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Long-Term Supplementation With Resveratrol Alleviates Oxidative Stress but Does Not Attenuate Sarcopenia in Aged Mice

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This study analyzed the capacity of resveratrol, a naturally occurring polyphenol, to reduce aging-induced oxidative stress and protect against sarcopenia. Middle-aged (18 months) C57/BL6 mice were randomly assigned to receive either a control diet or a diet supplemented with 0.05% trans-resveratrol for 10 months. Young (6 months) and middle-aged (18 months) mice were used as controls. Resveratrol supplementation did not reduce the aging-associated loss of muscle mass or improve maximal isometric force production, but it appeared to preserve fast-twitch fiber contractile function. Resveratrol supplementation did not improve mitochondrial content, the subcellular localization of cytochrome c protein content, or PGC1 protein content. Resveratrol increased manganese superoxide dismutase (MnSOD), reduced hydrogen peroxide, and lipid peroxidation levels in muscle samples, but it was unable to significantly reduce protein carbonyl levels. The data suggest that resveratrol has a protective effect against aging-induced oxidative stress in skeletal muscle, likely through the upregulation of MnSOD activity, but sarcopenia was not attenuated by resveratrol.

Key Words: Muscle atrophy—Oxidative stress—Sarcopenia—Mitochondria.

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ADVANCED age leads to sarcopenia, which is the loss of muscle mass and function (1). The causative factors of sarcopenia are multifactorial and include a progressive denervation of muscle fibers (2), an altered hormonal milieu (3), and a net loss of contractile proteins (4). Although no causal relationship has been established, skeletal muscle atrophy is often associated with an increased production of reactive oxygen species (ROS) (5), leading to an amplified oxidant load. An increase in oxidant exposure, in conjunction with the less effective endogenous antioxidant systems often present in aged animals (6), can lead to oxidative stress. Chronic oxidative stress left unchecked over time leads to the oxidation, and thus damage, of cellular macromolecules, including lipids (7), nucleic acids (8), and proteins (9). This progressive and cyclical oxidative assault on cellular organelles is believed to be an important contributor to the aging process (8) and is thought to be responsible for many of the pathologies associated with aging, including genomic instability, mitochondrial dysfunction, and chronic inflammation (8), all of which are associated with sarcopenia (10).

Supplementation with the naturally occurring phytoalexin, resveratrol (3,4',5-trihydroxystilbene), has recently been shown to protect against oxidative stress in rodent skeletal muscle (11,12) and to act as a systemic anti-inflammatory in vivo (13). Resveratrol protects against mitochondrial oxidative stress (14) and furthermore inhibits tumor necrosis factor–induced activation of NADPH oxidase subsequently rescuing endothelial function in diabetic mice (15). In skeletal muscle, resveratrol has been shown to upregulate components of the endogenous antioxidant system (11,12), reduce the oxidant load within the muscle environment, and consequently attenuate oxidative damage associated with muscle loading (12), unloading (11), and aging (11,12). Furthermore, resveratrol administration was able to significantly mitigate muscle atrophy in a mouse model of cachexia (16). Resveratrol's ability to directly scavenge free radicals resides in its phenol ring, and resveratrol has repeatedly been shown to be a potent scavenger of ROS in numerous cell types exposed to oxidants (17,18). However, its rapid first pass metabolism in mammals and thus relatively low bioavailability (19) may limit its role as a direct ROS scavenger in vivo. Consequently, in vivo, resveratrol's various health benefits can more likely be attributed to its ability to activate the NAD^+-dependent deacetylase, silent mating type information regulation homolog1, or sirtuin1 (Sirt1) (20).

Sirt1 has been shown to play a role in a variety of important physiological functions including inflammation (13), longevity (21), mitochondrial biogenesis (20), and the regulation of oxidative stress and antioxidant enzymes (21,22). Sirt1 is also activated with exercise (23) and under

conditions of nutrient deprivation (24), underscoring resveratrol's effectiveness as a caloric restriction mimetic. This is significant because both exercise (25) and caloric restriction (26,27) are proven countermeasures to combat both oxidative stress and sarcopenia. Furthermore, Sirt1 is a powerful positive mediator of peroxisome proliferator–activated receptor gamma coactivator 1 (PGC1α), which is considered to be the master regulator of mitochondrial function and biogenesis (28). PGC1α is decreased during atrophic conditions (29); however, muscle-specific overexpression of PGC1α confers protection against both denervation and fasting-induced skeletal muscle atrophy (29). Increasing PGC1 signaling has the potential to alleviate age-related mitochondrial alterations, which underlie a wide variety of diseases such as diabetes (30), neurodegeneration (31), and sarcopenia (10).

Although mitochondria are not the sole source of ROS within a cell, they are routinely viewed as the principle site of superoxide generation, which is the primary source of damaging ROS within muscle cells (32). Additionally, muscle mitochondria density is reduced with increased age (33), and the remaining mitochondria are known to undergo structural and functional alterations in skeletal muscles from aged animals (34,35). These changes occur concomitant to increases in oxidative stress (36). Resveratrol's ability to increase PGC1 signaling, via Sirt1 activation, could potentially provide a twofold mechanism with which to protect aged skeletal muscle against oxidative stress. First, resveratrol has been shown to act as a direct and indirect antioxidant, diminishing the likelihood of phospholipid oxidation and thus damage within the mitochondrial membrane preserving the integrity of the membranes and thus preventing "leaky" mitochondria. In addition, resveratrol has been shown to promote mitochondrial biogenesis and decrease mitochondrial oxidative stress in a Sirt1-dependent fashion (14,20), thus potentiating the possibility to replace damaged mitochondria in aged animals.

Acute resveratrol administration has been recently shown to reduce oxidative stress and improve functional outcomes in rodent skeletal muscle (11,12); however, the efficacy of chronic resveratrol supplementation as a countermeasure to combat sarcopenia has not been established. Thus, the purpose of the current investigation was to determine if long-term dietary supplementation, from middle age (18 months) through senescence, with moderate doses of resveratrol would effectively attenuate the loss of muscle mass and function that occurs in aged animals. It was hypothesized that resveratrol's ability to activate Sirt1 would consequently enhance the endogenous antioxidant system and increase PGC1 signaling, thereby reduce the release of ROS from mitochondrion. Furthermore, it was hypothesized that resveratrol supplementation would preserve muscle function in aged animals both by preservation of muscle mass and by providing a more favorable redox environment.

Methods

Experimental Protocol

Experiments were conducted on young adult (3 months), middle-aged (18 months), and aged (28 mo) C57BL/6 mice obtained from the National Institute on Aging colony (Harlan, Indianapolis, IN). The mice were housed in pathogen-free conditions at ~20°C. All mice had free access to food and water. Mice that were 18 months of age (middle aged) were put on either a control diet of normal mouse chow (AIN-76A Rodent Diet; Research Diets Inc., New Brunswick, NJ) or an identical diet containing 0.05% resveratrol (Research Diets Inc.). The dietary dosage was chosen to represent a low-to-moderate daily intake of resveratrol. Resveratrol was purchased from Orchid Pharmaceuticals (India). Mice that were 3 months and 18 months of age were used as control animals only. Aged animals were sacrificed at 28 months, after 10 months of being on either the control or resveratrol-supplemented diet. Immediately following sacrifice, skeletal muscles were dissected for use in biochemical analyses, mitochondrial isolation, and/or physiological analyses. All experimental procedures carried approval from the Institutional Animal Use and Care Committee from West Virginia University School of Medicine.

