate or who have impaired vision (in the presence of legally authorized representative - LAR) will be asked if any special provisions are required by them for the consent process, including having the consent document read to them. Information in the consent materials will then be presented orally to them. Participants will be encouraged to ask questions and discuss. Sufficient time will be allowed for questions to be asked and answered, both by the participant, and by the person obtaining consent to ensure the participant comprehends the consent information. The participant may then make their mark on the consent form to indicate a

willingness to participate. A copy of the signed and dated consent form will be given to participants (or their LAR), and the original document will be placed in participants' individual study files, which will be stored in a secure location. The study will be submitted for approval to the University of Florida's Institutional Review Board.

Screening Procedures

- **Telephone Pre-Screening.** Potential participants will undergo an initial telephone pre-screening to assess inclusion and exclusion criteria, which will include a brief medication and health history.

- **Screening Visit.** Individuals found to be potentially eligible to participate in this study will be invited to the Institute on Aging – Clinical and Translational Research Building (IOA – CTRB) for a short screening visit. At this visit, potential participants will complete the following procedures listed below:

    - Informed consent
    - Medical history questionnaire (inclusion/exclusion criteria)
    - Physical Exam (including height)
    - Vitals (blood pressure and heart rate)
    - Body weight, height and girth measurement
    - Blood draw for comprehensive metabolic profile (CMP) and a complete blood count and differential (CBC with differential).
    - Mini-Mental Status Exam (MMSE)
    - Center for Epidemiologic Studies Depression Scale (CES-D)
    - Short Physical Performance Battery (SPPB)

Following a review of the screening visit results by the study team and verification of the inclusion/exclusion criteria by the Principal Investigator, Clinical Research Director, or Compliance Officer, a study team member will call participants and inform them whether or not they are eligible to participate in this study.

Participants who are eligible to participate will be scheduled for their Baseline Visit within 30 days (± 7 days) of their Screening Visit.

If the person does not qualify for the study after screening, a study team member will explain the subject on call the reasons for not meeting the eligibility criteria outlined for the study. Research coordinator will then explain the subject how participation of ineligible people in the study might possibly affects the outcome of the research study and cause risks to the health of an individual.

6.2.2 Enrollment, Baseline, and/or Randomization

- **Baseline Visit 1.** If all study entry criteria are met at the in-person screening visit, participants will be scheduled to return to the clinic for two baseline visits prior to randomization. During the first baseline visit, eligible participants will be invited to the clinic to undergo the following study assessments, which are described in detail below:

- Contact and proxy contact information
- Consent to participate in the Tissue/Data Bank (optional)
- Vitals (blood pressure and heart rate)
- Body weight and girth measurement
- Blood draw for comprehensive metabolic profile (CMP), complete blood count (CBC w/differentials), and inflammation markers
- Short Physical Performance Battery (SPPB)
- Six-Minute Walk Test
- Muscle endurance on the Biodex Machine
- Activity monitor will be provided to wear during 7-day period

Participants will be scheduled for their Baseline Biopsy Visit within approximately seven (7) days of their Baseline Visit. If at the time of second baseline visit (which is when the activity monitor is returned) it is determined that the participant wore the activity monitor for less than 5 days, the participant will be asked to wear the activity monitor for an additional period of time. Only after completing the total requisite time of wearing the activity monitor, the participant's second baseline visit is marked complete.

- **Baseline Visit 2 (Muscle Biopsy)** Following this first baseline visit, participants will be invited to attend a second baseline visit to complete a muscle biopsy. Muscle biopsies will be performed by Bhanuprasad Sandesara, M.D., or other trained physician. Dr. Sandesara has performed numerous muscle biopsies for our previous studies, or by other trained medical professionals if necessary. Skeletal muscle samples will be obtained under local anesthesia from the *vastus lateralis* muscle using a percutaneous needle biopsy technique, as previously described by our group [62] (see **Appendix 1** for detailed description of procedure). The skin will be closed with steri-strips. Participants will be contacted by phone three days following this procedure, and will return for a wound check 10 days after the procedure date (see **Appendix 2** for detailed instructions and follow-up procedures for participants). Approximately 150-250 mg of muscle tissue is removed. Portions of the muscle will be immediately processed for permeabilized fiber high-resolution respiration measurements and a very small piece of tissue will be mounted in embedding medium and frozen in isopentane for future histochemical analysis. The remaining tissue will be immediately frozen in liquid nitrogen and stored at -80°C until analysis.

Randomization. Following completion of the Baseline Biopsy Visit, participants who satisfy all inclusion/exclusion criteria will be randomly assigned to receive resveratrol (1000 mg/day or 1500 mg/day) or placebo (vegetable cellulose). The randomization will be performed using the CTSI's standard methodology for double blind randomization and our Biostatistican, Jon Shuster, PhD, will oversee this process. The CTSI develops the cross reference between the participant's PIN number and randomized assignment, and provides it to UF's Investigational Pharmacy (Director: Susan Beltz, PharmD.). The list can be regenerated at any time, and is archived on the CTSI servers, and at the investigative pharmacy. The enrollment date will be defined and recorded on a case report form along with the allowable window between screening and randomization. The biostatistician has a secure copy of the master list and the SAS code including the seed number that generated it. If the program is rerun, it will produce the same randomization. Our standard method, used over

dozens of CTSI trials, is to employ random length permuted blocks, using a CTSI developed SAS algorithm. Further details of the lengths of the blocks were conveyed to the sponsor, but are withheld from the protocol, due to the remote possibility that it might lead to a partial unblinding.

Participants will be provided with up to a 40-day supply of study drug capsules at the Baseline Visit 2.

Participants will be asked to return to the clinic in approximately 30 days (± 7 days) for their 30-Day Visit.

6.2.3 Blinding

The statistician is not blinded, and does not have direct contact with participants. Also, the members of UF's Investigational Pharmacy are not blinded and do not have direct contact with participants. The statistician will serve as the study representative during the unblinded portion of the oversight committee's bi-annual review. All other study investigators and study team members who have direct contact with participants will be blinded to the participant's treatment assignment.

The study will be unblinded (possibly only for a specific subject) if the oversight committee indicates stopping the trial (for an individual or totally) is necessary due to adverse event frequency or severity. The Principal Investigator and/or any member of the oversight committee can break the blind through a written request to the Coordinator of the Investigational Drug Service. Following receipt of this request, the Investigational Pharmacist will provide the requested information in either electronic format or hard copy to the Principal Investigator, who will then provide this information to the oversight committee.

6.2.4 Follow-up Visits

30-Day Visit. Study compound compliance will be assessed by means of capsule count by a study team member. Additionally, the following procedures will be performed:

- Contact information will be updated, if necessary
- Vitals (blood pressure and heart rate)
- Blood draw for CMP and CBC w/differential
- Adverse events will be collected; NCI Toxicity Criteria will be used to determine severity
- Medical history update
- Product compliance

At the end of the visit, participants will be given a new supply of up to ~~a~~ 40-days ~~supply~~ of their randomly assigned study drugs, and will be asked to return to the clinic in approximately 30 days (± 7 days) for their 60-Day Visit. Participants will be asked to continue to follow the same dosing schedule as they did during Month 1. If this study visit is conducted late (outside of the allowable window), the target date for the 60-Day visit will be based on the date of the second baseline visit (BL2) which is when the participant started the study regimen.

60-Day Visit. Study compound compliance will be assessed by means of capsule count by a study team member. Additionally, the following procedures will be performed:

- Contact information will be updated, if necessary

- Vitals (blood pressure and heart rate)
- Blood draw for CMP and CBC w/differential
- Adverse events will be collected; NCI Toxicity Criteria will be used to determine severity
- Medical history update
- Product compliance

At the end of the 60- day visit, participants will be given a new supply of up to ~~a~~ 40-days ~~a 40-day supply~~ of their randomly assigned study drugs and will be asked to return to the clinic in approximately one month (± 7 days) for their 90-Day Visit. Participants will be asked to continue to follow the study regimen schedule.

6.2.5 Completion/Final Evaluation

90-Day Visit. Study compound compliance will be assessed by means of capsule count by a study team member. Additionally, the following procedures will be performed:

- Contact and proxy contact information
- Vitals (blood pressure and heart rate)
- Body weight and girth measurement
- Blood draw for comprehensive metabolic profile (CMP), complete blood count (CBC w/diff), and inflammation markers
- Short Physical Performance Battery (SPPB)
- Six-Minute Walk Test
- Muscle endurance on the Biodex Machine
- Activity monitor will be dispensed to wear during 7-day period
- Adverse events will be collected; NCI Toxicity Criteria will be used to determine severity
- Product compliance

If the previous study visit (i.e. 60 day visit) is conducted outside of the allowable window, the target date for the 90-Day visit will be based on the date of the second baseline visit (BL2) which is when the participant started the study regimen.

- **90-Day Visit 2 (Muscle Biopsy)**. Participants will be scheduled for this appointment within 1 week of their 90-Day Visit. Muscle biopsies will be performed by Bhanuprasad Sandesara, M.D., who has performed numerous muscle biopsies for our previous studies, or other trained medical professionals if necessary. Skeletal muscle samples will be obtained under local anesthesia from the *vastus lateralis* muscle using a percutaneous needle biopsy technique as previously described by our group [62] (see **Appendix 1** for detailed description of procedure). The skin is closed with steri-strips. Participants will be contacted by phone three days following this procedure, and will return for a wound check 10 days after the procedure date (see **Appendix 2** for detailed instructions and follow-up procedures for participants). Approximately 150-250 mg of muscle tissue is removed. Portions of the muscle will be immediately processed for permeabilized fiber high-resolution respiration measurements, and a very small piece of tissue will be mounted in embedding medium

and frozen in isopentane for future histochemical analysis. The remaining tissue will be immediately frozen in liquid nitrogen and stored at -80°C until analysis.

