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Resveratrol treatment reduces the appearance of tubular aggregates and improves the resistance to fatigue in aging mice skeletal muscles

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ABSTRACT

Resveratrol (RES) is a polyphenolic compound found in grapes, peanuts, and in some berries. RES has been reported to exhibit antioxidant, anti-inflammatory, anti-proliferative properties, and to target mitochondrial-related pathways in mammalian cells and animal models. Therefore, RES is currently advised as supplement in the diet of elderly individuals. Although it is hypothesized that some of RES beneficial actions likely arise from its action on the skeletal muscle, the investigation of RES effects on this tissue remains still elusive. This study reports the effects of a 0,04% RES-supplemented diet for six months, on the skeletal muscle properties of C57/BL6 aging mice. The analysis of the morphology, protein expression, and functional-mechanical properties of selected skeletal muscles in treated compared to control mice, revealed that treated animals presented less tubular aggregates and a better resistance to fatigue in an ex-vivo contraction test, suggesting RES as a good candidate to reduce age-related alterations in muscle.

1. Introduction

One of the most serious consequences of aging is its effect on skeletal muscle (Rosenberg, 1997). In fact, although aging affects many other tissues and organs, loss of muscle mass together with its impaired metabolic properties (Conley et al., 2000; Hollmann et al., 2007; Chabi et al., 2008) may have a negative impact on whole-body metabolism.

Muscle aging, is a multifactorial phenomenon also referred to as 'sarcopenia', characterized by a significant decline in muscular function and performance. Sarcopenia consists of a slow but progressive loss of muscle mass with advancing age, which results from a variety of both quantitative and qualitative changes, such as the progressive denervation, alterations in the excitation-contraction coupling (Ryall et al., 2008; Boncompagni et al., 2006; Marzetti and Leeuwenburgh, 2006), decrease in myofiber cross-sectional area (Murgia et al., 2017), loss of muscle fibers, and changes in fiber type (Murgia et al., 2017), with a progressive reduction of fast in favour of slow fibers (Murgia et al., 2017; Cristea et al., 2010).

One morphological characteristic that in recent years is attracting attention is the presence of tubular aggregates (TAs), which are accumulations of densely packed tubules arising from sarcoplasmic reticulum of striated muscles (Engel, 1964). In humans, TAs develop mainly in type II fibers (Engel et al., 1970), are associated with a wide variety of muscle disorders (Schiaffino, 2012) but also present in asymptomatic probands (Engel et al., 1970). Contrarily to what observed in humans, TAs in skeletal muscles of inbred strains mice are restricted to type IIB fibers, related to sex and age (Agbulut et al., 2000; Chevessier et al., 2004) and also in a variety of congenital myopathies (Schiaffino, 2012; Giacomello et al., 2015). Although the mechanisms responsible of their onset remain still unclear, it has been recently proposed that they arise from an altered proteostasis mechanism (Schiaffino, 2012), and from mitochondrial dysfunction (Vielhaber et al., 2001).

Overall, during aging skeletal muscle undergoes an exstensive modification of both morphological and biochemical profiles. Associated to these effects, a 'metabolic dysregulation' is also observed, with reduced sensitivity to insulin, impaired oxidative defence, and decreased mitochondrial function (Carter et al., 2015). In humans, insulin resistance has been associated with a misregulation of the ratio of oxidative type I to glycolytic type II muscle fibers, and decreased expression of genes involved in the regulation of mitochondrial activity (Hoeks and Schrauwen, 2012).

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In recent years, it has been proposed (Lopez-Lluch and Navas, 2016) that dietary restriction alleviates many of the age-related diseases, improves stress-resistance, and decelerates the functional decline in elderly individuals. As a consequence, the attention has been focused on the study of "dietary-restriction mimetic" compounds able to provide benefits similar to dietary restriction without a reduction of the caloric intake (Chung et al., 2012).

In this context, Resveratrol (3,5,49-trihydroxystilbene), a bioactive phenol found in grapevine, peanuts, and in some berries, has been described to be able to mimic dietary restriction, to extend the lifespan of diverse species from yeast to mammals (Baur et al., 2006; Narkar et al., 2008; Feige and Auwerx, 2008; Pearson et al., 2008), and most importantly, to counteract the effects of physical inactivity and aging by activating the protein deacetylase sirtuin-1 (Sirt1) and increasing PGC 1-alpha activity (Feige et al., 2008; Lagouge et al., 2006; Rodriguez-Bies et al., 2016). Moreover, RES has been described to alter the progression of type II diabetes, by enhancing insulin sensitivity in the skeletal muscle of aging mice (Baur et al., 2006; Lagouge et al., 2006).

Even though muscle specific effects of RES have been proposed as responsible of overall effects on the modulation of insulin sensitivity and resistance to obesity (Pearson et al., 2008; Lagouge et al., 2006), the investigation of its impact on skeletal muscle remains elusive. Recent reports on the effect of this molecule after long-term supplementation in mice show that RES exerts specific actions on the skeletal muscle tissue. Interestingly, RES has been proposed to reprogram muscle gene expression (Lagouge et al., 2006), improve the aerobic capacity of muscle (Lagouge et al., 2006), and counteract the high fat induced Myosin Heavy Chain (MyHC) switch (Hyatt et al., 2016). As a global effect, RES acts positively by improving resistance to fatigue (Rodriguez-Bies et al., 2016; Wu et al., 2013), exercise performance (Wu et al., 2013), biochemical profiles, and pathological responses in aging models (Murase et al., 2009). Although it results difficult to have a full picture of RES effects and potentialities, due to protocols that propose a wide range of doses and are mainly performed in short term, altogether these observations provide to RES the capability to moderate the age-related decline in physical performance.