Ex Vitro Muscle Physiological Analysis

Isometric muscle contractile properties were examined in the plantaris muscles of control and resveratrol-treated mice. The muscles were placed in an oxygenated ringers solution (137 mM NaCl, 4.7 mM KCl, 3.4 mM $CaCl_2$, 1.2 mM $MgSO_4$ 1 NaH_2PO_4, and 112 D-glucose). The Ringer solution was aerated with 95% O_2 and 5% CO (pH 7.4). The temperature of the solution was kept at 20°C. The distal end of the muscle was attached to a stationary Plexiglass plate, and the proximal end was fixed to the lever arm of a 300C dynamometer (Aurora Scientific, Aurora, Canada). The muscles were stimulated by passing a constant current through platinum plates that were positioned on either side of the muscle. Ex vivo isometric twitch and tetanic contractions were obtained using a Constant Current/Constant Voltage Stimulator (Aurora Scientific) that provided DC-square wave signals at stimulation current of 12 V, with a 200 μs pulse width. Muscles were adjusted to the optimal muscle length (L_o) by a micromanipulator that controlled the base position of the electrode clamp. L_o was established as the muscle length that produced maximal isometric twitch tension. L_o was periodically checked by the same procedure throughout each experiment. Force-frequency isometric force records were obtained by stimulating the muscle at 10, 20, 40, 50, 75, and 100 Hz, with 3 minutes of rest between each contraction. Physiological contractile measures included peak isometric twitch force (PT), time to peak twitch contraction time (CT), ½ relaxation time of twitch contraction (½ RT), and peak isometric tetanic force

(Po), as previously described (37). Following isometric contractions, the muscles remained in oxygenated Ringers for 5 minutes prior to the repeated stimulation fatigue protocol. Muscle fatigue was assessed by stimulating the muscle at 40Hz for 3 minutes with a duty cycle of 330 ms of stimulation followed by 660 ms of rest (38). The fatigue index was calculated as the difference in force from the average of the first three contractions to the average of the final three contractions. The contractile and fatigue measurements were analyzed off line using commercial software (DMI; Aurora Scientific).

Protein Isolation

Approximately 40 mg of gastrocnemius muscle from each animal was homogenized in 500 μL of RIPA buffer (1% Triton X-100, 150 mM NaCl, 5 mM EDTA, 10 mM Tris; pH 7.4) using a mechanical homogenizer, for use in the assessment of protein expression via immunoblotting. Muscle homogenates were then centrifuged at 1,000*g* for 5 minutes at 4°C. The resulting supernatant was collected and divided into two portions and frozen at −80°C either with or without a protease inhibitor cocktail containing 104 mM 4-[2-aminoethyl]-benzenesulfonyl fluoride hydrochloride, 0.8 mM aprotinin, 2 mM leupeptin, 4 mM bestatin, 1.5 mM pepstatin A, and 1.4 mM E-64 (Sigma-Aldrich, St. Louis, MO) added to it.

For all enzyme activity assays, and/or oxidative damage assessments, muscle samples were homogenized in the appropriate volume of either phosphate-buffered saline or the kit-specific buffer provide by the manufacturer. Protein concentrations for each sample were determined in duplicate using the DC Protein Assay Kit (Bio-Rad, Hercules, CA).

Mitochondrial Isolation

The vastus lateralis muscles were carefully removed while the mice remained under deep anesthesia (5% isoflurane/95% oxygen). Precautions were made to assure that the blood supply to the muscles remained intact until it was removed to prevent the artificial accumulation of oxidants. Mitochondria and mitochondria-free cytosolic muscle fractions were obtained using a commercially available mitochondrial isolation kit specifically designed for animal tissue (MITOISO1-1KT; Sigma-Aldrich). The fractions were obtained using sequential separation steps involving a protease digestion followed by separation of the fractions via centrifugation using slight modifications of the manufacture's recommendations. Briefly, the gastrocnemius muscle was placed on ice and minced in a 1.5-mL Eppendorf tube. Samples were washed and resuspended in an extraction buffer containing 0.25 mg/mL trypsin. After a 20-minute incubation period, albumin was added to a final concentration of 10 mg/mL to quench the proteolytic reaction. Samples were then washed and resuspended in extraction buffer and then gently homogenized with a Teflon pestle. The homogenate was then centrifuged at 600*g* for 5 minutes. The supernatant was transferred to a new tube and centrifuged at 11,000*g* for 10 minutes. This was repeated and the supernatant was centrifuged for 10 minutes at 11,000*g* and transferred to a new tube to assure a clean mitochondrial-free cytosolic fraction. The mitochondrial pellet was suspended in a sucrose storage buffer.

Manganese Superoxide Dismutase and Copper-Zinc Superoxide Dismutase Enzyme Activity Levels

Superoxide dismutase activity was measured in vastus lateralis muscle homogenate that was partitioned into a mitochondrial pellet and mitochondrial-free cytosolic fraction. Enzymatic activity was assessed using a colorimetric enzyme activity kit (Cayman Chemical Company, Ann Arbor, MI) following the manufacturer's guidelines. Copper-zinc superoxide dismutase (CuZnSOD) activity was obtained from the cytosolic fractions, and manganese superoxide dismutase (MnSOD) activity was measured using isolated mitochondria. All samples and standards were measured in duplicate. The assay was performed in a 96-well plate, and mitochondrial samples were treated with 10 μL of 12 mM potassium cyanide to inhibit any residual CuZnSOD activity. The absorbance of the resulting colorimetric changes was measured at a wavelength of 450 nm using a 96-well plate reader (Dynex Tech., Chantilly, VA). The samples were normalized to the protein concentration in each sample as assessed using a DC protein concentration assay (Bio-Rad).

Immunoblots

The protein content of CuZnSOD and MnSOD were measured in vastus lateralis total muscle homogenate. Cytochrome c protein content was measured in the cytosolic (mitochondrial-free) fraction of the vastus lateralis muscle, the corresponding mitochondrial fractions and total muscle homogenate. The protein content of Sirt1 and PGC1 were measured in gastrocnemius total muscle homogenates. β-Tubulin was used as a loading control for cytosolic fractions and total homogenate. A Ponceau stain was used to validate equal loading of the mitochondrial fraction. Thirty to 40 μg of protein were loaded into each well of a 4%–12% gradient polyacrylamide gel (Invitrogen, Carlsbad, CA) and separated by routine sodium dodecyl sulfate–polyacrylamide gel electrophoresis for approximately 1.5 hours at 20°C, followed by transfer to a nitrocellulose membrane for 70–120 minutes at 35 V. All membranes were blocked in 5% nonfat milk for 1 hour at room temperature. Membranes were then incubated in the appropriate primary antibodies, overnight at 4°C. Primary antibodies were diluted in Tris-buffered saline, with 0.1% Tween-20 (TBST) and 0.1% sodium azide. Membranes were then washed in 0.05% TBST followed by incubation in the appropriate dilutions of secondary antibodies (diluted in 5% nonfat milk in TBST) conjugated to horseradish peroxidase. Signals were developed

using a chemiluminescent substrate (Lumigen TMA-6; Lumigen, Southfield, MI) and visualized by exposing the membranes to X-ray films (BioMax MS-1; Eastman Kodak). Digital records were captured by a Kodak 290 camera, and protein bands were quantified using 1D analysis software (Eastman Kodak). Bands were quantified as optical density × band area and expressed in arbitrary units.

Sirt1 Activity

Sirt1 activity was measured using components of the Sirt1 Fluorimetric Drug Discovery Kit (AK -555; BIOMOL, Plymouth Meeting, PA). In short, endogenous Sirt1 activity was measured in total gastrocnemius muscle homogenates homogenized in ice-cold phosphate-buffered saline (pH 8.0) using the fluorescent deacetylase substrate and developer provided in the kit. Following homogenization, each sample was quantified using a DC protein concentration assay (Bio-Rad) and diluted to 2.5 μg/μL using the reagents supplied by the manufacturer. The fluorescent substrate in conjunction with 100 μM of the cosubstrate NAD^+ was incubated with 25 μL of each sample for 30 minutes at room temperature in a ½ volume 96-well white microplate. Following this incubation, 2mM nicotinamide (Sirt1 inhibitor) and the provided fluorescent developer were added to each well to stop the reaction and produce a fluorophore that is linearly related to Sirt1 activity. Resveratrol and Suramin were combined with the provided recombinant Sirt1 and were used as positive and negative controls, respectively. Additionally, a mouse liver homogenate was used as an additional positive control. The intensity of the fluorescent signal was detected using an excitation wavelength of 360 nm and an emission wavelength of 460 nm. Data are presented as fluorescent units normalized to the milligrams of protein present in each homogenate.

