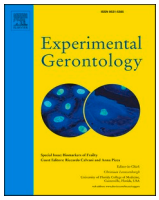
Experimental Gerontology 194 (2024) 112485

Contents lists available at ScienceDirect

Experimental Gerontology

journal homepage: www.elsevier.com/locate/expgero



Resveratrol impinges on retrograde communication without inducing mitochondrial biogenesis in aged rat soleus muscle

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ARTICLE INFO

Section Editor: Michal Masternak

Keywords:
Resveratrol
Rat soleus skeletal muscle
Mitochondrial biogenesis

ABSTRACT

The natural polyphenol resveratrol (RSV) might counteract the skeletal muscle age-related loss of muscle mass and strength/function partly acting on mitochondria. This work analysed the effects of a six-week administration of RSV (50 mg/kg/day) in the oxidative Soleus (Sol) skeletal muscle of old rats (27 months old). RSV effects on key mitochondrial biogenesis proteins led to un unchanged amount of SIRT1 protein and a marked decrease (60 %) in PGC-1α protein. In addition, Peroxyredoxin 3 (PRXIII) protein decreased by 50 %, which on overall suggested the absence of induction of mitochondrial biogenesis by RSV in old Sol. A novel direct correlation between PGC-1α and PRXIII proteins was demonstrated by correlation analysis in RSV and ad-libitum (AL) rats, supporting the reciprocally coordinated expression of the proteins. RSV supplementation led to an unexpected 50 % increase in the frequency of the oxidized base OH8dG in mtDNA. Furthermore, RSV supplementation induced a 50 % increase in the DRP1 protein of mitochondrial dynamics. In both rat groups an inverse correlation between PGC-1α and the frequency of OH8dG as well as an inverse correlation between PRXIII and the frequency of OH8dG were also found, suggestive of a relationship between oxidative damage to mtDNA and mitochondrial biogenesis activity. Such results may indicate that the antioxidant activity of RSV in aged Sol impinged on the oxidative fiber-specific, ROS-mediated, retrograde communication, thereby affecting the expression of SIRT1, PGC-1α and PRXIII, reducing the compensatory responses to the age-related mitochondrial oxidative stress and decline.

1. Introduction

Skeletal muscle (SM) is pivotal for movement, breathing, and circulatory functions. Muscle fibers' contraction requires ATP hydrolysis, obtained through the myosin adenosine triphosphatase (ATPase) activity, which can be histochemically characterized, leading to the identification of three distinct fiber types known as fast-glycolytic (type IIa), fast-oxidative glycolytic (type IIb), and slow-oxidative (type I) (Brooke and Kaiser, 1970). The fast-glycolytic and fast-oxidative glycolytic fibers are fast-twitch fibers, featuring fast myosin ATPase and sarcoplasmic reticulum Ca-ATPase activities. A mixture of both fast fiber types is present in fast-twitch muscles (extensor digitorum longus, EDL; gastrocnemius, G; vastus lateralis, VL; tibialis anterior, TA). On the other side, the slow-oxidative fiber type possesses slow myosin ATPase and sarcoplasmic reticulum Ca-ATPase activities, prolonged twitch duration and is mostly present in slow muscles as the soleus (Sol). Aging affects several functional features of SM as the percent distribution of the various fiber types, the mass, the total fibers number, and the single-fiber diameter (Larsson and Edström, 1986; Lexell et al., 1988). Other age-related alterations, contributing to decrease muscle endurance,

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https://doi.org/10.1016/j.exger.2024.112485
Received 29 February 2024; Received in revised form 22 May 2024; Accepted 8 June 2024