Follow-Up, 10 Day. After completion of the 90-Day Visit 2, participants will be scheduled to return to the clinic in approximately 10 days (± 3 days) for their Follow-Up 10 Day.
The following tests will be performed:

- Contact information will be updated, if necessary
- Vitals (blood pressure and heart rate)
- A blood draw will be taken for their CMP and CBC w/differential
- Medical history update
- Adverse events will be collected; NCI Toxicity Criteria will be used to determine severity

The participant will be asked to return to the clinic in approximately 20 days (± 1 week) for their Follow-Up 30 Day.

Follow-Up, 30 Day. The following tests will be performed:

- Contact information will be updated, if necessary
- Vitals (blood pressure and heart rate)
- Blood draw for CMP and CBC w/differential
- Medical update: review medication changes and changes in health
- Adverse events will be collected; NCI Toxicity Criteria will be used to determine severity

This will be the last visit of the study. For participants who cannot come to the visits, we will attempt to schedule home visits or phone calls.

7. SAFETY ASSESSMENTS

7.1 Specification of Safety Parameters

Below we provide an alphabetical list of potential adverse experiences that participants may experience during this trial, criteria for management, and modifications of intervention or participant assessments if an adverse event occurs.

Risks associated with biopsy: The muscle biopsy is associated with several potential risks. These include:

- discomfort during the muscle biopsy procedure,
- extremely low risk of allergic reaction to the local injection,
- scarring from the muscle biopsy skin incision,

- a possibility of a small area of numbness (about the size of a dollar coin) around the biopsy site,
- risk of bleeding,
- bruising, and
- risk of infection.

Protection against risks associated with muscle biopsy. The muscle biopsy procedure is performed by Dr. Sandesara or other trained physician and assisted by a nurse practitioner. Dr. Sandesara has extensive experience with this procedure, and has performed muscle biopsies in multiple studies conducted over the past five years. As in our pilot study, which was completed in preparation for this proposal, the muscle biopsy procedures are performed under sterile conditions using aseptic techniques. Local anesthesia is obtained using topical lidocaine (see **Appendix 1** for detailed description of this procedure). The risk of participants experiencing an allergic reaction to this injection is extremely low, and to reduce the risks of any potential adverse reaction occurring, participants will be queried prior to injection to ensure they to do not have a known allergy to lidocaine.

Many participants take anti-platelet therapy to prevent cardiac and cerebrovascular events. Only potential participants taking anti-platelet therapy with a low dose (i.e. $\leq$ 81 mg daily) of aspirin are included in the study. Participants who are taking warfarin or other anti-coagulant therapy will be excluded from this study (see **Appendix 2** for detailed instructions provided to participants before and after the muscle biopsy procedure).

Risks associated with the blood draw: The risks of drawing blood from a vein include discomfort at the site of puncture, possible bruising and swelling around the puncture site, rarely an infection, and, uncommonly, faintness from the procedure.

Protection against risks associated with the blood draw: Trained personnel and use of aseptic (sterile) technique minimize these risks. All assessment visits will be conducted at a central location, and all sessions will be conducted and supervised by a trained and certified research staff member, who will monitor potential adverse experiences and symptoms. A study physician is available on call, and contact numbers for emergency services are posted. Institutional and community EMS services will be activated if needed. If at any point during an assessment session, participants develop chest pain, shortness of breath, or dizziness, they will be instructed to rest and to contact the center and their physicians if these symptoms persist or recur.

Risks associated with blood pressure measurement: The risks of placing a blood pressure cuff on a participant's arms are that it may cause pinching or slight bruising.

Protection against risks associated with blood pressure measurement: All assessment visits will be conducted at a central location and all sessions will be conducted and supervised by a trained and certified research staff member, who will monitor potential adverse experiences and symptoms.

Risks associated with the six-minute walk test: The six-minute walk test may be associated with the risk of falling, the development of chest discomfort due to coronary ischemia, or dyspnea due to heart failure or lung disease. Rarely, falling during the six-minute walk test may result in a fracture.

Protection against risks associated with the six-minute walk test: Research staff who collect data have been trained in six-minute walk testing by other senior staff members with expertise in functional assessments, such as Dr. Todd Manini. Re-certification is performed by a post-doctoral fellow (under Dr. Manini's supervision) every six months. Study staff members are trained not to perform the six-minute walk test if they feel that testing is unsafe. In addition, participants are asked whether they feel the test is safe. Those who state it may be unsafe are not allowed to complete the test. During the testing sessions, a fully equipped defibrillator is available. All personnel associated with the study have received CPR training and training on management of acute events including syncope, chest pain, acute dyspnea, focal neurological symptoms, and abnormal vital signs. Portable defibrillators are available at the assessment site. Staff members are also trained to protect study participants against falling during testing. Additionally, a study physician is available on call, and contact numbers for emergency services are posted. Institutional and community EMS services will be activated if needed. If at any point during an assessment session, participants develop chest pain, shortness of breath, or dizziness, they will be instructed to rest, and to contact the center and their physicians if these symptoms persist or recur. In our experience conducting thousands of six-minute walk tests on individuals within various studies, the risk of falling is less than 1/500, and the risk of a fracture-associated fall is less than 1/2000. The risk of a significant cardiovascular event in association with the six-minute walk test is less than 1/3000.

Risks associated with SPPB measurement: Completion of the SPPB may be associated with the risk of falling, the development of chest discomfort due to coronary ischemia, or dyspnea due to heart failure or lung disease. Rarely, falling during the SPPB test may result in a fracture.

Protection against risks associated with SPPB measurement: Research staff who collect data have been trained in SPPB measurement and are certified by Dr. Manini, or our post-doctoral fellow, in conducting the SPPB measurement before they work with study participants. Re-certification is performed by Dr. Manini or a post-doctoral fellow every six months. During the testing sessions, a fully equipped defibrillator is available. All personnel associated with the study have received CPR training and training on management of acute events including syncope, chest pain, acute dyspnea, focal neurological symptoms and abnormal vital signs. Portable defibrillators are available at the assessment site. Staff members are also trained to protect against falling. Additionally, a study physician is available on call, and contact numbers for emergency services are posted. Institutional and community EMS services will be activated if needed. If at any point during an assessment session, participants develop chest pain, shortness of breath, or dizziness, they will be instructed to rest, and to contact the center and their physicians if these symptoms persist or recur.

Risks associated with Biodex measurement: There is risk of injury during exercise testing protocols on a Biodex. Participants may experience muscle fatigue, weakness, soreness and/or muscle pulls or tears.

Protection against risks associated with Biodex measurement: The risk of an event during exercise testing is minimized with a pretest review of the medical history, use of a highly trained staff, and well-defined emergency procedures. All tests are conducted in the presence of research staff with extensive experience in conducting exercise testing. Participants will be secured to the

seat with a series of seatbelt-like devices to prevent injury. These belts are easily released and can be moved easily if needed or desired. Prior to each test, a short period of practice will be allowed for familiarity and safety.

Risks associated with questionnaire administration: Participation includes a risk of loss of confidentiality of personal health information.

Protection against risks associated with questionnaire administration and loss of confidentiality: A number of methods are employed to maintain confidentiality of participants. First, questionnaire data are collected in secure spaces where the interview cannot be overheard. Secondly, only study investigators and key research staff (i.e. data manager and study programmers) have access to the study database. Third, participants are assigned a unique study identifier. Individual names will ultimately be removed from the study database and only the unique study identifier is used to distinguish participants in the database. Fourth, collected data are maintained in locked computer files and file cabinets, to which only study investigators have access. Collected data will be completely de-identified and used only for research purposes and analysis. Published data will not contain any individual identifiers. Finally, all research staff members have to receive HIPAA training and sign a confidentiality certificate every year.

Risks associated with resveratrol supplementation: Toxicity from the doses of resveratrol used in this study is likely to be low based on animal studies, and doses of 1000 mg/day have been well tolerated in previous safety trials in young and middle-age adults.[58-60] Additionally, a recent repeated-dosing study found that a dose of 200 mg of resveratrol at eight hour intervals for a three day period was well tolerated in older adults (age $\geq$ 65 years),[61] and doses of 1000 mg/day, 1500 mg/day, and 2000 mg/day of resveratrol supplementation were well tolerated in older adults during a four week intervention.[56] Based on the findings described above, we have chosen the test doses of resveratrol chosen for the proposed study to be 1000 mg/day and 1500 mg/day. Adverse effects of resveratrol have not been reported at these doses; however, long-term side effects are not known. Resveratrol is structurally similar to the synthetic estrogen diethylstilbestrol, and may have estrogenic activity. Resveratrol might also interact with blood thinners such as warfarin, and non-steroidal anti-inflammatory medications, thereby increasing the risk for bleeding. In our resveratrol pilot study, *The RIPE Trial*, resveratrol supplementation at 1000 mg/day had minimal effects on blood chemistries, and there were no significant differences in the number of participants reporting adverse or toxic events (e.g. headaches) across the three conditions (all p values > 0.05).

Risk management in participants using prohibited medication/interventions after study enrollment.

If the participant reports any change in medications, this information will be recorded in the participant's file (i.e., source documents and case report forms). If a participant reports use of any prohibited interventions as listed below and in sections 5.3.3. of the protocol (Prohibited Interventions) post-randomization, the participant will be withdrawn from the study.