Citrate Synthase Activity

Citrate synthase activity was measured in gastrocnemius whole-tissue extracts using a commercially available kit (CS0720; Sigma-Aldrich). Citrate synthase activity has been extensively used as a marker of mitochondrial mass (20,39) and is a suitable marker for use in whole-muscle homogenate given that it is located in the mitochondrial matrix; the enzyme's activity would be minimally affected by the homogenization procedure. Muscle samples were homogenized in ice-cold CelLytic MT Cell Lysis Reagent (C3228), and the resultant homogenate was centrifuged at 12,000*g* for 10 minutes to remove cellular debris. The resulting supernatant was used for the kinetic assessment of citrate synthase activity. The assay was performed in a 96-well plate as per manufactures instructions. Briefly, 8 μL of each muscle homogenate was added to a master mix containing the supplied assay buffer, 30 mM acetyl CoA solution and 10 mM 5,5'-Dithiobis (2-nitrobenzoic acid) solution. The reaction was initiated with the addition of 10 μL of oxaloacetic acid, and the formation of citric acid was determined spectrophotometrically at a wavelength of 412 nm at an interval of 90 seconds for 10 minutes. Net citrate synthase activity was calculated as the endogenous citrate synthase activity subtracted from total activity. All analyses were measured in duplicate, and the samples were normalized to micrograms of protein per microliter of muscle homogenate as determined by the DC Protein Assay Kit (Bio-Rad).

Hydrogen Peroxide Content

Hydrogen peroxide (H_2O_2) content in gastrocnemius total muscle homogenate was measured using a fluorescent assay according to the manufacture's recommendations (Cell Technology, Mountain View, CA). Briefly, 50 μL of control, unknown muscle samples, or H_2O_2 standards were mixed with 50 μL of the reaction cocktail provided in the kit and added to each well to initiate the reaction. The plate was then incubated in the dark for 10 minutes at room temperature. The intensity of the fluorescent signal was detected using an excitation wavelength of 530 nm and an emission wavelength of 590 nm. A linear regression was performed by plotting the resultant florescent intensities from the known standards and subsequently the unknown samples were fit to the corresponding linear equation. All analyses were measured in duplicate, and the samples were normalized to micrograms of protein per microliter of total muscle homogenate as determined by the DC Protein Assay Kit (Bio-Rad).

Lipid Peroxidation

Malondialdehyde (MDA) and 4-hydroxyalkenals (HAE) were measured using the methods and reagents from Percipio Biosciences, Inc., (BIOXYTECH LPO-586; Foster City, CA). Approximately 50 mg of gastrocnemius muscle was homogenized in 750 μL of ice-cold buffer containing phosphate-buffered saline (20 mM, pH 7.4) and 5 μL of 0.5 M butylated hydroxytoluene in acetonitrile per 1 mL of tissue homogenate. Assay reagents were added following the manufacturer's recommendations and have been previously described by our laboratory (12). Briefly, the muscle homogenate was centrifuged at 3000*g* at 4°C for 10 minutes, and the supernatant was carefully collected and used to quantify lipid peroxidation. The supernatant was subsequently incubated in the proper reagents at 45°C for 60 minutes, as per the manufactures instructions, and then centrifuged at 15,000*g* for 10 minutes. The absorbance of the resulting sample was read at 586 nm. All analyses were measured in duplicate, and the samples were normalized to micrograms of protein per microliter of total gastrocnemius muscle homogenate as determined by the DC Protein Assay Kit (Bio-Rad).

Protein Carbonyls

A colorimetric protein carbonyl assay (#10005020; Cayman Chemical Company) was used to determine the level of

Table 1. Descriptive Data

Descriptive Data	Young Control	Middle-Aged Control	Aged Control	Aged Resveratrol
BW (g)	26.5 ± 2.2	34.3 ± 2.3*	33.8 ± 3.9*	35.2 ± 2.3*
Gastrocnemius muscle wet weight (g)	0.141 ± 0.02	0.150 ± 0.01	0.126 ± 0.01*	0.130 ± 0.01*
Gastrocnemius muscle wet weight (g)/BW (g)	5.34 ± 0.59	4.26 ± 0.28*	3.74 ± .33*	3.71 ± 0.25*
Plantaris muscle wet weight (g)	0.020 ± 0.001	0.023 ± 0.004	0.180 ± 0.002*	0.019 ± 0.003
Plantaris muscle wet weight/BW (g)	0.80 ± 0.03	0.66 ± 0.04*	0.54 ± 0.02*	0.55 ± 0.02*
Food intake (g/day)	3.4 ± 0.18	3.8 ± 0.39	3.1 ± 0.27	3.2 ± 0.14
Resveratrol (g/kg/day)	n/a	60.8 ± 4.0	n/a	45.9 ± 3.9*

Notes: Animals were weighed prior to sacrifice, body weights (BW) are represented in grams. Gastrocnemius and plantaris muscles were dissected, immediately blotted, and weighed as an estimate of muscle size. The data are presented in grams or as ratio to the animal's body weight (mg/g). Food consumption was assessed for each experimental group and is reported as grams of food per day. Resveratrol was fed to aged mice for 10 months in a rodent chow that contained 0.05% trans-resveratrol. Resveratrol intake is reported as grams per day normalized to kilogram of body weight (g/kg/day). Significance was set at ($p \leq .05$), and all data are represented as mean ± *SE*.

*Denotes significantly different than young animals (aging effect).

protein oxidation in gastrocnemius muscle homogenates. All procedures were carried out according to the manufactures guidelines. In brief, ~50 mg of gastrocnemius muscle was homogenized in ice-cold phosphate-buffered saline (pH 6.7) containing 1 mM EDTA and subsequently centrifuged for 10,000*g* for 15 minutes at 4°C to obtain a clear supernatant. Four hundred microliters of the supernatant was subsequently transferred to two separate tubes, one to be used as a background control and one to be used to asses protein carbonyl formation. 2,4-Dinitrophenylhydrazine was added to the sample tube, and 2.5 M hydrochloric acid was added to each control tube. Both tubes were then incubated in the dark for 1 hour at room temperature. Following this incubation 20% trichloroacetic acid was added to all tubes and then briefly placed on ice. The samples were then exposed to a series of sequential acid incubations, centrifugations, and wash steps as per the manufactures directions. After the final wash, the pellets were resuspended in guanidine hydrochloride and transferred to the wells of a 96-well plate. The absorbance change was then measured at a wavelength of 360 nm using a plate reader. Following the readings, all samples were quantified using a DC protein concentration assay (Bio-Rad), and the results are presented as nanomoles of protein carbonyls normalized to the micrograms of protein present in each sample.

Statistics

Statistical analyses were performed using the SPSS 13.0 software package (SPSS, Chicago, IL). An analysis of variance was used to examine differences between the three age groups and additionally resveratrol supplementation. Bonferroni post hoc analyses were performed between significant means. A p value < .05 was considered statistically significant. Data are reported as mean ± *SEM*.

