Original Article

Muscular strength is unaffected by short-term resveratrol supplementation in aged mouse muscle

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Abstract

Background and Aim: Sarcopenia is the progressive loss of skeletal muscle mass and strength, due to aging. It represents a risk factor for physical frailty, reduced mobility, and increased incidence and severity of falls. Several biochemical mechanisms have been linked to sarcopenia such as aged-associated accumulation of oxidative damage that eventually can reduce muscular strength. Resveratrol supplementation has been shown to increase antioxidant content and reduce markers of oxidative stress and damage associated with age. However, the impact of resveratrol supplementation on functional deficits due to aging is less clear. Therefore, the aim of this brief investigation was to determine if short-term, low-to-moderate dose resveratrol supplementation would enhance strength in aged mouse skeletal muscle.

Methods: *In vivo* anterior crural muscle function and tibialis anterior total antioxidant capacity were analyzed in 21-month-old C57BL/6 male mice after 7 weeks of being fed a control diet (n=10) of standard rodent chow or a treatment diet (n=10) of standard rodent chow supplemented with resveratrol (0.05% w/w).

Results: The present study shows that 7 weeks of dietary resveratrol supplementation (0.05% w/w) did not alter twitch or peak isometric torque when compared with aged mice fed the control diet. Furthermore, resveratrol did not result in any detectable differences in the total antioxidant capacity of the tibialis anterior muscle.

Conclusion: Short-term, low-to-moderate dose resveratrol supplementation does not appear to enhance *in vivo* anterior crural strength in aged mouse skeletal muscle.

Key words: Aging, antioxidants, force, skeletal muscle, torque

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INTRODUCTION

Between maturity and old age, skeletal muscle mass and function are drastically reduced,[1] a process referred to as sarcopenia.[2,3] This process represents a risk factor for physical frailty, reduced mobility, and increased incidence and severity of falls.[4,5] Functional deficits due to sarcopenia are present across many species and characterized by reductions in absolute and normalized force/torque and power.[1] These reductions have been attributed to several potential causes that include: Loss of both fiber size and number,[6] intrinsic contractile dysfunction related to cross-bridge mechanics,[7,8] a net transition of type II fibers to type I fibers,[1] and a loss of high threshold motor units.[9] Reductions in muscle mass with age involve several cellular, molecular and biochemical changes[10] that ultimately results in the withdrawal of the anabolic stimuli and an up-regulation of the catabolic stimuli.[11] Research also suggests that underlying this imbalance, there are changes in oxidant stress and antioxidant enzyme activity that decreases skeletal muscle health.[10,12]

Several strategies have been developed to preserve muscle mass and function during healthy aging. One such strategy is the use of resveratrol (3,4′,5-trihydroxystilbene), a naturally occurring fat-soluble phytoalexin. In rodent skeletal muscle, resveratrol was reported to increase antioxidant content and reduce markers of oxidative

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stress and damage associated with age.[13-15] In parallel, these advantageous effects were also observed in aged muscle subjected to isometric exercise[13] or hindlimb suspension.[15] Moreover, supplementation of resveratrol in aged rodents, has reduced the markers of apoptosis[15,16] and increased sirtuin 1 activity,[14] a protein deacetylase involved in mitochondrial biogenesis.[17]

Despite these molecular adaptations, Jackson *et al*.[14] reported low-to-moderate dose resveratrol supplementation in mice for 10 months, from 18 to 28 months of age, did not improve isometric force production or reduce the loss of muscle mass. They also reported 18-month-old mice, classified as middle-aged, had similar force producing parameters and muscle-wet weights when compared to 28-month-old mice, which suggests the need to prevent age-related deficits before senescence by either increasing baseline muscular strength or attenuating its loss. And though Jackson *et al*.[14] observed long-term supplementation did not enhance force production in old muscle, it is possible that the potential benefits of resveratrol may have been missed due to the study's chronic treatment duration and/or extended length between testing periods. Therefore, the aim of this brief investigation was to fill this gap by determining if short-term, low-to-moderate dose resveratrol supplementation could improve function in middle-aged mice that had begun to experience reductions in muscular strength.

