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Resveratrol and exercise to treat functional limitations in late life: Design of a randomized controlled trial

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ABSTRACT

Skeletal muscle mitochondrial function declines with age and is a key factor in the maintenance of physical function among older adults. Research studies from animals and humans have consistently demonstrated that exercise improves skeletal muscle mitochondrial function in early and middle adulthood. However, mitochondrial adaptations to both acute and chronic exercise are attenuated in late life. Thus, there is an important need to identify adjuvant therapies capable of augmenting mitochondrial adaptations to exercise (e.g. improved mitochondrial respiration, muscle mitochondria biogenesis) among older adults. This study is investigating the potential of resveratrol supplementation for this purpose. The objective of this randomized, double-masked pilot trial is to evaluate the efficacy of resveratrol supplementation combined with a comprehensive supervised exercise program exercise for improving physical function among older adults. Moderately functioning, sedentary participants aged ≥60 years will perform 24 sessions (2 day/wk for 12 weeks) of center-based walking and resistance training and are randomly assigned to receive either (1) 500 mg/day resveratrol (2) 1000 mg/day resveratrol or (3) placebo. Study dependent outcomes include changes in 1) knee extensor strength, 2) objective measures of physical function (e.g. 4 m walk test, Short Physical Performance Battery), 3) subjective measures of physical function assessed by Late Life Function and Disability Instrument, and 4) skeletal muscle mitochondrial function. This study will provide novel information regarding the therapeutic potential of resveratrol supplementation combined with exercise while also informing about the long-term clinical viability of the intervention by evaluating participant safety and willingness to engage in the intervention.

1. Introduction

The maintenance of one's physical capabilities during older age is an essential part of healthy aging. Declines in physical abilities are associated with not only the onset of disability and the loss of independence but also with increased rates of morbidity and mortality [1–3]. As our group has shown previously, skeletal muscle mitochondrial function—including mitochondrial respiration, oxidative mitochondrial enzyme activity and muscle content of peroxisome proliferator-activated receptor γ coactivator-1 (PGC-1α)–declines with age and is a key factor in the maintenance of physical function among older adults [4,5]. These biological changes have direct implications for the maintenance of key physiologic variables – including skeletal muscle endurance and aerobic fitness – which mediate physical function. Thus, interventions that improve skeletal muscle mitochondrial function hold promise for preserving physical function among seniors.

Research studies from animals and humans have consistently demonstrated that exercise – particularly aerobic exercise – improves skeletal muscle mitochondrial function in early and middle adulthood [6–9]. These changes in muscle mitochondrial function track closely with changes in whole-body aerobic fitness [10]. However, several studies in recent years have indicated that mitochondrial adaptations to both acute and chronic exercise are attenuated in late life [11–14] as are improvements in skeletal muscle oxidative capacity [10]. Thus, age-related mitochondrial

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impairments do not appear to be completely abated by exercise alone. Accordingly, studies to identify efficacious adjuvants to exercise have the potential to improve older adult's response to exercise interventions.

Resveratrol has received considerable attention as a gene regulator affecting mitochondrial metabolism possibly by activating 5′ adenosine monophosphate-activated protein kinase (AMPK) as well as sirtuin 1 (SIRT1) and SIRT3 [15,16]. These beneficial effects on mitochondrial function have been demonstrated across multiple species – including humans, and have been associated with improvements on a variety of functional tasks and measures of healthspan in preclinical models [15,17–22]. Resveratrol appears to oppose the reductions in mitochondrial function associated with aging largely by affecting the expression of PGC-1α and associated genes. Because several studies using animal models of aging have reported that improvements in physical performance are greater in response to the combination of exercise and resveratrol than from either treatment alone, [19,23–25], we postulated that resveratrol supplementation has significant potential as an exercise adjuvant for preserving function among older adults. This study was designed to begin to test our central hypothesis that, when combined with chronic exercise, resveratrol improves functional outcomes among functionally-limited older adults in a dose-dependent manner. The objective of this randomized pilot trial is to refine and finalize elements critical to conducting a future, fully-powered randomized, controlled trial to definitively test our central hypothesis.