Results

Descriptive Data

All animals were weighed at the beginning and end of the experimental protocol. The data displayed in Table 1 are indicative of the final body weight of the animals immediately prior to sacrifice. The daily resveratrol dose was 60 mg/kg of body weight in 18-month-old animals and 46 mg/kg in 28-month-old animals. There was no significant difference between the body weights of aged control versus aged resveratrol-supplemented animals. Mice that were 18 and 28 months of age were significantly heavier than young animals that were 6 months of age (34.3 ± 2.3 vs 34.5 ± 3.9 vs 26.5 ± 2.1 g, $p \leq .05$). There was no significant effect of resveratrol administration on body weight. Conversely, both gastrocnemius and plantaris muscle wet weights were significantly greater in young animals compared with aged and middle-aged animals. The difference was even more prominent when muscle wet weights were normalized to their body weights, (Table 1). These results are indicative of sarcopenia; however, resveratrol showed no protective effect with regard to maintaining muscle mass during aging in either the plantaris or gastrocnemius muscles.

Silent Mating Type Information Regulation Homolog1 (Sirt1) Enzyme Activity and Protein Content.

Surprisingly, Sirt1 activity was 25% greater ($p \leq .05$) in gastrocnemius muscles from aged animals compared with their young counterparts. Gastrocnemius muscles from resveratrol-supplemented animals had a modest but additional increase in Sirt1 activity (2867 ± 108 vs 3243 ± 107 arbitrary fluorescent units/μg, $p \leq .05$; Figure 1A). Sirt1 protein content, as measured by immunoblotting, was similarly greater in muscles from aged animals, although to a much larger extent than the activity levels. Muscles from aged animals showed more than a twofold greater Sirt1 protein content compared with muscles from young animals. Resveratrol supplementation did not significantly increase Sirt1 protein content in muscles of animals that were 18 months of age, although the results approached statistical significance ($p = .063$; Figure 1B).

Peroxisome Proliferator–Activated Receptor Gamma Coactivator 1 (PGC1) Protein Content and Citrate Synthase Enzyme Activity

PGC1 protein content and citrate synthase activity were measured as indirect markers of oxidative metabolism and mitochondrial content, respectively. PGC1 protein content

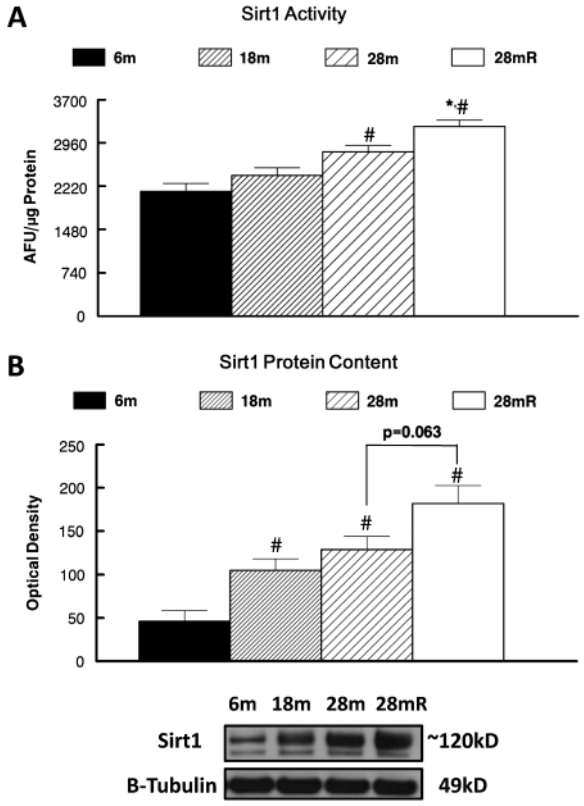

Figure 1. Silent mating type information regulation homolog1 (Sirt1) enzyme activity and protein content. (**A**) Sirt1 protein content was measured via immunoblotting in total gastrocnemius muscle homogenate. (**B**) Sirt1 enzyme activity was determined fluorometrically in gastrocnemius muscle homogenate. Data are expressed as arbitrary fluorescent units (AFU)/µg protein. 6 months = young control, 18 months = middle-aged control, 28 months = aged control, 28mR = aged resveratrol. Significance was set at $p \leq .05$, and all data are presented as mean ± *SE*. #$p \leq .05$, young control (aging effect). *$p \leq .05$, aged control versus age resveratrol (supplementation effect).

measured in total gastrocnemius muscle homogenate remained unchanged regardless of age or resveratrol supplementation (Figure 2A). Citrate synthase activity was ~20% lower in muscles from aged animals ($p \leq .05$), but resveratrol supplementation had no effect on the enzyme's activity (Figure 2B).

Subcellular Localization of Cytochrome c Protein

Cytochrome c protein content was measured, via immunoblotting, in the total, cytosolic (mitochondrial free), and mitochondrial fractions of vastus lateralis muscles as an estimation of both mitochondrial content and mitochondrial membrane permeability. Cytochrome c protein content in both the total homogenate and the mitochondrial fractions remained unchanged regardless of age or resveratrol supplementation (Figure 3A). Cytochrome c present in the mitochondrial-free cytosolic fraction was ~10-fold greater in muscles from both mice that were 18 months of age and 28 months of age, regardless of resveratrol supplementation ($p \leq .05$), suggesting increased mitochondrial membrane permeability and perhaps an increased likelihood of mitochondrial dysfunction present in mice aged 18 months of age and 28 months of age (Figure 3A).

SOD Enzyme Activity and Protein Content

Superoxide dismutase activity was measured in vastus lateralis muscles fractioned into mitochondrial and cytosolic (mitochondrial-free) fractions. MnSOD activity was assessed in isolated mitochondria, and CuZnSOD activity was measured in the corresponding mitochondrial-free cytosolic faction. Total homogenates from the vastus lateralis muscle was used to determine the protein content of each SOD isoform. MnSOD activity was similar in mitochondria isolated from vastus lateralis muscles from mice that were 18 months of age relative to young mice that were 6 months of age. Resveratrol supplementation increased MnSOD activity in muscles from animals that were 28 months of age by an additional ~40% ($p \leq .05$; Figure 4A). There was no

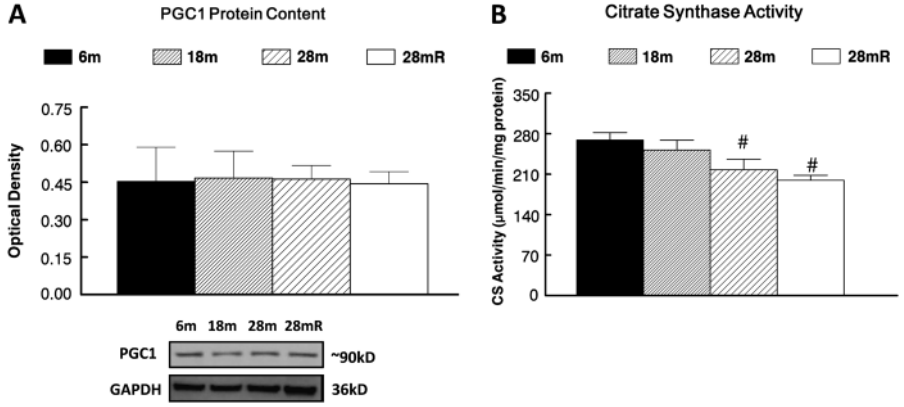

Figure 2. PGC1 protein content and citrate synthase enzyme activity. (**A**) PGC1 protein content was measured via immunoblotting in gastrocnemius muscle homogenate. (**B**) Citrate synthase enzyme activity was measured kinetically in homogenates from gastrocnemius muscles. Data are expressed as micromoles per minute per microgram of protein. 6m = 6 months old, young adult control; 18m = 18 months of age, control; 28m = 28 months of age, control; 28mR = 28 months of age, resveratrol treated. Significance was set at $p \leq .05$, and all data are represented as mean ± *SE*. #$p \leq .05$, young control (aging effect). *$p \leq .05$, aged control versus age resveratrol (supplementation effect).