MATERIALS AND METHODS

Animals and resveratrol supplementation

Twenty one month aged, C57BL/6 male mice were obtained from the National Institutes of Health, National Institute of Aging repository, housed in groups of 5, supplied with food and water *ad libitum*, and maintained in a room at 20°C–22°C with a 12-h photoperiod. All procedures were approved by the Georgia State University Institutional Animal Care and Use Committee. Mice (n = 10) were provided standard rodent chow supplemented with 0.05% (w/w) resveratrol (ChromaDex, Irvine, CA, USA) for 7 weeks prior to testing. This dietary dosage has previously been used by others[13,14] to represent a low-to-moderate daily intake of resveratrol. Control animals (n = 10) were fed the standard rodent chow (Purina Diet 5001, Indianapolis, IN).

In vivo analysis of muscular function

Contractile function (i.e. torque-frequency relationship) of the left anterior crural muscles (tibialis anterior, extensor digitorum longus and extensor hallucis muscles) was determined as previously described.[18,19] Briefly, mice were anesthetized with isoflurane (1.5% isoflurane and 400 mL O_2/min) and placed on a temperature controlled platform to maintain core body temperature between 35°C and 37°C. The left knee was clamped and the left foot was secured to an aluminum "shoe" attached to the shaft of an Aurora Scientific 300B servomotor (Aurora, ON, Canada). Sterilized needles were inserted through the skin for stimulation of the left common peroneal nerve. Stimulation voltage and needle electrode placement were optimized with 5–15 isometric contractions (200 ms train of 0.1 ms pulses at 300 Hz). Following optimization, contractile function of the anterior crural muscles was assessed by measuring isometric torque as a function of stimulation frequency (20–300 Hz). Mice were then euthanized with an overdose of isoflurane, followed by cervical dislocation.

Total antioxidant capacity

The right tibialis anterior muscle was rapidly frozen in liquid nitrogen and stored at −80°C until analysis of total antioxidant capacity. Total antioxidant capacity was determined using a total antioxidant capacity kit per manufacturer's instructions (Abcam, Cambridge, UK). Briefly, the muscle was homogenized in ice-cold phosphate-buffered saline using a Dounce homogenizer, incubated in reaction buffer to reduce Cu^{2+} for 1.5 h at room temperature. Reduced Cu^{+} was chelated with a colorimetric probe and absorbance measured at 570 nm. The results are expressed as trolox equivalents according to a trolox standard curve.

Statistical analysis of data

The torque-frequency relationship was modeled with the following equation:

$$f(x) = \min + (\max - \min)/(1 + [x/EC_{50}]^{n}). \quad \text{Eq. (1)}$$

Where x is the stimulation frequency, minimum and maximum are the smallest (i.e. twitch) and largest (i.e. peak tetanic) respective forces estimated. EC_{50} is the stimulation frequency at which half the amplitude of force (max – min) is reached and n is the coefficient describing the slope of the steep portion of the curve.

An independent t-test was utilized to detect differences between groups in body weight, isometric torque parameters and total antioxidant capacity. Statistical significance was set at an α-level of 0.05. Values are presented in mean ± standard error. All statistical testing was performed with SigmaPlot (Systat Software, Inc. San Jose, CA, USA).

RESULTS

Supplementation had no effect on body weight (P = 0.29) [Table 1]. Further, no differences were detected in normalized twitch (P = 0.09) or peak (P = 0.30) isometric torque values, or the twitch to peak torque ratio (P = 0.17) [Figure 1a-c].

The steep portion of the torque-frequency curve was not modified ($P \geq 0.14$) by resveratrol supplementation, as evident by no significant shift (i.e. rightward or leftward) in the curve [Figure 1d]. Moreover, there were no differences in the slope coefficients ($P = 0.84$) or EC_{50} ($P = 0.31$) between the groups [Table 1]. Resveratrol supplementation also did not alter ($P = 0.16$) the muscle's total antioxidant capacity [Figure 2].

DISCUSSION

Loss of skeletal muscle mass and strength are common consequences of aging. This progressive process, described as sarcopenia, represents a risk factor for frailty, loss of independence and physical disability.[5] Sarcopenia is associated with the steady-state accumulation of oxidative damage that can eventually result in the loss of various cellular processes.[12] It has recently been reported that resveratrol supplementation can provide some protection against oxidative stress in aged rodent skeletal muscle.[13-15] However, it is less clear how resveratrol supplementation impacts function in aged skeletal muscle. Here, we show that *in vivo* strength is not altered after short-term, low-to-moderate dose resveratrol supplementation in middle-aged mouse skeletal muscle.