Aimed at gathering new insights on the effects provided by RES treatment during aging, our experimental plan envisaged a 6-month supplementation of 0,04% RES in the diet of 12 months old C57/BL6 mice. According to previous studies (Dutta and Sengupta, 2016), the mice age at the beginning of treatments corresponds to middle aged individuals, approximately to 55 years old humans, and at the end of the treatment, 18 months, corresponds approximately to 65 years old humans. We analyzed the morphological properties, the modulation of protein expression, and the functional-mechanical characteristics of selected skeletal muscles in treated mice compared to a control group fed with a standard diet. Our data show that RES reduces the appearance of tubular aggregates (TAs), counteracts the MyHC switch and improves the resistance to fatigue in an ex vivo test. Taken together, our results further confirm RES as a supplement to the diet useful for reducing some of the age-related alterations to skeletal muscle tissue.

2. Materials and methods

2.1. Animals and treatment

The experimental procedures used throughout all the experiment were in accordance with the European legislation on the use and care of laboratory animals (EU Directive 2010/63) and were approved by the Ethics Committee of the University of Siena and from Ministero della Salute, Italy. Animals were fed a chow diet and distilled water ad libitum, and were maintained on a regular cycle (12-h light/dark) at room temperature. C57/BL6 mice aged 12 months have been randomly divided in two groups: one group fed for 6 months with a standard diet supplemented with 0,04% RES as previously reported by Baur et al. (2006), and a second group of mice fed with a standard diet. The reported results are the product of three independent experiments involving 15 animals per group for a total of 90 animals. In each experiment, after the 6-month treatment period, control and treated mice were subdivided in 3 groups. One group of mice was sacrificed and the tibialis anterior and gastrocnemius muscles were harvested for protein expression, histological and immunofluorescence analyses. One group was dedicated to the ex vivo analyses of Exstensor Digitorum Longus (EDL) and soleus muscle contractile performance, and a third group was subjected to a treadmill endurance test.

2.2. Antibodies

Primary antibodies used in the present work are listed in Table S1.

2.3. Cryostat sectioning

Tibialis anterior muscles were dissected from mice, directly frozen in isopentane cooled in liquid nitrogen, and cryoprotected with Tissue-Tek II OCT compound (Miles Inc., USA). Transverse sections, 8 μm thick, were cut with a Leica cryostat (CM 1850, Leica Microsystem, Austria).

2.4. Immunofluorescence staining

Sections were fixed with 3% para-formaldehyde, blocked with 0.2% BSA and 5% goat serum in PBS to avoid non-specific binding of the antibodies, and incubated with primary antibodies overnight in a humidified chamber at 4 °C. The sections were extensively washed with PBS-BSA 0.2% and incubated with antimouse and antirabbit Cy2 or Cy3 conjugated secondary antibodies (1:500; Jackson ImmunoResearch Laboratories, West Grove, PA) for 1 h at room temperature, washed with PBS-BSA 0.2%. Sarcolemma staining was performed with Wheat Germ Agglutinin (WGA) labeled with AlexaFluor-488 (1:500; Life Technologies), in PBS-BSA 0.2% for 30 min at room temperature and extensively washed. Samples were mounted with Mowiol (Sigma Aldrich, Italy) added with 0.025% DABCO (Sigma Aldrich, Italy) as antifading agent. The specimens were analyzed with a confocal microscope (LSM510, Zeiss, Jena, Germany).

2.5. Succinate dehydrogenase (SDH) stain

Slides were directly placed in freshly prepared SDH solution (Cobalt Chloride 2%, Natrium Succinate 0.2 M, MTT Thiazolyl Blue 1 mg/ml in Tris-HCl buffer 0.2 M, pH 7.4) from −80 °C and let to incubate 15–20 min. The enzymatic reaction was stopped by quickly dipping the slides in ddH_2O, the slides where air-dried in dark and mounted. Images were acquired with an Axioplan 2 microscope (Zeiss, Jena, Germany) equipped with a Micro-MAX digital CCD camera (Princeton Instruments, Trenton, USA). SDH activity was quantified with ImageJ free software (http://imagej.nih.gov/ij/) by converting images to greyscale and the grey intensity was calculated for each fiber.

2.6. Toluidine blue stain

Tissue sections were incubated for 2 min in 0.1% toluidine Blue solution, extensively rinsed in distilled water and then dehydrated with 95% and absolute ethanol alcohol, followed with two changes of xylene and mounted. Images were acquired with an Axioplan 2 microscope (Zeiss, Jena, Germany) equipped with a Micro-MAX digital CCD camera (Princeton Instruments, Trenton, USA). Cross sectional area (CSA) of fibers was determined with ImageJ software from the photographs of the sections stained with Toluidine Blue or from images used to determine SDH activity, depending on the experiment.