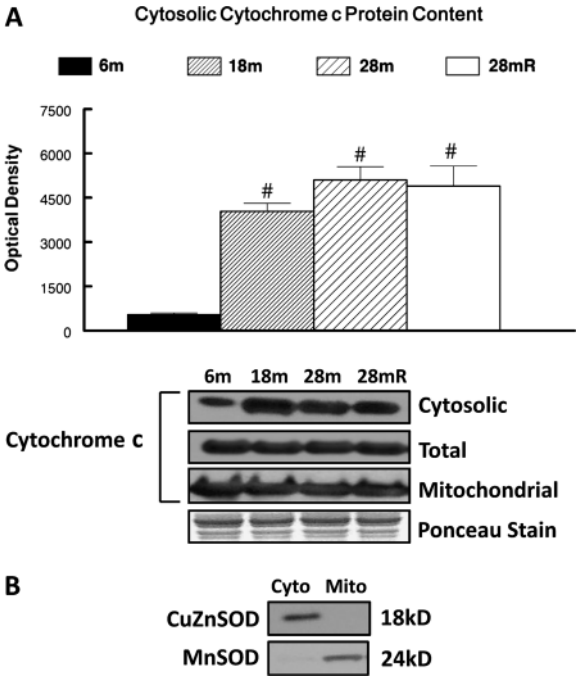

Figure 3. Subcellular cytochrome c protein content. (**A**) Cytochrome c protein content was measured, via immunoblotting, in the total, cytosolic (mitochondrial-free) and mitochondrial fractions of vastus lateralis muscles as an estimate of both mitochondrial content and mitochondrial membrane integrity. (**B**) Immunoblots of the mitochondrial form of SOD (MnSOD) and the cytosolic form of SOD (CuZnSOD) to illustrate the purity of the tissue fractions. 6m = 6 month old, young adult control; 18m = 18 months of age, control; 28m = 28 months of age, control; 28mR = 28 months of age, resveratrol treated. Significance was set at $p \leq .05$, and all data are represented as mean ± *SE*. #$p \leq .05$, young control (aging effect). *$p \leq .05$, aged control versus age resveratrol (supplementation effect).

aging or supplementation effect on MnSOD protein content (Figure 4B). CuZnSOD activity was significantly increased in gastrocnemius muscles from 28-month-old animals compared with gastrocnemius muscles from young 6-month-old animals (121 ± 6.3 vs 73.8 ± 6.6 U/mL/mg, $p \leq .05$). Resveratrol supplementation significantly decreased CuZnSOD activity in animals that were 28 months of age, by 33% ($p \leq .05$), reducing the enzyme activity level to that of young animals (Figure 4C). There was no change in CuZnSOD protein content with resveratrol supplementation; however, aging significantly increased CuZnSOD protein content in both animals aged 18 and 28 months of age compared with their young counterparts that were 6 months of age ($p \leq .05$; Figure 4D).

Hydrogen Peroxide (H_2O_2) Concentrations

H_2O_2 was assessed in gastrocnemius muscle homogenates as an indicator of oxidant load during aging. H_2O_2 levels were ~80% higher in muscle homogenates from gastrocnemius muscles from animals aged 28 and 18 months of age when compared with gastrocnemius muscle homogenates from young animals (1.88 ± 0.11 vs 1.80 ± 0.25 vs 1.05 ± 0.12 μmols H_2O_2/μg protein, $p \leq .05$). Resveratrol supplementation significantly reduced H_2O_2 levels in gastrocnemius muscle homogenates from 28-month-old mice compared with gastrocnemius muscle homogenates from 28-month-old control animals (1.52 ± 0.10 vs 1.88 ± 0.11 μmols H_2O_2/μg protein, $p \leq .05$). This represented a 24% ($p \leq .05$) decrease in H_2O_2 concentrations of muscle homogenates from resveratrol-supplemented animals, but the muscle homogenates from aged resveratrol animals still had significantly higher levels of H_2O_2 when compared with young muscle homogenates (Figure 5A).

Lipid Peroxidation

Malondialdehyde (MDA) and 4-hydroxyalkenals (HAE) were assessed in whole-muscle homogenates as indicators of oxidative damage, specifically as markers of muscle lipid peroxidation. Lipid peroxidation was increased in aged animals that were 28 months old as compared with their young counterparts that were 6 months of age (0.25 ± 0.04 vs 0.44 ± 0.05 μM [MDA/HAE]/mg protein, $p \leq .05$). Muscles from animals that were 18 months of age did not show elevated markers of lipid peroxidation when compared with muscles from young animals. Resveratrol supplementation was able to partially attenuate the increase in lipid peroxidation that occurred due to aging. Gastrocnemius muscles from resveratrol-supplemented animals that were 28 months of age had a 20% reduction in MDA and HAE levels compared with control animals that were 28 months of age (0.35 ± 0.02 vs 0.44 ± .05 μM [MDA/HAE]/mg protein, $p \leq .05$; Figure 5B).

Protein Oxidation

Protein carbonyl formation was measured in gastrocnemius muscle homogenates as an indicator of protein oxidation caused by oxidative stress. Muscles from mice that were 28 and 18 months of age showed significantly greater concentrations of protein carbonyl formation compared with muscles from their young counterparts (7.43 ± 1.3 vs 7.10 ± 1.2 vs 4.63 ± 0.67 nmol/mL/μg, $p \leq .05$). Resveratrol supplementation was unable to mitigate protein oxidation in 28-month-old animals, as measured by carbonyl formation (Figure 5C).

Ex Vitro Physiological Analyses

Isometric muscle contractile properties were examined in the plantaris muscles of control and resveratrol-treated mice (Figure 6). The force-frequency curve was shifted significantly rightward in resveratrol-supplemented aged mice compared with aged control mice (Figure 6A). This rightward shift is indicative of a faster muscle phenotype. These results were completely unexpected given that previous literature has shown that dietary resveratrol supplementation improves muscular endurance (20) and increases mitochondrial biogenesis in skeletal muscle (20,40), which would likely be