Table 1: *In vivo* isometric torque parameters

	Aged control	Aged resveratrol
Body weight (g)	33.8±0.8	32.3±1.2
Min_{meas} (Nmm/kg)	19.2±1.7	22.7±1.1
Max_{meas} (Nmm/kg)	88.4±3.3	93.5±3.4
Min_{estim} (Nmm/kg)	17.8±1.5	21.0±1.0
Max_{estim} (Nmm/kg)	89.2±3.5	91.8±3.3
Twitch/peak torque	0.22±0.02	0.24±0.01
EC_{50} (Hz)	81.4±2.2	78.3±2.1
n coefficient	5.9±0.3	5.8±0.2

Values are means±SE. The minimum (min) and maximum (max) torques measured and estimated represent twitch and peak isometric torques, respectively. Twitch/peak torque is the twitch to peak isometric torque ratio. EC_{50} is the stimulation frequency at which half of the rise in amplitude of torque occurred. The *n* coefficient describes the slope of the steep portion of the torque-frequency curves depicted in Figure 1d. SE: Standard error, EC: Effective concentration

As a result of aging, normalized *in vivo* peak isometric torque declined by 20% when compared to what we have previously reported in 2–5 months old mice with similar body weights.[18] This reduction is slightly lower than the 25% drop reported by Ryan *et al*.[13] in peak plantar flexor force. However, these differences are likely due to variations in age, in the present study mice were 21 months old whereas the mice Ryan *et al*.[13] used were 27 months old. To determine if resveratrol supplementation could attenuate these age related strength deficits, we supplemented mouse diets with 0.05% (w/w) resveratrol for 7 weeks. Supplementation did not significantly improve muscular function (e.g. twitch or peak isometric torque, the twitch to peak torque ratio) when compared to the aged control mice. These results are similar to what others have demonstrated when using the same dose of resveratrol, but for different

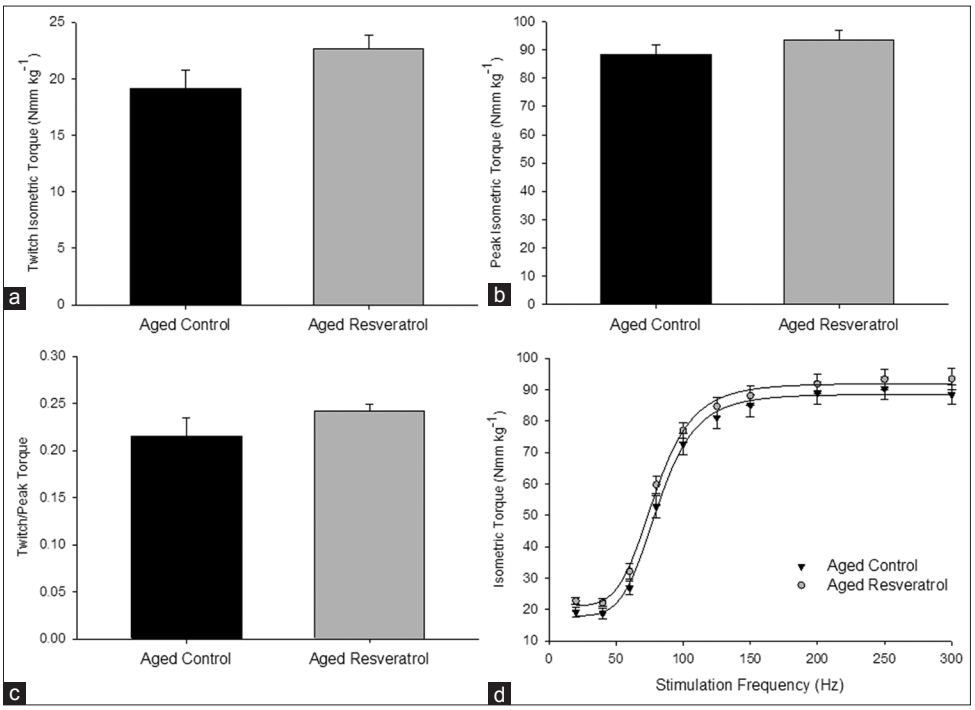

Figure 1: *In vivo* isometric torque analysis. Isometric torque was measured in the anterior crural muscles of aged control and resveratrol mice. (a) Twitch isometric torque. (b) Peak isometric torque. (c) Twitch/peak torque is the twitch to peak isometric torque ratio. (d) Isometric torque as a function of stimulation frequency modeled with Eq. (1), listed in methods