Prohibited Interventions

- Active treatment for cancer, stroke, peripheral vascular disease, coronary artery disease, myocardial infarction, congestive heart failure (stage III or IV), valvular

heart disease, major psychiatric disease, severe anemia, liver or renal disease, diabetes, severe osteoarthritis, blindness or deafness, fracture in upper or lower extremity, upper or lower extremity amputation, or Parkinson's disease; - *Safety Reasons*

- Hard Exercise (i.e., running, bicycling, etc.) ≥ 30 min/day on 5 or more days per week; - *Scientific Reasons*
- Use of anabolic medications (e.g. growth hormones or testosterone), anticholinesterase inhibitor (e.g. Aricept), or anticoagulant therapies (aspirin use > 81 mg/day; aspirin use ≤ 81 mg/day is permitted); -*Safety Reasons*
- Dietary supplementation of grape seed extract or ginko biloba; - *Scientific Reasons*
- Consumption of any dietary supplements containing resveratrol, quercetin, or *P. cuspidatum; Scientific Reasons*
- Use of other Investigational products; - *Safety and Scientific Reasons*
- Dietary supplementation with CoQ10 or alpha-lipoic acid. - *Scientific Reasons*

If the participant reports changes in health, medications, or lifestyle other than the interventions listed above, the study physician will evaluate the safety of participants to continue in the intervention based on these reported changes. In these circumstances, the role of the study physician will be to provide recommendations to the study team regarding whether or not the participant should continue in the study based on reported changes in health, medication use, or lifestyle.

Adverse events will be graded according to Common Terminology Criteria for Adverse Events (CTCAE) v 4.03. In compliance with the Federal Registry, 21 CFR 312.32, effective April 1, 2008, we will use the following definitions for adverse event reporting:

Adverse event means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

Life-threatening adverse event or *life-threatening suspected adverse reaction*. An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

Serious adverse event or *serious suspected adverse reaction*. An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: Death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasia or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, "reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the adverse event. Suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

Unexpected adverse event or *unexpected suspected adverse reaction*. An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended. For example, under this definition, hepatic necrosis would be unexpected (by virtue of greater severity) if the investigator brochure referred only to elevated hepatic enzymes or hepatitis. Similarly, cerebral thromboembolism and cerebral vasculitis would be unexpected (by virtue of greater specificity) if the investigator brochure listed only cerebral vascular accidents. "Unexpected," as used in this definition, also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs, or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

Protection against risks associated with resveratrol supplementation: Participant safety will be our highest priority, the study participants will be encouraged to call the study team member or investigators if they experience adverse event. We will monitor potential adverse events associated with resveratrol consumption by blood work analysis (complete metabolic panel and complete blood count) and National Cancer Institute (NCI) criteria during all study visits: baseline, day 30, day 60, day 90, as well as at both 10-day Post-Biopsy and 30 day follow-up visits (see **Table 1**). Under aseptic conditions, using standard venipuncture techniques, 15 ml samples of blood will be drawn and analyzed for metabolic profile (Albumin, Albumin/Globulin Ratio (calculated), Alkaline Phosphatase, ALT, AST, BUN/Creatinine Ratio (calculated), Calcium, Carbon Dioxide, Chloride, Creatinine with GFR Estimated, Globulin (calculated), Glucose, Potassium, Sodium, Total Bilirubin, Total Protein, Urea Nitrogen as well as for a complete blood count (WBC, RBC, Hemoglobin, Hematocrit, MCV, MCH, MCHC, RDW, Platelet Count, MPV and Differential (Absolute and Percent - Neutrophils, Lymphocytes, Monocytes, Eosinophils, and Basophils). These tests will be performed by a CLIA Certified Labratory. If a blood marker falls in the abnormal range, the study physician will be consulted and will determine whether it is safe for the participant to continue in the trial.

The study physician and Investigators will evaluate adverse events for seriousness, expectedness, severity, and relationship to study intervention on a weekly basis. A grading scale based on the latest NCI criteria for adverse events will be used (see Appendix Common Terminology Criteria for Adverse Events Version 3.0). The primary mechanism for ensuring participant safety will be clinical observation of symptoms. The study will be conducted and supervised by trained physicians, psychologists, and exercise physiologists certified in CPR, who will monitor potential adverse experiences and symptoms. During each visit, subjects will log any health-related problems or symptoms they are experiencing. These sheets will be reviewed by study staff before allowing the

participant to continue. Community health services will be contacted immediately if warranted by the participant's symptoms.

7.2 Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

As noted above, we will monitor potential adverse events associated with resveratrol consumption by blood work analysis (complete metabolic panel and complete blood count) and National Cancer Institute (NCI) criteria during all study visits, **baseline, day 30, day 60, day 90**, as well as at both10-day Post-Biopsy and 30 day follow-up visits (see **Table 1**). All assessment visits will be conducted at a central location, and all sessions will be conducted and supervised by a trained research staff, who will monitor potential adverse experiences and symptoms.

7.3 Adverse Events and Serious Adverse Events

Adverse Event – An adverse event (AE) is defined as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. An adverse event (also referred to as an adverse experience) can also be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporally associated with the use of a drug, without any judgment about causality. An adverse event can arise from any use of a drug (e.g., off label use, use in combination with another drug) and from any route of administration, formulation or dose, including overdose.

Serious Adverse Event (SAE) - Any AE that results in one or more of the following outcomes:

- Death
- A life-threatening event
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant disability/incapacity
- A congenital anomaly or birth defect
- An important medical event based upon appropriate medical judgment

Serious adverse events (SAEs) that are unanticipated and/or possibly related to study participation are reported to the NIH within 48 hours of learning of the event, to the IRB and Independent Safety Board (oversight committee) within five business days of learning of the event. If an event, incident, experience, or outcome is life-threatening or fatal, the IRB must be notified by phone within 24 hours.
Any unexpected fatal or life-threatening suspected adverse reactions will be reported to the FDA no later than 7 calendar days after the initial receipt of the information. For SAEs that are not fatal or life-threatening, the time frame for submitting an IND safety report to FDA is no later than 15 calendar days after the investigator-sponsor determines that the suspected adverse reaction or other information qualifies for reporting.

Minor events will be reported to the University of Florida's Health Science Center IRB at the time of annual review, as well as to the oversight committee on a bi-annual basis. Both the principal investigator and the study staff will review the study weekly and examine reports of adverse incidents and reports of study participant recruitment and follow-up. Throughout the course of the study, information regarding issues deemed critical to the study or to the safety of research participants will be provided to the principal investigator and primary mentors. As a result of

receiving this critical information, a meeting to discuss this critical information may be convened. Information deemed critical would include:

- serious and non-serious adverse events that may occur,
- suspicion of scientific fraud or misconduct,
- any other issues which may warrant protocol changes or modifications.

Monitoring of both AEs and SAEs will be achieved via administration of structured questionnaires. Standardized forms for referring and treating study participants who experience adverse events will be in place.

- Classification of AE Severity – Adverse events will be graded according to Common Terminology Criteria for Adverse Events (CTCAE) v 4.03. The study physician will be responsible for all trial-related medical decisions and the review of AEs throughout the study. The physician will assess AEs and will ensure that they are recorded in detail. Adverse events, whether in response to a query, observed by study team, or reported spontaneously, will be reported on the appropriate CRF and/or source document.

AE Attribution Scale – All adverse events (AEs) will also be categorized according to the likelihood that they are related to the study intervention. Specifically, they will be labeled either definitely, probably, possibly, or unrelated to the study intervention. The classification of potential relationship to the intervention is shown in **Table 2**.

Table 2. Adverse Event (AE) Classification

Adverse Event (AE) Classification	
Definite.	Temporal pattern + known or expected AE response pattern + confirmed by stopping the intervention + reappearance of AE on re-challenge
Probable.	Temporal pattern + known or expected AE response pattern + confirmed by stopping the intervention + could not be explained by participant's clinical state
Possible.	Temporal pattern + known or expected AE response pattern + could have been produced by a number of other factors
Unknown.	Relationship for which no evaluation can be made
Not related.	AE for which sufficient information exists to indicate that the cause is unrelated to the study intervention

As noted above, we will also monitor potential adverse events associated with resveratrol consumption by blood work analysis (complete metabolic panel and complete blood count) and National Cancer Institute (NCI) criteria during all study visits: baseline, day 30, day 60, day 90, as well as at the both10-day Post-Biopsy and 30 day follow-up visits (; see **Table 1**). If a blood marker falls in the abnormal range, the study physician will be consulted and will determine whether it is safe for the participant to continue in the trial.

7.4 Reporting Procedures

Study progress and safety will be reviewed bi-weekly by the Principal Investigator. Progress reports, including participant recruitment, retention/attrition, and AEs will be provided to an

Independent Monitoring Committee for bi-annual reviews. An annual report will be compiled and will include a list and summary of AEs. In addition, the annual report will address (1) whether adverse event rates are consistent with pre-study assumptions; (2) reason for dropouts from the study; (3) whether all participants met entry criteria; (4) whether continuation of the study is justified on the basis that additional data are needed to accomplish the stated aims of the study; and (5) conditions whereby the study might be terminated prematurely. The annual report will be signed by the chair of the Independent Monitoring Committee and may be forwarded to the IRB, NCCIH, FDA, and industry collaborator, who will only provide study product. The IRB and other applicable recipients will review the progress of this study on an annual basis.

As noted above, all SAEs that are unanticipated or that may possibly be related to study participation are reported to the NIH within 48 hours of learning of the event, to the IRB and Independent Safety Board (oversight committee) within five business days of learning of the event. If an event, incident, experience, or outcome is life-threatening or fatal, the IRB must be notified by phone within 24 hours.
Any unexpected fatal or life-threatening suspected adverse reactions will be reported to the FDA no later than 7 calendar days after the initial receipt of the information.

Relevant data and safety information obtained for each study participant will be verified against the original source documents by the primary study coordinator after each visit, plus the data will be spot checked by the program manager or compliance office. As noted above, any identified discrepancies will be discussed with the Principal Investigator and reviewed at weekly meetings. The frequency of data review for this study differs according to the type of data, and is summarized in **Table 3**.