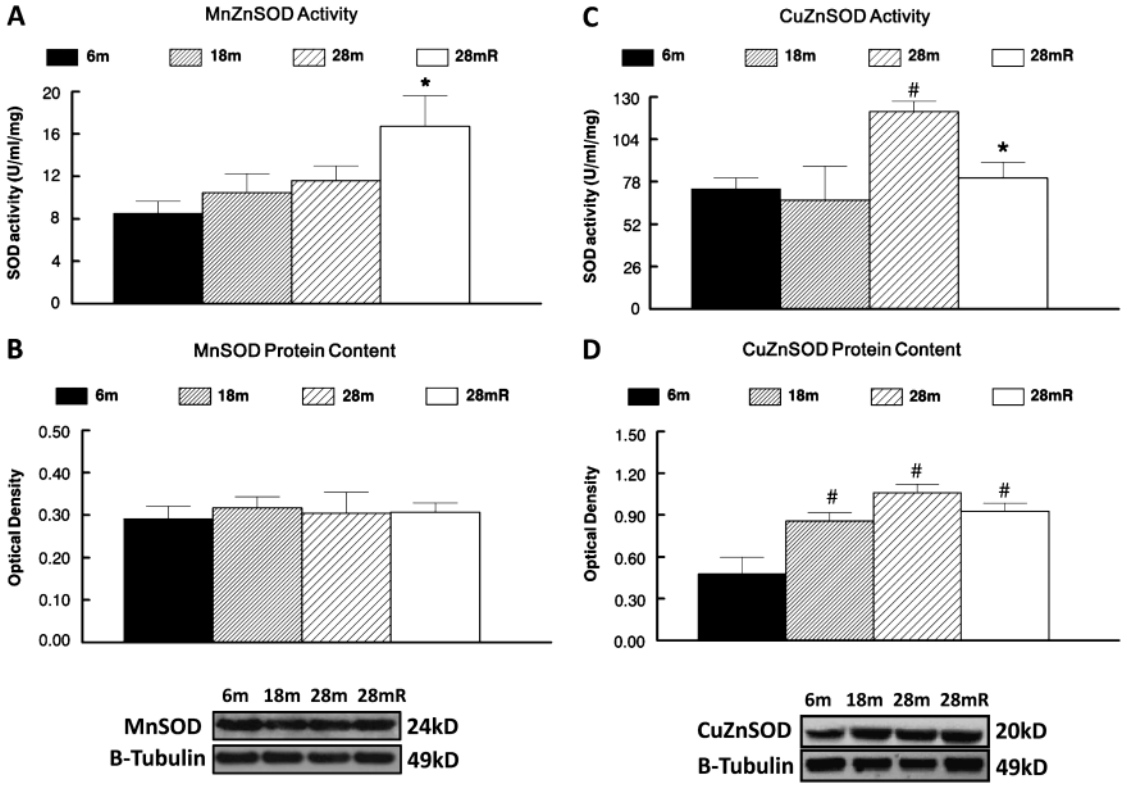

Figure 4. Isoform-specific superoxide dismutase activity and protein content. (**A**) MnSOD activity was assessed colorimetrically in mitochondria isolated from vastus lateralis muscles. Data are expressed as units per milliliter per microgram of protein. (**B**) MnSOD protein content was analyzed via immunoblotting in isolated mitochondria of vastus lateralis muscles. (**C**) CuZnSOD activity was assessed colorimetrically in the mitochondria-free cytosolic fraction from vastus lateralis muscles. Data are expressed as units per milliliter per microgram of protein. (**D**) CuZnSOD protein content was analyzed via immunoblotting in the mitochondria-free cytosolic fraction of vastus lateralis muscles. 6m = 6 month old, young adult control; 18m = 18 months of age, control; 28m = 28 months of age, control; 28mR = 28 months of age, resveratrol treated. Significance was set at $p \leq .05$, and all data are represented as mean ± *SE*. #$p \leq .05$, young control (aging effect). *$p \leq .05$, aged control versus age resveratrol (supplementation effect).

consistent with a shift in the force-frequency curve leftward to represent a slower muscle phenotype. Additionally, CT and ½ RT were analyzed to determine if resveratrol had any effect on the twitch properties of plantaris muscles (Figure 6B). Plantaris muscles from animals that were 18 months of age had significantly shorter contraction times than did muscles from control animals that were 28 months of age (32.8 ± 2.1 vs 39.6 ± 1.4 ms, $p \leq .05$). Similarly, muscles from resveratrol-supplemented animals also showed increased contraction times compared with 28-month-old control animals (34.7 ± 0.8 vs 39.6 ± 1.4 ms, $p \leq .05$), eliminating any aging effect between middle-aged (18 months) and aged (28 months) resveratrol-supplemented animals. These data are consistent with the rightward shift in the force-frequency curve of muscles from resveratrol-supplemented animals (Figure 6A). No differences were found in the ½ RT (Figure 6B) or the twitch to tetanus ratio (P_t/P_o) of plantaris muscles with respect to age or resveratrol supplementation (Figure 6C). Lastly, a modified Burke protocol (41) was implemented to assess muscle fatigue in plantaris muscles. The fatigue protocol consisted of 3 minutes of 120 electrically evoked contractions at 40 Hz. Each contraction was for 1.0 seconds with a 33.3% duty cycle. The pulse duration was 200 μs. There was no difference between the fatigue index of mice that were 18 months of age and 28 months of age, and furthermore, resveratrol was unable to improve fatigue resistance in plantaris muscles from either age group (Figure 6D).

Discussion

Oxidative stress is believed to be a common underlying mechanism potentiating many of the factors leading to muscle loss with aging (42). There appears to be inherent alterations in the endogenous antioxidant defense systems that occur with aging in skeletal muscle (6,43). Although there is an incongruence in the literature as to whether antioxidant enzyme activities increase, or decrease, with aging; there is an abundance of evidence suggesting that despite potential increases in enzymatic activity and/or the content of the antioxidant enzymes, the antioxidant defense system in aged individuals can be more easily overwhelmed by oxidants leading to oxidative stress and consequent oxidative damage (11,44). Increased oxidant production and/or an attenuated capacity to buffer oxidants may result in reductions in muscle function due to both

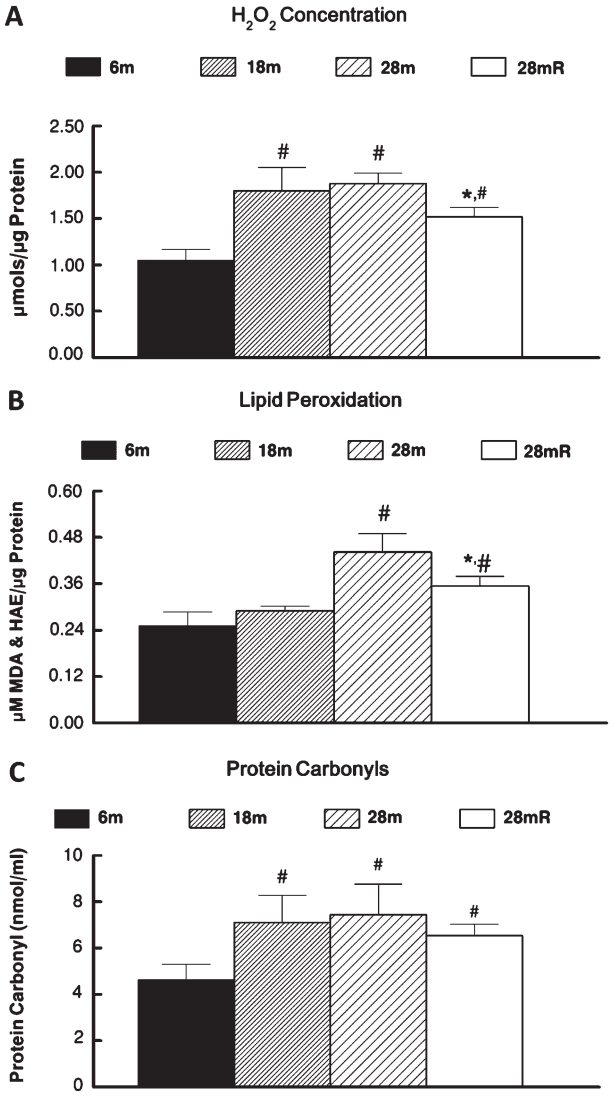

Figure 5. Resveratrol attenuated increases in hydrogen peroxide (H_2O_2) concentration and lipid peroxidation associated with aging but did not prevent protein carbonyl formation. (**A**) H_2O_2 concentrations were determined fluorometrically in gastrocnemius muscle homogenate. Data are expressed as micromoles per H_2O_2per microgram pf protein. Significance was set at ($p \leq .05$), and all data are represented as mean ± *SE*. (**B**) MDA and HAE levels were evaluated as a combined marker of lipid peroxidation and expressed in micromolar (MDA/HAE) per microgram of protein. (**C**) Protein carbonyl formation was analyzed as a marker of protein oxidation in gastrocnemius muscle homogenate. Data are expressed as nanomoles per milliliter. 6m = 6 month old, young adult control; 18m = 18 months of age, control; 28m = 28 months of age, control; 28mR = 28 months of age, resveratrol treated. Significance was set at $p \leq .05$, and all data are represented as mean ± *SE*. $^{\#}p \leq .05$, young control (aging effect). $^{*}p \leq .05$, aged control versus age resveratrol (supplementation effect).

muscle atrophy and also functional decrements that go beyond the linear relationship between muscle cross-sectional area and force generation (45,46). Given that sarcopenia is the loss of muscle mass and function with aging (1), it stands to reason that there may be a possible link between increases in oxidative stress with aging and the progression of sarcopenia.