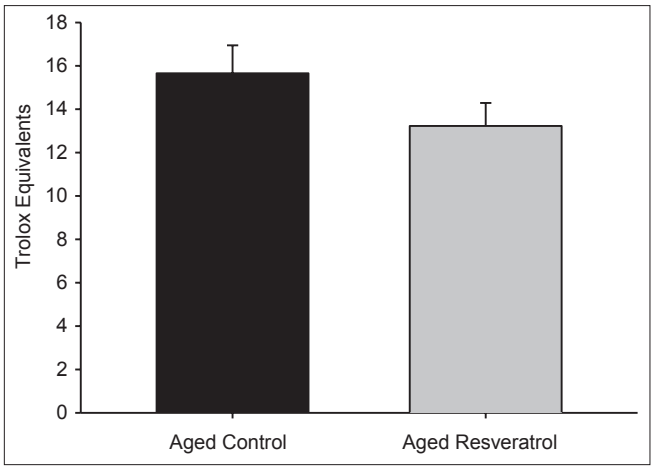

Figure 2: Total antioxidant capacity analysis. Total antioxidant capacity was measured in the tibialis anterior muscle of the aged control and resveratrol mice and expressed as trolox equivalents

treatment durations.[13,14] Ryan *et al*.[13] reported resveratrol supplementation for 7–10 days was unable to influence *in vivo* peak isometric torque of the plantar flexors in 27 months old mice. Likewise, they also demonstrated that 10 months of resveratrol supplementation from middle age through senescence did not increase *ex vivo* twitch or peak isometric force, or alter the twitch to peak force ratio of mouse plantaris muscle.[14]

Though twitch and peak isometric strength were unchanged, Jackson *et al*.[14] reported 10 months of supplementation decreased twitch contraction time and caused a rightward shift in the force-frequency curve in aged plantaris muscle when compared to aged controls, indicative of properties consistent with a faster muscle profile. To the contrary, they did not see any changes in twitch ½ relaxation time or fatigue resistance. In the present investigation, 7 weeks of resveratrol supplementation in aged mice did not result in a shift in the toque-frequency curve or alter any parameters of the curve (i.e. slope coefficient, EC_{50}). The differences in the force/torque-frequency curves in the present study and that of Jackson *et al*.[14] likely reflect supplement duration (7 weeks vs. 10 months) and the final age at which the mice were tested (21 vs. 28 months).

Although we report resveratrol does not affect strength of the anterior crural muscle group, others have shown it up-regulates components of the endogenous antioxidant system, reduces oxidant load within the muscle environment and consequently attenuates oxidative damage.[13-15] In fact, both short-[13,15] and long-[14] term resveratrol supplementation increased manganese superoxide dismutase activity, reduced hydrogen peroxide concentrations and attenuated lipid peroxidation levels in aged skeletal muscle of rodents. However, it appears that resveratrol's ability to reduce the oxidant environment is dependent on up-regulating specific enzymatic antioxidants rather than the antioxidant system as a whole. In support of this, we used a nonenzymatic assay that measured small molecule (e.g. ascorbate, uric acid, Vitamin E) and protein (e.g. albumin, transferrin) antioxidants, and were unable to detect any differences in aged muscle after 7 weeks of supplementation. Furthermore, 10 months of resveratrol supplementation did not reduce the levels of oxidative stress in aged muscle measured by protein carbonyl formation.[14] These results suggest that resveratrol supplementation reduces markers of oxidative stress (e.g. hydrogen peroxide) associated with age by increasing particular enzymatic antioxidants (e.g. catalase), but its effects on nonenzymatic antioxidants and global levels of oxidative stress remain to be validated.

Limitations of the study

Although it is difficult to determine if the mice in the present study would be classified as sacropenic, it is seemingly apparent that resveratrol supplementation does not increase strength in aged mice (≥21 months) regardless of the treatment duration (i.e. short- or long-term).

CONCLUSION

Here we demonstrate that short-term, low-to-moderate dose resveratrol supplementation does not enhance *in vivo* muscular strength in middle-aged mice which is in agreement with Jackson *et al*.[14] who reported similar findings *ex vivo* after long-term supplementation in older mice. However, others have reported resveratrol increases aerobic endurance[17] and reduces fatigue associated with repetitive isometric exercise[13] in young mice, and attenuates force deficits after hindlimb suspension in aged rats.[15] Therefore, as a whole, these results indicate that resveratrol's therapeutic potential is dependent on supplement duration, age and the type of stress placed on the muscle (e.g. atrophy, sarcopenia, exercise). Future investigations will need to determine the effects of higher doses and how resveratrol supplementation impacts recovery or regeneration in other stressful situations, such as contraction- or trauma-induced injury models.

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