2. Methods

2.1. Study design/methods

This study is a three-arm, randomized, double-masked pilot trial to evaluate the safety and efficacy of combining orally-ingested resveratrol with physical exercise for improvement of physical function among older adults at risk for becoming disabled. The study design allows for the assessment of which dosage/placebo, when coupled with exercise, improves skeletal muscle mitochondrial function, skeletal muscle function, walking speed, lower-extremity function, self-assessed physical function, or self-assessed health status. Following study entry, participants are randomly assigned to receive resveratrol supplementation at 500 mg/day, 1000 mg/day, or placebo. In addition to taking the study supplement, all participants are asked to participate in the center-based multi-model exercise intervention (Fig. 1). Participant safety is overseen by a comprehensive study team—including the principal investigator, study physician, study staff, and an appointed Data and Safety Monitoring Board. The study was registered at www.clinicaltrials.gov prior to participant recruitment (NCT02523274), and all participants provide written informed consent based on documents approved by a university Institutional Review Board.

2.2. Participants

The study team is recruiting up to 60 (n = 20/group) sedentary older men and women ≥65 years of age with objective signs of functional limitations. Inclusion criteria include a long-distance (400 m) corridor walk test time of >290 s indicating functional limitations and moderate to low aerobic fitness, [1], and a sedentary lifestyle defined as <150 min/wk of moderate physical activity as assessed by the Community Healthy Activities Model Program for Seniors (CHAMPS) questionnaire [26].

Persons currently consuming a resveratrol supplement, with absolute contraindications to exercise training, [27], or with other medical conditions that would preclude safe participation are excluded.

2.3. Screening, randomization and masking

Interested individuals initially complete a pre-screening interview by phone. Participants deemed eligible based on the pre-screening are invited to an in-person screening visit. During this visit, potential participants are first asked to give their informed consent and are then screened for study entry criteria. Initial screening procedures include a review of their medical history, physical activity habits, medication use, cognitive function (assessed by the Mini Mental State Exam), [28], and a physical exam performed by a study physician. The study physician reviews collected information relevant to potential participant's health and makes a recommendation on each individual's suitability to participate in this trial.

Participants are then asked to complete the long-distance corridor walk test to evaluate functional status and aerobic fitness. If all study entry criteria are met, participants return to the clinic research center for baseline assessments prior to randomization. During this visit, participants are asked to complete validated study questionnaires, including the Late-Life Disability Instrument [29] and provide a fasting blood sample for evaluation of clinical safety lab values. Participants also complete the Short Physical Performance Battery (SPPB), a 6-minute walk test, and assessments of muscle strength. Finally, the study coordinator provides participants with a wearable physical activity monitor and dietary intake form to objectively evaluate baseline physical activity and dietary habits. Staff members then assign the participant using permuted block randomization stratified by age (i.e 65–75, >75 years) and gender. Participants who agree to participate in the muscle biopsy procedure are scheduled for a separate visit prior to initiation of study interventions.

2.4. Muscle biopsy

Willing participants undergo a percutaneous skeletal muscle biopsy as previously described [30]. Samples are collected under 2% lidocaine local anesthetic using a six-gauge needle with suction applied. Samples are snap frozen in liquid nitrogen and stored at −80 °C for later analysis. Participants currently taking anti-platelet or anti-coagulant medications, with conditions which reduce wound healing, or with a known allergy to lidocaine are excluded from the biopsy procedure.

2.5. Assessments

Study outcomes include measures of walking speed, lower-extremity function, exercise capacity, skeletal muscle strength, self-assessed physical function, and skeletal muscle mitochondrial function as described below. Physical function assessments will be assessed at baseline, week 6 and week 12, while mitochondrial function will be assessed at baseline and week 12 (Table 1).

2.5.1. Walking speed

Walking speed is assessed by asking the participants to walk at their usual pace over a 4 m course. Participants are instructed to stand with both feet touching the starting line and to start walking after a specific verbal command. Timing begins when the command is given, and the time needed to complete the entire distance is recorded. The faster of two walks is used. The reliability of the 4 m walk test is excellent – with an intraclass correlation coefficient (ICC) > 0.9 [31].