The source of increased ROS present in aged skeletal muscle most likely originates in the mitochondria. Age-related mitochondrial alterations underlie a wide variety of diseases such as diabetes (30), neurodegeneration (31), and sarcopenia (10). The premise behind this relationship is based on the theory that with advanced age there are more dysfunctional mitochondria present within aged skeletal muscle cells (47). The high level of carbonylation that occurs in skeletal muscle mitochondrial proteins with increasing age (48) may potentially contribute to mitochondrial dysfunction in muscle cells. These defective mitochondria contain "leaky" electron transport chains and thus more oxidants are produced leading to oxidative stress (49). The process is cyclic; with more mitochondrial uncoupling, there are more pro-oxidants present to further damage vulnerable membrane phospholipids (7,49). Consequently, superoxide generation from individual mitochondrial derived increases with advanced age because the decline in skeletal muscle mitochondrial content found in human aging is due to a reduction in the number but not the size of the existing mitochondria (33).

Supplementation with the naturally occurring polyphenol, resveratrol, has the potential to alleviate oxidative stress in aged skeletal muscle via activation of Sirt1, by favorably augmenting endogenous antioxidant enzymes (22), suppressing inflammation (13), reducing mitochondrial oxidative stress (14), and enhancing mitochondrial function (20) and biogenesis (40). The present study sought to determine the efficacy of long-term resveratrol supplementation to alleviate oxidative stress from middle age through senescence and to attenuate the progression of sarcopenia.

As expected, sarcopenia was present in both plantaris and gastrocnemius muscles from 28-month-old animals (Table 1). Interestingly, although muscles from middle-aged mice that were 18 months of age animals were also significantly heavier than those from aged animals, when normalized to body weight, muscles from middle-aged animals already showed a relative decrease in muscle mass when compared with muscles from young animals. Counter to our hypothesis, resveratrol supplementation was unable to attenuate the loss of muscle mass or relative muscle mass in aged animals nor did dietary resveratrol supplementation affect animal body weight (Table 1). This was surprising since previous work in our laboratory using an acute administration of resveratrol that was a lower daily dosage (12.5 mg/kg/day) than the current study (60 mg/kg of body weight in 18-month-old animals and 46 mg/kg in 28-month-old animals) was protective against unloading-induced muscle atrophy (11). In addition, other work has shown that lower doses of resveratrol (22 mg/kg/day) than that which was used in the current study was protective against metabolic diseases (40,50). The mode and duration of administration or the potential for acute and chronic administration of resveratrol to produce different results as well as the different experimental species involved in the investigations may

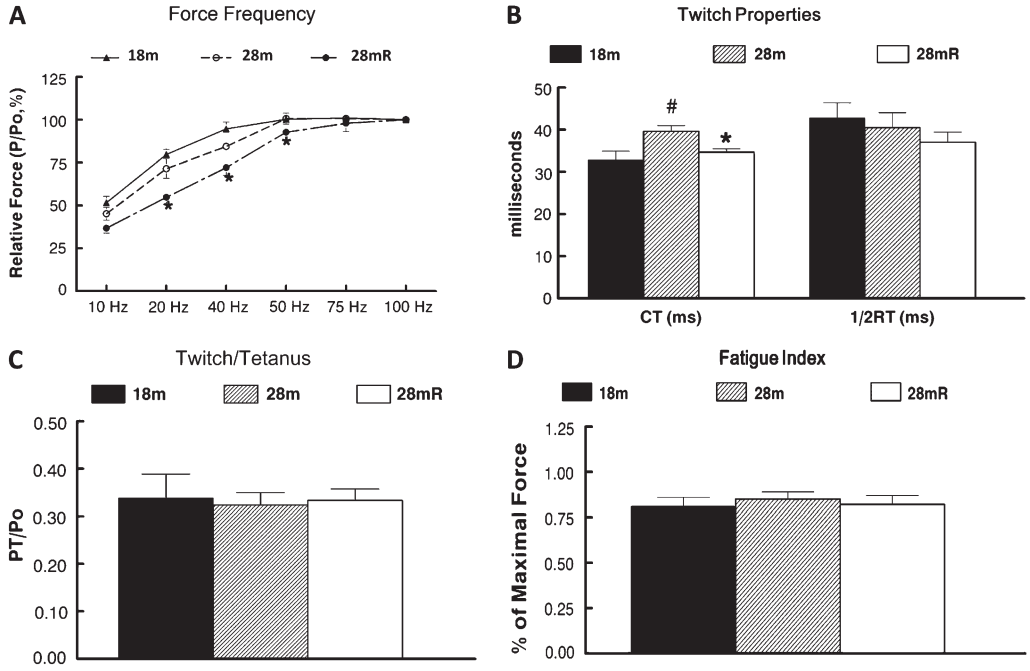

Figure 6. In vitro muscle physiological analysis. Isometric muscle contractile properties were examined in the plantaris muscles of control and resveratrol-treated mice. (**A**) Graphical depiction of a force-frequency curve illustrating the maximal force produced in plantaris muscles at a given frequency. Data are presented as the force production at a given stimulation frequency relative to maximal force of that muscle. (**B**) Contraction time (CT) and ½ relaxation time (½ RT) were analyzed to determine twitch properties in plantaris muscles. Data are presented as a unit of time (milliseconds). (**C**) The twitch to tetanus ratio (P_t/P_o) was assessed in plantaris muscles. Data are presented as a ratio of twitch force to maximal force. (**D**) A modified Burke protocol was implemented to assess muscle fatigue in plantaris muscles. Data are presented as a measure of fatigue index, calculated as a percent change from the first to last contraction (120th). 6m = 6 month old, young adult control; 18m = 18 months of age, control; 28m = 28 months of age, control; 28mR = 28 months of age, resveratrol treated. Significance was set at $p \leq .05$, and all data are represented as mean ± *SE*. *$p \leq .05$, aged control versus age resveratrol (supplementation effect).

explain the incongruent findings. Additionally, the mechanisms of aging-induced atrophy and unloading-induced muscle atrophy may occur via distinct cellular mechanisms and thus account for the seemingly contradictory findings.

Resveratrol supplementation moderately, but significantly, enhanced Sirt1 activity (Figure 1A) in gastrocnemius muscles from aged animals compared with muscles from aged animals on the control diet. There was a tendency for Sirt1 protein levels to be elevated by dietary resveratrol supplementation; however, these results did not reach statistical significance ($p = .063$; Figure 1B), and this is consistent with literature showing that supplementation with resveratrol primarily acts via increasing Sirt1 activation, not protein content (40). Somewhat unexpectedly, both Sirt1 activity and protein levels increased with aging (Figure 1A and B). We had originally theorized that Sirt1 activity would be decreased in aged animals in light of the fact that upregulation of Sirt1, with resveratrol, can protect against certain facets of aging, although it does not appear to be able to alter life span (51,52). However, recently Sirt1 has been found to be upregulated in heart muscle in response to both exogenous stress and aging (53), suggesting that Sirt1 may be elevated in muscles of aged animals, in an attempt to confer protection against endogenous and exogenous stressors, and these results are in agreement with the findings of the current study. Nevertheless, this elevation in Sirt1 protein and activity was insufficient to protect skeletal muscle against sarcopenia.

Sirt1 is a known positive regulator of PGC1 (20) and as such has the potential to influence pathways involved in mitochondrial biogenesis and oxidative metabolism. Long-term resveratrol supplementation did not increase total PGC1 protein content in aged gastrocnemius muscles nor did total PGC1 protein content change with aging (Figure 2A). Given that total PGC1 protein levels may not be sensitive to changes in the actual functional capacity of PGC1, the enzymatic activity of citrate synthase was also analyzed as a marker of oxidative metabolism and mitochondrial content. Citrate synthase activity was similarly unaffected by resveratrol supplementation; however, citrate synthase activity was sensitive to aging because it was significantly decreased in skeletal muscle from aged animals (Figure 2B). Similarly, cytochrome c release from the mitochondria to the cytoplasmic space was increased in muscles from aged and middle-aged animals, but this was not ameliorated with resveratrol supplementation (Figure 3A). This suggests that resveratrol did not protect mitochondria from age-associated increases in permeability. It should be noted that in the current investigation, the subcellular localization of cytochrome c was used as an indicator of mitochondrial membrane integrity and content, although traditionally cytochrome c release is used as a hallmark of apoptosis (53).