Table 3. Frequency of Data Review

Data type	Frequency of review	Reviewer
Participant accrual (adherence to protocol regarding demographics, inclusion/exclusion)	Weekly	Principal Investigator
Compliance to treatment	Bi-Weekly	Principal Investigator
Adverse event rates (injuries)	Bi-Weekly	Principal Investigator
・Participant Accrual ・Out of range (non-clinically significant) laboratory data; ・Compliance to treatment ・Adverse events and adverse event rates.	Bi-Annually	Study Monitoring Board

This study will be stopped prior to its completion if: (1) the intervention is associated with adverse effects that significantly impact the risk-benefit ratio, (2) study recruitment or retention becomes futile, (3) any new information becomes available during the trial that necessitates stopping the trial; or (4) if oversight committee indicates stopping trial is necessary due to adverse event frequency or severity.

7.5 Follow-up for Adverse Events

All reported adverse events will be followed until they are *resolved or considered stable.* The study coordinator will document the severity of any reported adverse event based on a conversation with the participant or their emergency contact. Whenever possible, medical records will be obtained to provide additional information regarding the nature of any reported adverse event.

7.6 Safety Monitoring

A Data and Safety Monitoring Plan (DSMP) will be implemented, with the following procedures to ensure data and participants' safety:

- Study progress and safety (e.g. cumulative table of adverse events and serious adverse events) will be reviewed monthly (and more frequently if needed) by the principal investigator.

- Progress reports, including participant recruitment, retention/attrition, and adverse events will be provided to an Independent Monitoring Committee for bi-annual reviews. The Data and Safety Monitoring Committee for this study will consist of an established board, which has reviewed all studies conducted within the University of Florida's Pepper OAIC during bi-annual conference calls for the past seven years. This board consists of the following individuals: (1) Stephen Kritchevsky, Ph.D., Chair, an epidemiologist who has been involved in research for many years, (2) Jing Cheng, Ph.D., a biostatistician who has been involved with a number of clinical trials, and (3) John Meuleman, M.D., a physician who has been involved in the conduct of clinical research for many years.

- We will also compile an annual report, which will include a list and summary of adverse events. In addition, the annual report will address:

    1) Whether adverse event rates are consistent with pre-study assumptions;
    2) Reasons for dropouts from the study;
    3) Whether all participants met entry criteria;
    4) Whether continuation of the study is justified on the bases that additional data are needed to accomplish the stated aims of the study; and
    5) Conditions whereby the study might be terminated prematurely.

The annual report will be signed by the chair of the Independent Monitoring Committee and will be forwarded to the IRB. The IRB and other applicable recipients will review the progress of this study on an annual basis.

The Data and Safety Monitoring Plan will also require that Dr. Anton report all significant serious adverse events (SAEs) that may be possibly related to the study participation to the NIH within 48 hours of learning of the event, and to the IRB and Independent Safety Monitoring Board within five business days of learning of the event. In addition, any unanticipated SAEs that may increase risk to participants or potential participants will be reported to the NIH within 48 hours and to the IRB within five business days of Dr. Anton learning of the event.

8. INTERVENTION DISCONTINUATION

Participants may be removed from the study for the following reasons: (1) Unacceptable toxicity (≥ grade 2 toxicity) related to administration of resveratrol using the NCI toxicity criteria; (2) participant voluntarily decides to withdraw; (3) participant non-compliance with the study protocol; or (4) intercurrent disease, medication use, or condition that would affect the assessment of the participant's status. With their permission, participants will continue to be followed if study intervention is discontinued. This study will be stopped prior to its completion if: (1) the intervention is associated with adverse effects that significantly impact the risk-benefit ratio, (2) study recruitment or retention becomes futile, (3) any new information becomes available during the trial that necessitates stopping the trial; or (4) if the oversight committee indicates that discontinuation of the trial is necessary due to adverse event frequency or severity.

9. STATISTICAL CONSIDERATIONS

9.1 General Design Issues

A parallel three arm design of placebo (A) vs. standard dose resveratrol (B) vs. high dose resveratrol (C) will be used to test the primary hypothesis that the unweighted average of the target population means B and C is the same as that of A. This will be tested at p=0.05 (two-sided) by a Satterthwaite-corrected contrast. The analysis does not require equal variances in the three target populations, nor does it require that the means for B and C are the same. As our dependent variable, we will use the difference between the post-test value (90 days) and baseline. The primary and secondary aims and hypotheses are listed below.

Specific Aim 1. To examine the effects of resveratrol treatment on mitochondrial function (State 3 and 4 respiration on permeabilized muscle fibers, COX, CS enzyme activities and mitochondrial DNA content) in muscle samples of older adults.
Hypotheses. Compared to placebo, resveratrol supplementation will increase mitochondrial respiration (State 3=**primary outcome**, State 4), COX, CS enzyme activities, and mitochondrial DNA content in muscle specimens from the *vastus lateralis*.

Specific Aim 2. To evaluate the effects of resveratrol supplementation on levels of PGC-1α, AMPK, and sirtuins (SIRT1 and SIRT3) in muscle samples of older adults.
Hypotheses. Compared to placebo, resveratrol treatment will increase PGC-1α (primary outcome), AMPK, SIRT1, and SIRT3 levels in muscle specimens obtained from the *vastus lateralis*.

Exploratory Aims. Explore the effects of resveratrol supplementation on measures of physical function, metabolic risk factors, and changes in physical activity in older adults.
Hypotheses. Compared to placebo, resveratrol supplementation will (1) increase walking speed, (2) improve physical performance, (3) increase resistance to muscle fatigue, (4) reduce established metabolic risk factors (i.e. diastolic blood pressure and blood levels of insulin and glucose), and (5) increase levels of spontaneous physical activity.

The proposed study will be the first to test whether resveratrol improves muscle mitochondrial function, and the mechanisms that underlie this potential improvement in older adults. An important exploratory goal is to determine whether the hypothesized biological changes in the mitochondria

translate to improved physical function and reduction in metabolic risk profile. The long-term goal of this line of research is to examine biological mechanisms of resveratrol in human muscle, and to understand how these adaptations improve human health. The investigative team has expertise in all aspects of this multi-disciplinary research and a history of collaboration, making this team uniquely qualified to conduct this study.

9.2 Sample Size and Analysis Methodology

A total of 60 participants will be randomized into one of three conditions: (1) 1000 mg/day resveratrol (n = 20), (2) 1500 mg/day resveratrol (n = 20), or (3) vegetable cellulose (n = 20). Conservatively, presuming a repeated measures correlation for a participant is 0.7 (baseline explains about 50% of the variation at the post test), a study of 18 completers in each of the three groups has 80% power to detect a difference of 0.69 standard deviations in this contrast. Secondarily, we shall contrast each pair of treatments using a two-sided *t*-test (Satterthwaite-corrected for potentially unequal standard deviations) with 80% power, to detect a difference of 0.81 standard deviations. All comparisons will be accompanied by point and 95% confidence intervals for effect sizes.

From a parallel study, we estimate that baseline STATE 3 mitochondrial respiration (Primary Outcome) will have a mean of about 120 and a standard deviation of about 40. Hence 0.69 σ (0.81 σ) represents sensitivity to better than a 25% (30%) change from baseline for STATE 3 mitochondrial respiration, respectively.

Robustness of Methods absent missing data: Based upon our experience, STATE 3 is not expected to be outlier prone, making the t-test highly robust against non-normality, even for moderate size samples such as in this trial. In clinical studies, where the comparison is between a placebo and an active treatment, comparing changes between treatments from baseline to a post-test, it is expected that the variability in the control group (pure replication) will be smaller than that in the experimental group (replication plus treatment). The Satterthwaite method deals with this contingency, whether or not it really occurs in our trial. Of course, the most important aspects of the analysis are the point estimates and 95% confidence intervals for the mean differences between the three pairs of treatments. The Satterthwaite method will be used for these as well.

Missing Data: Since the analysis is primarily based on the second biopsy, missing data represent a potential problem.

Sensitivity analysis for Baseline Imbalance: Using baseline BMI, Baseline STATE 3, and gender, but not treatment, we shall develop a prediction equation via a linear model for the final STATE 3 value. Next, we shall use the predicted value for those missing the final STATE 3 value, and conduct permutation t-tests for each pair of treatments, replacing missing values by the predicted value. The type of bias that this corrects for is that missing values by be disproportionate in the arms according to the baseline value. For example, high baseline BMI may have more missingness on one arm while low BMI has more missingness on another. While this analysis corrects for interaction between treatment and missingness for baseline covariates, it does not account for missingness that develops during the study based on treatment vs. control issues. For example, if the treatment is associated with some subjects getting a headache and this leads to withdrawal from the study, this sensitivity analysis will not cover that contingency.

Sensitivity analysis for withdrawals for patient related study events, recognizing the events may not be measurable. First, we shall try to get a follow-up biopsy on every subject, ideally at the end of month 3, whether or not the subject completes the study medication program. Second, we shall ask the subject to provide reasons for withdrawal, based on a brief questionnaire. Third, we shall compare the withdrawal rates for the three treatment groups via an exact chi-square analysis. While an approximately equal withdrawal rate is not necessarily indicative of resolution, it is a reasonable assessment to make. Finally, using repeated measures mixed models methods, we shall compare the BMI trajectories for withdrawals vs. non withdrawals, stratified for treatment as a surrogate to the question as to whether the event of withdrawal is related to how the subject is doing on the trial. The month one and month two data will be used, as that will be missing for month 3.

Secondary analyses derived from the biopsy will be analyzed as above, but the study is powered strictly around STATE 3. Analysis on secondary variables that have repeated measures will utilize linear mixed model analysis of variance with unstructured covariance for repeated measures. Sensitivity analyses will be done via t-tests, utilizing the Satterthwaite correction for changes from baseline to three months, with last observation carried forward for missing data.

Occurrence of side effects will be evaluated by Fisher's Exact test.