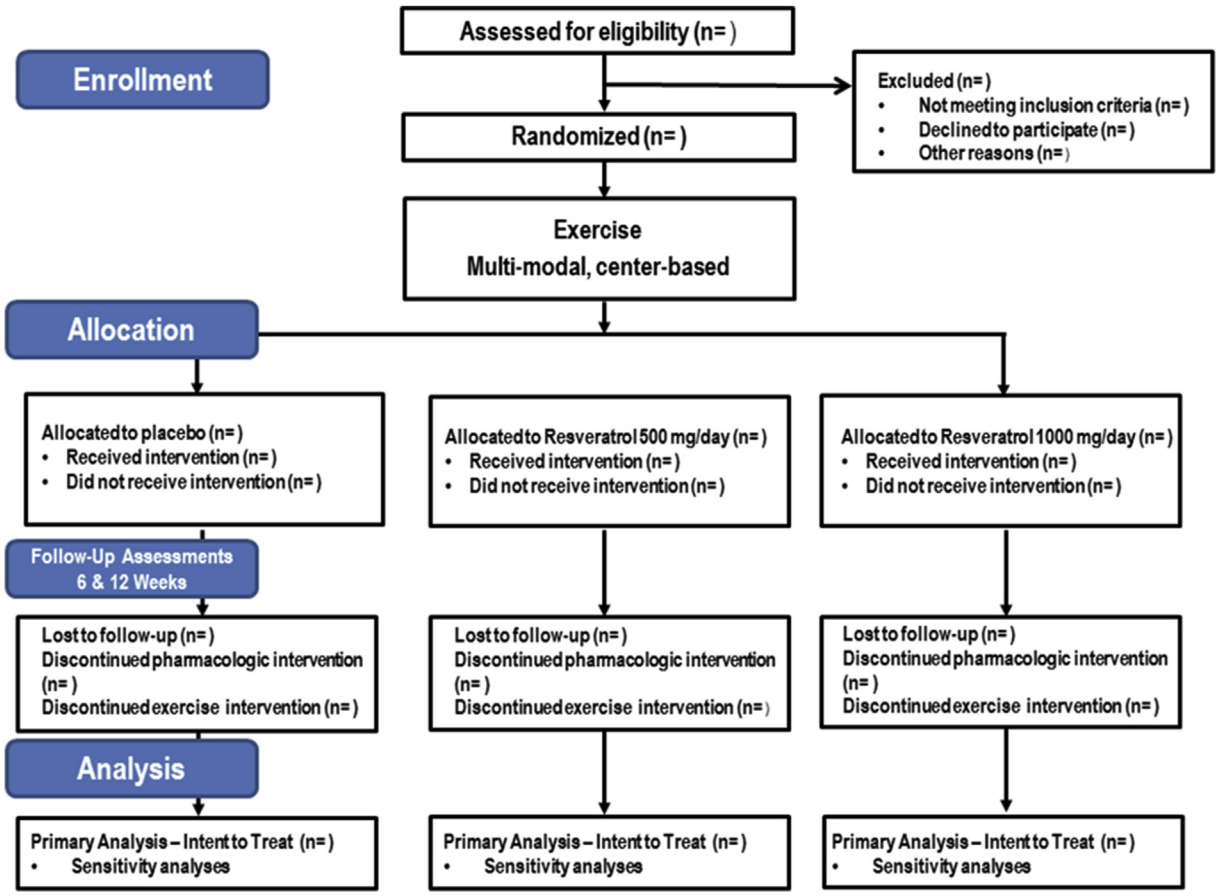

Fig. 1. Overview of study design according to CONSORT format.

Table 1
Data collection summary by study visit.

Study Phase	Pre-randomization		Randomization	
Visit description (FU = follow-up, CO = close-out)	Screen	Baseline	FU	CO
Visit number	1	2	3	4
Visit week	−2	0	6	12
Informed consent, review inclusion/exclusion criteria	x			
Personal interveniw, medical history, MSSE	x			
Monitor vital signs	x	x	x	x
Physical exam, Long-distance corridor walk	x			
Randomization		x		
Late-Life Disability Questionnaire, SPPB		x		x
Blood collection		x	x	x
Muscle function, 6-min walk		x	x	x
Muscle biopsy[a]		x		x
Collect home physical activity data		x		x
Dispensing, pill counts, assess adverse experiences		x	x	x

[a] Baseline biopsy procedure performed at separate visit.