Taken as a whole, these data imply that while Sirt1 activity was enhanced with resveratrol supplementation, it was not increased enough to enhance PGC1 signaling and thus had no measurable influence on mitochondrial membrane integrity, mass, or metabolism. The discrepancies in our findings compared with others whom have found resveratrol supplementation to enhance mitochondrial biogenesis in a PGC1/Sirt1-dependent mechanism are likely due to the higher dosages implemented in those studies (20,40). The low-to-moderate dosage chosen in the current investigation was intended to translate to a realistic human daily dosage and furthermore be low enough that it would not have unintended negative consequences given that high doses of resveratrol have been shown to potentiate apoptosis (54), a potential contributor to sarcopenia (55).

Long-term resveratrol supplementation was successful at mediating endogenous antioxidant enzymes and markers of oxidative stress and oxidative damage in muscles from aged animals. Specifically, resveratrol administration significantly increased MnSOD activity, while having an opposing effect on CuZnSOD activity by significantly decreasing it in vastus lateralis muscles (Figure 4A and C). On the other hand, resveratrol supplementation did not alter either SOD isoform at the protein level (Figure 4B and D). CuZnSOD activity is known to be increased with aging and in other conditions eliciting elevated levels of oxidative stress (56), so it seems plausible that resveratrol's ability to reduce the muscle's oxidant load by reducing H_2O_2 concentrations (Figure 5A) may have prevented the aging-induced increase in CuZnSOD activity, resulting in a younger antioxidant profile.

The ability of resveratrol to induce MnSOD is a commonly accepted result. Although the exact molecular signaling pathways responsible for the mediation of MnSOD has not been fully established, it is believed that resveratrol augments Sirt1 activity which in turn upregulates MnSOD in a FOXO-dependent manner (57). Although FOXO3a protein abundance was not affected by resveratrol supplementation in this study (Supplementary Figure 1), we do not know if FOX03a acetylation was altered in the muscles of resveratrol-treated mice. Acute resveratrol supplementation has previously been shown to enhance MnSOD at both a gene (12) and a protein (11) level in addition to enhancing the enzyme's activity, suggesting that there may be differential signaling with regard to MnSOD regulation between acute and chronic exposure to resveratrol. It should be noted that overexpression of MnSOD has been shown to be an effective countermeasure for both oxidative damage and mitochondrial dysfunction (58), but it has not been shown to be effective at improving longevity (58). Similarly, transgenic mice with significantly decreased MnSOD activities show marked increases in oxidative damage within skeletal muscle but do not show diminished survival (59) or alleviating from age-related pathologies (36). Therefore, the role that MnSOD plays in relation to skeletal muscle aging and age-associated pathologies warrants further investigation.

The reduction of H_2O_2 concentrations in gastrocnemius muscles from aged animals supplemented with resveratrol appears to have assuaged the accumulation of the lipid peroxidation by-products malondialdehyde (MDA) and 4-hydroxyalkenals (HAE). This is consistent with recent data from our laboratory and others that have shown that resveratrol protects against H_2O_2-mediated lipid peroxidation in vivo (11,12) and in vitro (60). Although the muscle oxidant load (H_2O_2) was reduced in tissue homogenates and the level of lipid peroxidation was attenuated in aged gastrocnemius muscles, resveratrol supplementation did not protect skeletal muscle against aging-induced protein oxidation as measured by protein carbonyl formation (Figure 5C). This observation differs from recent rodent studies in both diabetic (61) and cancer (62) models showing that resveratrol could diminish protein oxidation in vivo. It is probable that the results of the current study are more representative of the effects that resveratrol can have under basal conditions in aged animals and it may prove to be more protective under perturbations that involve excessive stress, such as disease states and/or injuries resulting in chronic inflammation, or under muscle wasting conditions, such as cachexia or hind-limb suspension.

Given that resveratrol supplementation has been shown to improve muscular endurance (20) and increase mitochondrial biogenesis in skeletal muscle (20,40), it was hypothesized that the primarily fast-twitch plantaris muscles from supplemented animals would show an improved resistance to fatigue. Presumably, these muscles would show a shift in muscle characteristics that would be more in line with a slower muscle phenotype. This did not turn out to be the case, and in fact, resveratrol supplementation not only conferred no protection against muscle fatigue (Figure 6D), it in fact shifted the force-frequency curves of the plantaris muscles rightward (Figure 6A), indicating that the muscles had properties more consistent with a faster muscle profile. One possible explanation for this phenomenon is that resveratrol supplementation, by retarding oxidative stress and damage, may have prevented the atrophy and/or loss of type II fibers that is known to occur in muscle from aged animals (63) and thus was able to preserve a younger muscle profile. Furthermore, although muscle twitch characteristics primarily reflect the calcium transients in the muscle fibers and may or may not reflect mitochondria content, we cannot rule out the possibility that resveratrol may have also decreased mitochondria volume or number in fibers that shifted to a faster contractile phenotype. However, this possibility seems unlikely because neither citrate synthase, a marker for mitochondrial content, nor the muscle's fatigue index decreased in muscles from resveratrol-treated animals. Another possibility, although not addressed in the current study, is that resveratrol could improve the capacity of aged muscles to regenerate. In a recent study, resveratrol stimulated muscle precursor cell proliferation in a Sirt1-dependent

manner (64), further expanding the mechanisms by which resveratrol supplementation may improve muscle mass and function by enhancing muscles regenerative capacity, which has been shown to be depressed with aging (65,66) and in certain pathological conditions.

Taken as a whole, the results of the current study indicate that long-term dietary supplementation with moderate doses of resveratrol may prove to be beneficial by upregulating MnSOD and thus reducing the oxidant load present in the skeletal muscle environment and hence preventing some measures of oxidative damage. However, the current experimental protocol proved to be insufficient to enhance mitochondrial integrity or content. It is possible that a greater increase in Sirt1 protein and/or activity may be needed before a change in mitochondrial function will be observed.

Another possibility is that nuclear factor E2–related factor 2 (Nrf2) may have an important role in regulating oxidative stress in aged skeletal muscle from resveratrol-treated mice. Nrf2 is a transcription factor that binds to the response element of various antioxidant target genes to increase their transcription and, ultimately, to improve the cell's resistance to oxidative stress. Recent studies have shown resveratrol to confer protection against both mitochondrial and cellular oxidative stress in an Nrf2-mediated manner (67). This is significant because recent data have shown that Nrf2 is downregulated in skeletal muscles from old sedentary humans (68). Although not addressed in the current investigation, it is possible that resveratrol may increase Nrf2 activity and/or abundance, thereby leading to reduced oxidative stress in sarcopenic muscles.

The effects of resveratrol may be to improve the quality of health by increasing resistance to disease and to various biological stresses (40,50) but not to improve life span per se (52,69). Although it is clear from the current literature that reductions in oxidative stress and/or damage do not always alleviate age-associated pathologies (36,58,59), it is still probable that reductions in oxidative stress may improve the regenerative outcome and restorative potential of skeletal muscle in acute situations in which high levels of oxidative stress may occur, such as chronic illness and or injury. Further work is needed to determine if resveratrol has the potential to be an effective therapeutic agent to treat muscle functional decrements associated with elevated oxidative stress in aged individuals or in conditions of muscle loss, such as prolonged bed rest, presumably through a pharmacological pre-conditioning effect resulting in an improved redox status associated with these conditions.

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Supplementary Material

Supplementary material can be found at: http://biomed.gerontologyjournals.org/

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