9.3 Definition of Populations

Intent-to-treat population for the primary outcome is synonymous to those getting baseline and three-month biopsies, whether or not they adhere to the treatment. Every effort will be made to bring back participants for their three month (post-treatment) biopsy visit, regardless of their levels of adherence. Intent-to-treat for serial measurements will include those with at least one of their follow-up visits. As above, we shall make every effort to get all follow-up visits completed, regardless of adherence.

The per protocol (PP) population will consist of all patients in the ITT population who are not major protocol violators. Specifically, PP population will consist of individuals who did not take any prohibited therapies during the trial, had $\geq$ 90% adherence with prescribed regimen based on pill counts, and completed all baseline and post-treatment assessments.

The gold standard for clinical trials is by intent-to-treat. An analysis of the prognostic significance of actual dose consumed over the active period has value since it will allow for further analysis of dose effects. This will be a secondary analysis, and will allow for determination of whether higher consumption while on active medication results in greater changes to outcome variables. For this study, a completer analysis will be performed on the target population of older adults (age $\geq$ 65 years).

9.4 Interim Analyses and Stopping Rules

No interim analyses are planned.

To determine the short and long term toxicities associated with administration of two doses of resveratrol when compared to a placebo in adults aged ≥ 65 years. The incidence, type, and severity of adverse events will be recorded during all phases of treatment and follow-up according to the Schedule of Activities shown in **Table 1**.

A single grade IV toxicity or two grade III toxicities will trigger an off cycle review by the DSMB, who can recommend suspension, amendment, or termination of the trial.

Toxicity due to this therapy is expected to be low. A description of each of the following study endpoints will be made at the conclusion of the subjects' accrual and 90 day post-treatment assessment:

A. The number of subjects experiencing grades 1-5 adverse events according to Common Terminology Criteria for Adverse Events (CTCAE) v.3.0; the nature of these events; and the timing of their occurrence.
B. The presence and magnitude of detectable change in blood chemistries.

Participants may have their treatment discontinued (permanently or temporarily) but remain on study for the following reasons; (a) unacceptable toxicity (≥ grade 3 toxicity) related to administration of resveratrol, using the toxicity criteria outlined in **Table 4**; (b) participant non-compliance with the study protocol; or (c) intercurrent disease, medication use, or condition that would affect the assessment of the participant's status. Of course, a participant is free to withdraw from the study at any time.

The study physician (Dr. Bhanu Sandesara) will advise on whether or not he believes the participant should be removed or temporarily suspended from the study based on the toxicity criteria listed below.

Table 4. Dosage adjustment scheduled based on changes in blood chemistry values or participant report of clinically important symptoms.

Lab	Value	Placebo	Resveratrol 1000mg/day	Resveratrol 1500mg/day
WBC (n/µL)	< 3,000	Temporary Stop	Temporary Stop	Temporary Stop
Platelets (n/µL)	< 50,000	Temporary Stop	Temporary Stop	Temporary Stop
Creatinine Clearance (mL/min)	< 30	Temporary Stop	Temporary Stop	Temporary Stop
AST, ALT	> 2.0x ULN	Temporary Stop	Temporary Stop	Temporary Stop
Hematocrit	< 27%	Temporary Stop	Temporary Stop	Temporary Stop

Lab	Value	Placebo	Resveratrol 1000mg/day	Resveratrol 1500mg/day
Clinically Important Symptoms	Yes	Temporary Stop	Temporary Stop	Temporary Stop

The oversight committee will review the number of intercurrent events by condition, and will determine if the statistician should review the events by condition and/or whether the blind should be broken. The statistician will then report the findings of these analyses to the oversight committee and NCCIH. Based on this information, the oversight committee, in conjunction with NCCIH, will determine whether the study should continue.

9.5 Outcomes

9.5.1 Primary Outcome

From a previous study, we estimate that baseline STATE 3 (Primary Outcome) will have a mean of about 120, and a standard deviation of about 40. Hence 0.69 σ (0.81 σ) represents sensitivity to better than a 25% (30%) change from baseline for STATE 3, respectively. Data on this outcome measure will be collected through a muscle biopsy procedure at the second baseline visit and at the second follow-up visit. Muscle biopsies will be performed by Bhanuprasad Sandesara, M.D. who has performed numerous muscle biopsies for several previous studies. Skeletal muscle samples will be obtained under local anesthesia from the *vastus lateralis* muscle using a percutaneous needle biopsy technique as previously described by our group.[62] The skin will be closed with steri-strips. Participants will be contacted by phone three days following this procedure, and will return for a wound check one week after the procedure date. Approximately 150-250 mg of muscle tissue is removed. Portions of the muscle will be immediately processed for permeabilized fiber high-resolution respiration measurements and a very small piece of tissue will be mounted in embedding medium and frozen in isopentane for future histochemical analysis. The remaining tissue will be immediately frozen in liquid nitrogen and stored at -80°C until analysis.

Mitochondrial functional properties, such as respiration and level of uncoupling, will be determined immediately on freshly isolated tissues (not in isolated mitochondria). This technique is highly innovative, and more reflective of the true function of mitochondria in a complex biological sample at a given time. This technique is therefore a very powerful tool to correlate mitochondrial bioenergetics with intervention studies (e.g. pharmacological, genetic, nutritional). The use of saponin-permeabilized tissues also drastically reduces the amount of sample needed (10-20 mg). Importantly, this technique ensures the comprehensive evaluation of *all* mitochondria within a sample, independent of size, location, and functional status, and in their natural intracellular position and assembly. Hence, this method assesses an integrated mitochondrial respiratory function, electron transport chain, and oxidative phosphorylation capacity, as well as ability for specific substrate utilization (i.e. fatty acids). The instrumentation utilized for respirometry is the high-resolution Oxygraph-2k, including acquisition and analysis software (Oroboros, Innsbruck, Austria). Mitochondrial DNA (mtDNA), a quantitative measure of intact, functioning mitochondria, will be isolated and quantified as previously described.[63] Briefly, total DNA will be prepared from 20 mg of muscle tissue using a NucleoSpin Tissue Kit (Machinary-Nagel) and stored at -20

ºC. The CS enzyme, the rate-limiting step in the Krebs cycle, is a quantitative measure of oxidative metabolism in intact, functioning mitochondria.[64] The COX enzyme, which is the last enzyme in the respiratory electron transport chain (Complex IV) of the mitochondria, enables ATP synthase to synthesize ATP, and is highly correlated with overall mitochondrial enzyme function, which can be measured reliably.[65] Several unique features permit small sample size and high signal resolution, such as low limit of detection of flux (1 pmol s^{-1} ml^{-1}), oxygen impermeable and inert materials, and analytical algorithms for chemical and instrumental background calibrations. We have successfully performed this method on human tissues of all ages.

Method for measuring respiration. Initially, 5-10 mg of freshly collected sample is washed in 1 ml ice-cold isolation medium. Tissues are separated to form thin muscle-fiber bundles, and permeabilized in isolation medium containing 50 μg ml^{-1} saponin, at 4°C. The permeabilized tissue is blotted dry, weighed, and added to the chamber. Respiratory function of mitochondria and flux ratios are determined using the following titration protocol (final concentrations and assessment in parenthesis).[66]

9.5.2 Secondary Outcomes

Resveratrol supplementation is also hypothesized to increase the following secondary outcomes: (1) PGC-1α (primary outcome), AMPK, SIRT1, and SIRT3 levels in muscle specimens obtained from the *vastus lateralis*. See **Table 4** below:

Table 5. Evaluation of the Effect of Resveratrol on Outcome Measures

	Measures	Comments
Highest priority for Aim #1	Respiration, COX and CS, Mt DNA content	Respiration analysis will also provide information on the ability to metabolize fatty acids. COX and CS are quantitative measures of oxidative metabolism. CS correlates with mitochondrial volume. COX correlates with overall mitochondrial function.
Highest priority for Aim #2	PGC-1α, SIRT1, SIRT3, AMPK	PGC-1α promotes mitochondrial biogenesis and improves muscle performance. SIRT1 is mainly implicated as an activator of PGC-1α, with only select reports on SIRT3, a mitochondrial sirtuin.
Exploratory Aim #3	Functional assessment or walking speed, muscle fatigue and SPPB	We and others have shown that CS, COX, and PGC -1α, correlates very well with functional assessments of walking speed.

The secondary outcomes listed in the table above will be assessed at the second baseline visit, and at the second follow-up visit. The Exploratory outcomes related to changes in physical function will be assessed at the first baseline visit, and the first follow-up visit.

10. DATA COLLECTION AND QUALITY ASSURANCE

10.1 Data Collection Forms

Study team members in the Institute on Aging (IoA) will obtain informed consent from participants during their initial screening visit. All questions and concerns will be discussed before participants sign the consent form. Subsequently, the research team will conduct a screening visit with each participant to determine if he or she is appropriate for the study. All protocol activities and consent forms will be approved by the University of Florida's Health Science Center's IRB.

Prior to beginning data collection, all study coordinators undergo training and are certified by a post-doctoral fellow who is trained by Drs. Anton, Leeuwenburgh, and Manini on administration of all study procedures, except muscle biopsy (procedures for muscle biopsy described below). A detailed checklist for each data collection element is developed and used to ensure all study coordinators are appropriately trained and able to conduct study procedures. 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 short physical performance battery (SPPB). The post-doctoral fellow or senior study coordinator re-certifies coordinators every six months to ensure continued adherence to the protocol. Any study coordinator who is found to not adhere to all aspects of the protocol is required to complete additional training followed by re-certification.

All research staff will complete the protections of human participants in research training, which is required by The University of Florida institutional review board (IRB) and National Institutes of Health (NIH). 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.