2.5.2. Lower-extremity function

The SPPB is based on a timed short distance walk (4 m), repeated chair stands and balance test. This test is reliable [35] and valid for predicting institutionalization, hospital admission, mortality and disability [36–39]. Each task is scored by the research coordinator from 0 to 4, with 4 indicating best level of performance and 0 the inability to complete the test. A summary score (0–12) is then calculated.

2.5.3. Exercise capacity

Exercise capacity of participants is assessed using the six-minute (6-min) walk test, a safe and reliable test of aerobic endurance in older persons and those with cardiovascular conditions [40,41]. This test has strong reproducibility, with intra-subject coefficients of variation averaging < 10%, and has a modest correlation with peak VO_2 [42]. Participants are asked to walk as far and fast as possible for 6-min on a 60 m track.

2.5.4. Skeletal muscle strength

Knee extensor strength is a key determinant of physical function among older adults [43]. Unilateral isokinetic strength of the knee extensors of the dominant limb is assessed by dynamometer

(Biodex Medical Systems, New York, NY) as published previously [30,44,44].

2.5.5. Self-assessed physical function

Self-assessed functional status is documented using the Late Life Function and Disability Instrument [45,46]. The instrument includes 16 tasks representing a broad range of disability indicators that assesses both frequency of doing a task and perceived limitation. The instrument uses a scale from 0 to 100, with higher scores indicating higher levels of function. The scale has strong concurrent and predictive validity with physical performance [29].

2.5.6. Mitochondrial outcome measures

Mitochondrial respiration is determined immediately on freshly saponin-permeabilized muscle fibers as published previously [5] using the high-resolution Oxygraph-2k (Oroboros, Innsbruck, Austria). Skeletal muscle expression PGC-1α, mitochondrial DNA content, as well as citrate synthase (CS) and cytochrome C oxidase (COX) activities are determined using well-standardized, validated methods with excellent test re-test reliability [47]. Other mitochondrial measures will be considered as tissue availability allows.

2.6. Interventions

2.6.1. Exercise intervention

Each study arm employs a 2 days/week, center-based exercise intervention. Each session begins with a brief warm-up followed by 30 min of walking. According to ACSM/AHA guidelines, [48,49], aerobic exercise intensity is monitored using the Borg CR10 (0–10) scale for physical exertion. Participants are initially instructed to walk at a moderate intensity, equivalent to a 5–6 on the CR10 scale. Participants are encouraged to, as possible, incorporate brief periods of vigorous walking (7–8 on CR10 scale) with a target goal of achieving at least 10 min of vigorous walking per session. Sessions also include 30 min of strength exercises followed by a cool-down period consisting of flexibility and balance exercises. Resistance exercises are performed in a progressive manner using standard isotonic resistance training equipment at intensities between moderate (5–6) and vigorous (7–8), according to published guidelines [49]. Both lower- and upper-body exercises are performed with the primary emphasis on the lower extremity. We have included resistance training because (1) it is a clinically-recommended component of rehabilitation interventions and (2) it also improves skeletal muscle mitochondrial function [50] – even enhancing responses to aerobic exercise [51].

2.6.2. Resveratrol supplementation

Participants are randomly assigned to receive, in a double-masked fashion, either 500 or 1000 mg/day of encapsulated resveratrol or a vegetable cellulose-based placebo (Reserveage Organics, Gainesville, FL). The high dose of 1000 mg/day was chosen based on our group's 12-week resveratrol pilot study demonstrating that this dose of resveratrol was safe for older adults [52]. This dose of resveratrol has also been shown to improve markers of metabolic function (i.e., blood glucose) in overweight, older adults [53]. The low dose of 500 mg/day was previously shown to be sufficient for improving mitochondrial function in healthy, obese men [17] and allows for comparison with published human studies of resveratrol and exercise to date [54,55]. The resveratrol supplement and placebo are provided in two daily 500 mg doses.