A number of methods are employed to maintain the participant's confidentiality. First, questionnaire data are collected in a quiet, secluded location where the interview cannot be overheard. Second, only study investigators and key research staff (i.e. data manager and study programmers) have access to the study database. Third, participants are assigned a unique study identifier. Individual names will ultimately be removed from the study database and only the unique study identifier is used to distinguish participants in the database. Fourth, collected data are maintained in locked computer files and file cabinets, to which only study investigators have access. Collected data will be used only for research purposes, and published data will not contain any individual identifiers. Finally, all research staff members have to complete NIH and HIPAA training on the protection of private health information on an annual basis. Thus, the risk of adverse experiences or violations of confidentiality will be minimized by utilizing only qualified individuals to conduct the study (the staff in University of Florida's Institute on Aging and Clinical Translational Science Institute). Appropriate attention to detail in the experimental setting will be emphasized. Prior to study initiation, procedures for protecting the confidential nature of participant data collected will be reviewed, and all questions or concerns will be clarified at this time. These procedures will be reviewed throughout the study.

10.2 Data Management

Participant data, which will be collected on IRB approved forms or source documents created specifically for this study. This information will then be entered into a secure database (i.e., REDCap) by the study coordinator and/or data management staff member. This database was developed by Vanderbilt, and has over 50,000 active user groups. It is FDA CFR 21 compliant, and has full audit capabilities. It exports to SAS, EXCEL, STATA, R, and SPSS. It has field and user level control for read or write privileges. The University of Florida Clinical and Translational Institute maintains REDCap, and has onsite and offsite daily back-up, with secure encrypted data entry and automatic editing. It has the capability of producing printed case report forms for auditing purposes.

The database server where the participant data is stored is hosted in an enterprise datacenter. The database is only accessible from the webserver that accesses it, and a tools server for database maintenance. There are firewalls in place to keep unnecessary traffic away from the database server. The application requires unique usernames and passwords that are stored in a one-way salted hash (SHA256).

10.3 Quality Assurance

10.3.1 Training

Prior to beginning data collection, all study coordinators undergo training and are certified by a post-doctoral fellow who is trained by Drs. Anton, Leeuwenburgh, and Manini on administration of all study procedures, except muscle biopsy (procedures for muscle biopsy described below). A detailed checklist for each data collection element is developed and used to ensure all study coordinators are appropriately trained and able to conduct study procedures. 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 SPPB. Either one of the investigators, or a post-doctoral fellow, will re-certify coordinators every six months to ensure continued adherence to the protocol. Any study coordinator who is found to not adhere to all aspects of the protocol is required to complete additional training followed by re-certification.

10.3.2 Quality Control Procedures

A number of quality control procedures will be used to ensure the validity and integrity of the data, and the safety of all participants involved in the study.

The Principal Investigator or designated study team member will be responsible for coordinating meeting times of the Independent Safety Board (oversight committee). Only blinded study team members (i.e., study biostatistician) will have access to information discussed during Closed Session meetings and the Safety Board's Closed Session report. Relevant data and safety information obtained for each study participant will be verified against the original source documents by the primary study coordinator. The coordinator will review all of the participants' questionnaires and data collection forms prior to ending each clinic visit. Forms must be completed neatly and accurately, and every question should be answered. Written responses to any items on the questionnaires/ forms should be legible. After reviewing the forms, the reviewer's initials should be written in the form header (top of first page) as a confirmation that a review was done.

Prior to data entry, all forms (100%) will be spot checked by a second coordinator and a research assistant. An acceptable error rate will be 1% or fewer missing or unidentifiable fields. Any identified discrepancies will initially be discussed between the coordinators to resolve. If the discrepancies cannot be resolved by the coordinators and research assistants, then these will be discussed with the Principal Investigator at weekly meetings. At these meetings, data collection procedures will be reviewed to determine if any improvements can be made. Records of the findings from data entry review and the ways by which spot check irregularities were resolved will be documented in the participant's source document and subsequently stored in participant binder. This information will be used to determine if the error rate is acceptable. If error rates fall above this range after discussion in team meetings, then additional training will be provided to the appropriate study team members. If necessary, a full review of data collection procedures will be completed to identify the potential causes of errors.

The data entry screens are designed to mirror the paper data collection forms to allow smooth flow from item to item and thereby minimize error with data entry. Verification of participant identifiers and visit numbers are incorporated into the data entry system, in addition to gross range checking of fields. Internal comparisons of the entered data to detect missing records or suspicious or invalid data will be performed on a monthly basis and approximately 10% of the entered records will be reviewed. These comparisons include logical consistency checks of data within and across forms/questionnaires. An acceptable error rate will be 1% or fewer inconsistencies between entered data and data collected on study forms. When inconsistencies are detected, these errors will be discussed at weekly study meetings, and data collection and entry procedures will be reviewed to determine if any improvements can be made. Records of the inconsistencies and the improvements made to check errors will be documented in the weekly meeting report and stored with the study binder. This information will be used to determine if the error rate is acceptable. If error rates fall above this range after discussion in team meetings, then additional training will be provided to the appropriate study team members. If necessary, a full review of the data entry process will be completed to identify the potential causes of entry errors.

To ensure participant confidentiality, the data obtained on all participants will be de-identified in the study database and any PHI will be stored in locked file cabinets.

10.3.3 Metrics

We have established protocols for each method, and detailed protocols will be made available upon request. We are committed to establishing and maintaining quality assurance standards, in part by maintaining and calibrating instrumentation for all assays and techniques. Volumetric equipment and analytical balances are professionally calibrated annually, and validated monthly (sample handling). The BioTek Synergy™ HT multidetection microplate reader is validated monthly (sample preparation and enzymatic activity assays). The more specialized OROBOROS Oxygraph-2k instrument is calibrated prior to use for high resolution respirometry according to the manufacturer's recommendations. The Applied Biosystems 7300 Real-Time PCR System (Life Technologies™) is professionally serviced annually, and calibrated prior to use (Mt DNA content). Where appropriate, reference controls and/or cross samples are included in our methods to determine inter- and intra-analysis reliability.

10.3.4 Protocol Deviations

All deviations from this protocol will be logged in a cumulative table and reported to the IRB on a yearly basis during the Continuing Review or during Study Closure. A Major Deviation is defined as an unapproved temporary change in previously approved research activities, implemented without IRB approval, and potentially adversely affecting subjects' rights, welfare, or safety, willingness to continue study participation, and/or integrity of research data. Major Deviations will be reported to the IRB within five (5) days of discovery and added to the study's Cumulative Deviation Log. A Minor Deviation is defined as an unapproved temporary change in previously approved research activities, implemented without IRB approval, which *does not* adversely affect subjects' rights, welfare, or safety, willingness to continue study participation, and/or integrity of research data. Minor Deviations are also added to the study's Cumulative Deviation Log, and submitted to the IRB at Continuing Review/Study Closure.

10.3.5 Monitoring

Relevant data and safety information obtained for each study participant will be verified against the original source documents by the primary study coordinator after each visit. Additionally, the data will be spot checked by the program manager or compliance office. As noted above, any identified discrepancies will be discussed with the Principal Investigator and reviewed at weekly meetings. The frequency of data review for this study differs according to the type of data and is summarized in **Table 3.**

11. PARTICIPANT RIGHTS AND CONFIDENTIALITY

11.1 Institutional Review Board (IRB) Review

This protocol and the Informed Consent Form, as well as any subsequent modifications, will be reviewed and approved by the University of Florida's IRB, which is responsible for oversight of the study. The consent form will be separate from the protocol document.

11.2 Informed Consent Forms

Study team members in the Institute on Aging (IoA) will obtain informed consent from participants during their initial screening visit. All questions and concerns will be discussed before participants sign the consent form. A signed consent form will be obtained from all potential participants before any procedures are administered. Subsequently, the research team will conduct a screening visit with each participant to determine if he or she is appropriate for the study. All protocol activities and consent forms will be approved by the University of Florida's Health Science Center's IRB prior to study initiation.

If the potential participants are illiterate or have impaired vision the information in the consent materials will be presented orally to them (in the presence of legally authorized representative - LAR). Participants will be encouraged to ask questions and discuss to ensure they comprehend the consent information. The participant may then make their mark on the consent form to indicate a willingness to participate. A copy of the signed and dated consent form will be given to participants (or their LAR), and the original document will be placed in participant's individual study files, which will be stored in a secure location.

In accordance with the NIH and the Office of Human Research Protection (OHRP) guidelines, the informed consent will contain the following elements:

1. A statement that the study involves research
2. An explanation of the purposes of the research
3. The expected duration of the subject's participation
4. A description of the procedures to be followed
5. Identification of any procedures which are experimental
6. A description of any reasonably foreseeable risks or discomforts to the subject
7. A description of any benefits to the subject or to others that may reasonably be expected from the research
8. A disclosure of appropriate alternative procedures or courses of treatment, if any, that might be advantageous to the subject
9. A disclosure of audio taping of cognitive test administrations to allow for ongoing review of the technician's skills related administration of the battery
10. A statement describing the extent to which confidentiality of records identifying the subject will be maintained
11. An explanation of whether any compensation and any medical treatments are available if a research-related injury occurs and, if so, what these consist of, or where further information may be obtained
12. An explanation of whom to contact for answers to pertinent questions about the research and research subjects' rights, and whom to contact in the event of a research-related injury to the subject
13. A statement that participation is voluntary, refusal to participate will involve no penalty or loss of benefits to which the subject is otherwise entitled, and the subject may discontinue participation at any time without penalty or loss of benefits, to which the subject is otherwise entitled
14. Anticipated circumstances under which subject participation may be terminated by the investigator without regard to the subject's consent
15. Any additional costs to the subject that may result from participation in the research
16. The consequences of a subject's decision to withdraw from the research and procedures for orderly termination of participation by the subject
17. A statement that significant new findings developed during the course of the research, which may relate to the subject's willingness to continue participation, will be provided to the subject
18. The approximate number of subjects involved in the study
19. Specifically for the biological samples repository, the consent will describe the operation of the repository, the conditions under which data and specimens will be released to recipient-investigators, the specific types of research to be conducted, the procedures for protecting the privacy of subjects and maintaining the confidentiality of data (i.e. recipient-investigators will not be provided access to the identities of donor subjects).

11.3 Participant Confidentiality

A number of methods are employed to maintain confidentiality of participants. First, questionnaire data are collected in quiet, secluded spaces where the interview cannot be overheard. Second, only study investigators and key research staff (i.e. data manager and study programmers) have access to the study database. Third, participants are assigned a unique study identifier. Individual names will ultimately be removed from the study database and only the unique study identifier is used to distinguish participants in the database. Fourth, collected data are maintained in locked computer files and file cabinets, to which only study investigators have access. Collected data will be de-

identified in our dataset and will be used only for research purposes and analysis. Published data will not contain any individual identifiers. Finally, all research staff members have to complete HIPPA training on the protection of PHI annually. Thus, the risk of adverse experiences or violations of confidentiality will be minimized by utilizing only qualified individuals to conduct the study (the staff in University of Florida's Institute on Aging and Clinical Translational Science Institute). Appropriate attention to detail in the experimental setting will be emphasized. Prior to study initiation, procedures for protecting the confidential nature of participant data collected will be reviewed and all questions or concerns will be clarified at this time. These procedures will be reviewed throughout the study.

11.4 Study Discontinuation

The study may be discontinued at any time by the IRB, the NCCIH, the OHRP, the FDA, or other government agencies as part of their duties to ensure that research participants are protected. Specific reasons why this study may be stopped prior to completion include the following: (1) the intervention is associated with adverse effects that significantly impact the risk-benefit ratio; (2) study recruitment or retention becomes futile; (3) any new information becomes available during the trial that necessitates stopping the trial; and (4) if the oversight committee indicates stopping the trial is necessary due to adverse event frequency or severity.

12. COMMITTEES

This study will be monitored by an Independent Monitoring Committee, which will consist of an established board, which has reviewed all studies conducted within the Pepper Older Americans Independence Center during bi-annual conference calls for the past seven years. Specifically, this board consists of the following individuals: (1) Stephen Kritchevsky, PhD, Chair, an epidemiologist who has been involved in research for many years, (2) Jing Cheng, Ph.D., a biostatistician who has been involved with a number of clinical trials, and (3), John Meuleman, M.D., a physician who has been involved in the conduct of clinical research for many years.

13. PUBLICATION OF RESEARCH FINDINGS

Any presentation, abstract, or manuscript resulting from the study findings will be made available for review by the NCCIH prior to submission.

14. REFERENCES

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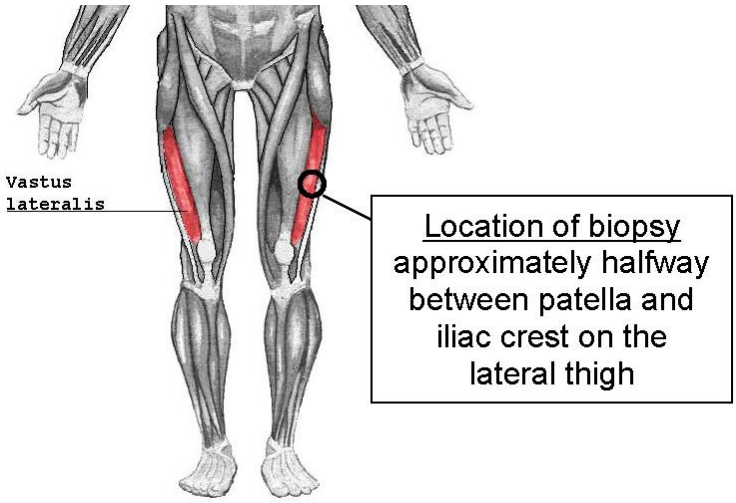
15. SUPPLEMENTS/APPENDICES

Appendix 1 – Muscle Biopsy Procedure

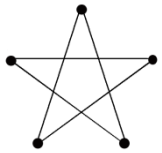
Appendix 2 – Participant Instructions For Muscle Biopsy Procedure

Appendix 1
MUSCLE BIOPSY PROCEDURE

1.1 Muscle Biopsy Procedure – Vastus Lateralis

- Have the participant lie comfortably in a reclined position with both legs outstretched. Support calves/feet with pillows to maintain relaxed position.
- Check the patient binder to see which leg was tested during the strength measurement or MRI. Perform the biopsy on this leg. Otherwise it is PI, or participant preference.
- Ask participant if they are allergic to latex, and if so, use alternative material gloves.
- The muscle biopsy will be obtained approximately midway between the patella and iliac crest on the lateral thigh. See figure below. This distance was chosen so each participant will receive the biopsy at the same relative length of their lower extremity.
- 

- The site can be marked with pen, ink, or by pressing into the skin to leave an indention.
- Do not allow the participant to watch the procedure.
- If necessary, an area of skin can be shaved to prevent hair from entering the incision and improving adhesion of the sterile bandage.
- The skin is cleaned with ChloraPrep solution. Swab, starting from the anticipated biopsy site in a circular motion extending to a diameter of approximately 4-5 inches.
- Record procedure start time.
- Inject 1 to 2 ml of 2% lidocaine intradermally and subcutaneously. Allow a brief period (30-60 seconds) for the skin to numb before injecting the remaining volume more deeply. Insert the needle to just below the fascia, aspirate to assure that needle is not in a vessel, inject a small amount of lidocaine starting below the fascia and continue while drawing the needle straight up through the subcutaneous tissue. Repeat in a star pattern. See figure below:



- Wait 8 to 10 minutes after injection of the lidocaine before proceeding, to ensure maximal participant comfort.

- **Scalpel skin test**: Using a no. 11 blade scalpel, gently touch the scalpel to the anticipated site and ask the patient if they can feel anything where touched. It may help to have the patient close their eyes. If they can feel the scalpel, allow more time and retest in 5 minutes. If the participant can still feel the blade touch, consider switching to carbocaine

- When the participant is properly numbed, make a small (5 mm) incision in the skin down through the muscle fascia. It may make only a small nick

- Insert the closed trocar through the incision, and through the fascia, advancing the trocar into the muscle belly. The trocar window extends 2 cm from the tip. To obtain proper suction it is necessary to have the whole opening below the fascia.

- Once in position the surgeon places their thumb over the hole atop the inner cannula. The sterile assistant will state "up" when ready to apply suction. The surgeon will raise the inner cannula 1 inch, opening the trocar window. The assistant will immediately apply suction through a 60 ml syringe, drawing tissue into the cutting chamber as the surgeon advances the inner cannula in a rapid guittione-like action. Be sure the trocar does not advance deeper in the leg with this action.

- To maximize tissue yield, rotate the trocar 90 degrees without removing from the leg, and repeat the suction procedure two more times for a total of four separate muscle samples.
- The needle is passed to the sterile assistant who removes the accumulated tissue on a sterile gauze with use of the forceps and a needle plunger. The tissue is quickly placed on an iced receptacle such as a weight boat or saline-soaked gauze.

- If the yield is insufficient you may re-insert the trocar up to three times through the same hole in the fascia but at slightly different angles in the numbed area, as long as the participant is comfortable and consents to repeated passes.

- Hold firm direct pressure over incision site for at least 5 minutes or longer until external bleeding has ceased.

- Approximate the incision edges and close the incision with a sterile liquid bonding agent (such as Dermabond) or butterfly bandage. This will help prevent bleeding and scarring.

- Apply a small dab of triple antibiotic over the incision (once glue is dry, if using). Apply a single folded sterile gauze on top of the incision, and cover with Tegaderm.

- Wrap with elastic wrap and place an ice bag on the incision site for 15 to 30 minutes to reduce swelling and pain, unless prohibited by the study protocol.

- Study coordinator: Record procedure stop time.

- Study coordinator: Record approximate amount of 2% lidocaine injected.

- Study coordinator: Record whether or not there were any adverse reactions during or post procedure.

- Study coordinator: Record whether or not any muscle tissue sample was collected.

- Study coordinator: The total amount of time that the participant will be in the clinic for this procedure is approximately 45 minutes to 1 hour. This includes about 15 minutes of interaction with the study physician for the site preparation and the biopsy itself; 10 to 15 minutes to wait for the lidocaine to take effect; about 15 minutes for icing the area after the biopsy; and 15 minutes for a snack/recovery. Be sure to offer the snack as soon as possible after the biopsy.

1.2. Recommended Tray/Room setup

Tray setup must be done 45 minutes to 15 minutes prior to biopsy and out of sight of the research participant. The biopsy tray should be prepared, covered until use, and used during the procedure away from the participant's field of observation.

Sterile field must be maintained during tray setup. Sterile gloves must be worn, or sterilized ring (sponge) forceps (handle non-sterile, tips sterile) must be used when handling sterile items.