2.7. Safety

Numerous safety procedures were put in place to ensure participant safety. For instance, potential adverse events for study related activities and interventions are explained to each participant by trained study personnel during the informed consent process. Participants are encouraged to notify study staff immediately if they have any adverse experiences that could be related to the study interventions. Adverse experiences are monitored by study coordinators in a masked fashion at each study visit and as reported. Interventionists also monitor adverse events as they are reported as well as any potential events that occur during performance of the exercise intervention. Clinical blood tests (i.e. CBC, metabolic panel, coagulation markers) are performed at each clinic assessment visit and are utilized to monitor potential hematologic and metabolic abnormalities in response to the interventions (Table 1).

2.8. Statistical analyses

The primary analysis will follow an “intent-to-treat” model in which participants are grouped according to randomization assignment. Because this study is a pilot, caution will be taken in the interpretation of hypothesis tests – as the relatively small sample size may create an imbalance in pre-randomization covariates [56]. These factors will be considered in the interpretation and presentation of study findings. Mixed effects models will be used to determine the effects of the interventions on study outcomes. Differences in mean outcome measures between intervention groups will be estimated with the baseline outcome measure, age, gender, visit, and the intervention group assignment included in the model. Hypothesis tests for intervention effects at assessment visits will be performed using contrasts of the 6- and 12-week intervention means. Overall comparisons between groups for the outcome measure across follow-up visits will be obtained using a contrast to compare average effects across follow-up visits. Corrections for comparison of multiple outcomes will not be utilized given the pilot nature of the study. Baseline characteristics of participants who do and do not have follow-up measures will be compared to determine if there were any differences among these groups. Maximum likelihood will be used to obtain tests for fixed effects. It will account for the possibility that missing outcomes are dependent on observed covariates or previously observed outcomes [57]. Sensitivity of results to missing outcomes will be investigated through the use of multiple imputation [58] or propensity scores [59].

3. Discussion

Our long-term goal is to develop interventions that optimize the beneficial effects of exercise training on physical function among older adults at risk for becoming disabled. The maintenance of one's physical capabilities during older age is an essential part of healthy aging. Declines in physical abilities are associated with not only the onset of disability and the loss of independence but also with increased rates of morbidity and mortality [1–3]. To date, physical exercise is the only intervention with consistent evidence supporting its use in treating age-related functional limitations. However, despite strong adherence to training, clinically-significant improvements in physical function are not obtained by many older adults [60,61]. Thus exercise may be, as proposed previously,[62] essential but insufficient for maintaining function among many seniors. Alternative strategies are therefore needed to improve the therapeutic efficacy of exercise in physically-limited older adults.

Several pre-clinical studies have recently demonstrated that resveratrol potentiates beneficial physiologic effects of exercise in rodents. Notably, Hart et al. [23] speculated that resveratrol may have enhanced post-training improvements in aerobic capacity by

simultaneously activating different molecular pathways related to mitochondrial function. This hypothesis was subsequently confirmed by Menzies et al. [19] using SIRT1-deficient mice and a cell culture model of differentiated myotubes [19]. This study demonstrated that while resveratrol and exercise each stimulated mitochondrial biogenesis in a SIRT1-independent manner, the combination of resveratrol and exercise demonstrated a SIRT1-dependent synergistic effect.

Importantly, we recently published findings indicating that age-related declines in walking speed are strongly associated with impairments in mitochondrial function and indices of mitochondrial biogenesis [4,5]. Accordingly, we propose to evaluate the efficacy of resveratrol supplementation combined with multi-modal exercise training as an adjuvant to exercise training for improving physical function among older adults at risk for becoming disabled. The study described here will provide important feasibility and early efficacy data regarding the benefits of resveratrol supplementation and exercise in our target population. The study will also enable us to refine the study protocol and procedures prior to conducting a fully-powered trial. As such, it should be noted that the study protocol may be modified during the trial to optimize study procedures (recruitment, safety, etc) based upon information obtained during the trial. Due to this and other limitations (e.g. relatively small sample size; single site design), it will be important not to over-interpret the results of this pilot study. Nonetheless, this study will inform the design of an efficient and definitive full-scale trial to determine if resveratrol potentiates the beneficial effects of exercise training among our target population. Importantly, both aspects of the intervention can be easily disseminated and implemented in clinical practice; thus, if our hypothesis is correct, the combination intervention may hold significant promise for improving physical function and mobility among older adults.

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