Assemble the following items for the tray:

(2) non-fenestrated sterile drapes (one for lining tray and one for draping over set-up tray)
(1) fenestrated sterile drape
1. (1) biopsy needle (all parts-trocar, cannula, plunger. Numbers must match) [sterilized]
2. (1) standard bore 27-33" IV extension tubing
(1) Sterile 3-way stopcock (if assistant preference)
3. (1) tweezers [sterilized] if not packaged with biopsy needle
4. (5-8) sterile 3x3 or 4x4 gauze pads
5. (1) No. 11 safety scalpel
6. (1) 23 g 1¼" safety needle
7. (1) 16 g 1" safety needle
8. (1) 60 cc syringe
9. (1) 10 cc syringe
10. (1) pack of sterile skin glue such as Indermil or Dermabond (kept in refrigerator in Sample Processing Room)
11. (1) sterile medium butterfly bandage (if not using glue)
12. (1) sterile transparent dressing (tegaderm) appropriate for needed coverage
13. (1) Bottle of 2% buffered lidocaine containing at least 10ml
14. (1) Sterile alcohol pad

1.3 Two-person tray assembly:

1. The non-sterile assistant dons exam gloves. The sterile assistant dons sterile gloves.
2. The non-sterile assistant opens 1 non-fenestrated drape, pulling the corners of the packaging back so the sterile person can remove the item without touching the outside of the packaging.
3. The sterile person unfolds the drape and places the drape over the tray, blue side down, without touching the tray with their gloves.
4. The non-sterile assistant continues to open each sterile item in the manner previously described, and the sterile person places them on the sterile field.
5. The sterile person assembles the biopsy needle and assures the pieces fit together, then attaches the tubing, stopcock (if using) and 60 ml syringe.
6. The sterile person attaches the 16G draw needle to the 10ml syringe.
7. The non-sterile assistant cleans the stopper of the lidocaine with a sterile alcohol pad, allowing the alcohol to evaporate.
8. The non-sterile assistant then inverts the bottle and holds it steady while the sterile assistant draws up 10ml of lidocaine.
9. The sterile person activates the safety device on the needle and directly discards the needle in an open sharps container. The 23G needle is then attached to the syringe to maintain sterility and avoid leakage
10. The sterile person unfolds the 2nd non-fenestrated drape and lays it over tray, blue side up.
11. Holding the tray by the drape covered edges, the sterile person transports the tray to a tray holder in the biopsy room. The non-sterile assistant may be needed to open doors.

1.4 One-person tray assembly:

Lidocaine will not be drawn up with this assembly so make sure that it's available in the biopsy room
With exam gloves on:

1. Open 1 non-fenestrated drape, and, handling only the corners, lay it on sanitized surgical tray (blue side up).
2. Open each pack of sterile instruments or dry supplies and drop on to draped tray; make sure that items are not handled directly.

Don surgical gloves (keeping gloves sterile at all time)

3. Maintaining the sterile field, reposition biopsy tray contents and assemble needles.
4. Unfold second non-fenestrated drape and lay over tray, blue side down.
5. Holding the tray by the drape covered edges, the sterile person transports the tray to a tray stand in the biopsy room.

1.5. Materials in the Room

(2) chloroprep swabs
(2) pairs of sterile surgical gloves-for surgeon
15. (2) pairs of sterile surgical gloves-for biopsy assistant
16. (1) box of exam gloves (S/M/L) for non-sterile assistant
(1) bottle of 2% lidocaine (to be stored in processing lab per DOH exclusion when not in use)
(1) bottle of 1% carbocaine (to be stored in processing lab per DOH exclusion when not in use)
17. (1) spray bottle of Gebauer's Pain Ease (nonflammable instant topical anesthetic)
18. (1) alcohol prep pad
19. (1) pouch triple antibiotic
20. (1) tegaderm
21. (1) sterile butterfly bandage
22. (1) pack of bandaids
23. (1) roll of lab tape
24. (1) box of sterile gauze pads
25. (2) instant ice packs
26. (2) elastic (ACE or Coband) wrap
27. (2) standard bore 27-33" IV extension tubing
(1) biopsy needle (all parts-trocar, cannula, plunger. Numbers must match) [sterilized]
28. (1) scissors [sterilized]
29. Pillow cases
30. Blue absorbent leak-proof pads (chux)
31. Exam shorts

Appendix 2
PARTICIPANT INSTRUCTIONS FOR MUSCLE BIOPSY PROCEDURE

2.1 Procedure Description

You will report to the Institute on Aging located at 2004 Mowry Rd, Gainesville, FL for a muscle biopsy from your thigh. This procedure involves first numbing the area with a local anesthetic, performed by a licensed physician experienced in this technique, and then using a needle about the size of a pen to collect a small amount of muscle tissue, approximately equal to the size of the head of a pencil eraser. You may feel some numbing pain during the local anesthetic, but you should not feel pain during the biopsy. You may feel pressure, but please tell the physician if you feel pain. This is a safe procedure, but may cause minor swelling and localized soreness and bruising. Please tell us now if you are allergic or sensitive to local anesthetics.

After the tissue sample is taken, you will receive detailed instruction on how to care for the incision site. A study staff member will call you in 1-4 days following the procedure, and will do a brief 10 minute phone interview regarding any problems that you may be having related to the procedure.

2.2 Risk Description

During the procedure you may feel pressure as the needle is inserted into the muscle. You might feel discomfort, but pain rarely occurs. You should notify the physician or nurse practitioner if you have any pain during the procedure. The potential risks of this procedure include infection, mild muscle soreness, and swelling. You might also have a small scar on the upper thigh from the procedure that will fade over time. Nerve damage causing injury to a small number of muscle fibers (<0.01%) could occur, but no observable or measurable changes in muscle strength or muscle function have been previously reported for this procedure. The potential for mild soreness and swelling is 50%. The procedure is done under sterile conditions, but there is a slight risk of infection any time the skin is broken. To minimize the risk of bruising and infection, pressure dressings might be applied, and only disposable sterile materials will be utilized. Some people may be sensitive or allergic to the local anesthetics, and the research team should be informed if you have such an allergy or sensitivity. If you have any of the following conditions please let the study staff know:

- High risk of bleeding *due to the medications you are taking*
- High risk of bleeding, *such as low blood counts and* Von Willebrand's Disease
- High risk of *skin* scarring
- Allergies to *numbing medication (e.g.* lidocaine*)*
- History of poor wound healing

2.3 Preparation Instructions for Participant

7 days prior to biopsy

Please avoid the following medications for 1 week before the procedure:

- Coumadin (also known as Warfarin, Jantoven, Marevan, and Waran)
- Plavix (also known as Clopidogrel, Clopilet, and Ceruvin)
- Aggrenox (also known as Aspirin with Dipyridamol)
- Ticlid (also known as Ticlopidine)
- Agrylin or Xagrid (also known as Anagrelide)
- Pradaxa (Dabigatran)
- Aspirin (Bayer, Ascriptin, Excedrin, Bufferin, Anacin, Ecotrin, BC Powder)
- Aspirin containing products (Alka-Seltzer, others)
- NSAIDs (such as Aleve, Ibuprofen, Advil, Naproxen, Indomethicin, or other anti-inflammatories)

48hrs prior to biopsy

Please avoid strenuous exercise for the 48 hours before the procedure.

24hrs prior to biopsy

Please do not eat or drink anything, except your usual prescription medications and water from midnight the night before the procedure, until after the procedure has taken place.

Please shower with antibiotic soap the night before or morning of the biopsy, as you will have bathing limitations after the biopsy.

Please shave the expected biopsy area (if known) the night before to reduce the risk of razor burn if done at the time of the biopsy.

MUSCLE BIOPSY
2.4. DISCHARGE INSTRUCTIONS for Participant

Prior to discharge, the participant who has had a muscle biopsy procedure will be instructed in the care of the biopsy site and made aware of any complications that might occur. The participant will acknowledge that (s)he has been instructed and that (s)he understands these instructions by signing the discharge instruction sheet.
Name: _____ Date: _____

Discharge instructions given by: _____

Please follow these discharge instructions for your comfort and safety.

1. Although it is very rare, infection (presenting as redness, pus from wound, increasing pain) can occur due to the nature of this procedure. If you should develop any of these symptoms call us immediately. To prevent infection, keep the site clean and dry for at least 24 hours. You should not shower for 24 hours and should not take a bath for 72 hours.

2. You should not experience excessive bleeding or bruising. A few drops of blood on the bandage is expected. If excessive bleeding occurs, call us immediately. To prevent bleeding and/or bruising, keep the bandage clear for 72 hours. If steri-strips were used, they will come off on their own. Use a light bandage over them if needed.

3. Do not do any strenuous activity or exercise for 24 hours after your procedure.

4. Do not take any aspirin or ibuprofen for at least three days after your procedure. You may take Tylenol or the medicine prescribed today for any discomfort. If you have pain at the biopsy site, you may apply ice every two (2) hours for 20 minutes until the pain subsides.

5. If you do have a problem during office hours, please contact your study coordinator, your study investigator, or the physician / physician assistant (PA) who performed the biopsy listed below:

   Study Coordinator: _____ Phone:
   Study Physician/PA: _____ Phone:
   Principal Investigator: _____ Phone:

6. Wait 72 hours (3 days) after the muscle biopsy to restart any of the drugs affecting platelets, bleeding and/or bruising that were stopped only for the muscle biopsy.

If you have an emergency related to this procedure after normal office hours, please go to the hospital emergency room. Please notify the hospital staff of the muscle biopsy procedure, and provide the principal investigator and the study physician/PA contact information (see above).

I have read and understand these discharge instructions and have reviewed them with the study staff.

_____ _____
Participant's signature Date

_____ _____
Signature of Person administering instructions Date

2.5 Follow-up Information for Participant

Although complications after the biopsy procedure are rare, you will be contacted by phone regarding such complications, as some may be serious. To ensure your health and safety, you will be contacted 24 hours after your procedure, 3 – 5 days after your procedure, and will complete an in person assessment visit 10 days after the procedure.

At the 24-hour contact, you will be asked questions about:

- Signs of infection (redness, pus, or excess heat)
- Excess discomfort
- Excess bruising or bleeding
- Problems related to the bandage/Dermabond (rash, excess itching)
- Pain relieving drugs taken for pain related to the procedure
- Any other problems or concerns with the procedure

At the 3-5 day contact, the original dressings should be removed by this point. The expectation is that most discomfort should have subsided as well, so you will be asked questions about:

- The appearance of the incision
- Any lingering discomfort or other issues pertaining to the procedure

At the 10 day in person contact, you will be asked questions about:

- Any further complications following the procedure

If you have any questions regarding the procedure or would like to contact us for any reason, please call the Institute on Aging at (352) 273-9212 between the hours of 7:30 AM and 4:30 PM, or Dr. Stephen Anton after hours at (352) 871-0398.