include reduction in capillary density and blood supply, impairment of glucose transport and substrate availability, reduced rate of phosphocreatine repletion, decrease in mitochondrial number and in activity of oxidative enzymes. Such changes markedly impact on aerobic metabolism, reducing fatty acid oxidation and metabolism of glucose and lipids (Holloszy and Coyle, 1984). Therefore, the increase in muscle fatigability occurring with age is likely due also to the decline in mitochondrial content and function, impairing muscle oxidative and endurance capacity (Thompson, 2022). Dysfunctional mitochondria are considered major contributors to muscle function decline since the organelles are the main cell source of energy. In particular, the susceptibility of the slow twitch Sol muscle to aging had been clearly demonstrated through the age-related decrease in the activity of the mitochondrial enzymes citrate synthase and complex IV, passing from young rats (7–10-month-old) to senescent animals (35-month-old). Such enzyme decline was specific of Sol and fast oxidative regions of red gastrocnemius (RG) muscle, with no change in the fast glycolytic region of white gastrocnemius (WG), thus unveiling the sensitivity of Sol to aging, which had been underscored before (Carter et al., 2010). Other previous studies on the age-related mitochondrial alterations in rat Sol reported different changes, affecting organelle structure and functions, as a marked decrease in content of mitochondrial DNA (mtDNA) and of the histone-like protein of mtDNA, mitochondrial transcription factor A (TFAM) (Picca et al., 2014) as well as a reduction in mitochondrial biogenesis (Pesce et al., 2010; Gadaleta et al., 1998). In recent years, consciousness about the great promotion of healthy aging due to the regular consumption of various nutrients present in fruits, vegetables and other natural products has constantly and largely grown. In particular, such products carry an additional value beside their nutritional content that is their ability to be engaged in signaling pathways leading to responses able to counteract different stresses affecting cells and tissues. Several among such natural compounds have multifaceted features, also including a clear geroprotective potential that makes them favoured over other approaches aiming to delay aging and to prevent or retard the overt onset of age-related pathologies (Musillo et al., 2021). The natural polyphenol resveratrol (RSV), abundant in red grapes, berries, and peanuts has gained a very large resonance because of its pleiotropic actions (Schrauwen and Timmers, 2014). RSV's multiple therapeutic effects include antioxidant, antimicrobial, cardioprotective, anti-tumour, anti-diabetes, anti-obesity and anti-aging activities (Camins et al., 2009; Liuzzi et al., 2011; Zhang et al., 2021). In particular, although not all the various molecular mechanisms elicited by RSV have yet been fully clarified, several positive effects of the molecule on metabolic health resemble those induced by the up to now most efficacious treatment for delaying aging namely calorie restriction. The huge number of studies about RSV ability to preserve and/or restore metabolic health endangered by aging processes has led to the identification of the molecule as one of the most promising calorie restriction mimetic (Lam et al., 2013; Madeo et al., 2019). RSV is also very efficacious in safeguarding skeletal muscle functionality since it can promote mitochondrial biogenesis, improve muscle fatigue resistance, and reverse sarcopenia in older people (Qu et al., 2021). Mitochondria are among the targets of RSV beneficial effects, since the natural compound enhances mitochondrial function through different pathways. As for rodent SM, RSV has been reported to induce an enhancement of mitochondrial oxidative capacity through increased expression of electron transport chain components, driving to an improved energy utilization in gastrocnemius (G) and Sol of adult mice (Price et al., 2012). Furthermore, RSV has been shown to promote mitochondrial biogenesis in adult rodent non oxidative fibers from G skeletal muscle through the activation of the biogenesis master regulator peroxisome proliferator activated receptor-gamma coactivator-1α (PGC-1α). Such RSV-related activation is obtained through the sirtuin 1 (SIRT1)-dependent deacetylation of PGC-1α and modulation of the expression of PGC-1α target genes in the muscle, thus originating the SIRT1- PGC-1α axis (Lagouge et al., 2006). Previous reports have led to the conclusion that RSV triggers tissue-specific effects (Lagouge et al., 2006; Handy et al., 2023) and that, as for SM, the response might be different analyzing fast-twitch muscles as TA (Niu et al., 2021) or whole G (Muhammad and Allam, 2018) versus the oxidative RG. In fact, in a previous study, the potential efficacy of treating old rats (27 months old) with short-term (6 weeks), RSV (50 mg/kg/day) was investigated in the glycolytic WG and oxidative RG muscles, finding different results depending on the tested part of the G muscle (Joseph et al., 2013). Furthermore, another issue that deserves a thorough consideration deals with the efficacy of RSV treatment in old subjects namely whether positive effects might be induced by RSV administration begun late in life and for a short-term period. We decided to administer RSV for the same short time and at the same dosage used in (Joseph et al., 2013), which had been shown effective in altering mitochondrial protein expression with long-term treatment, but well below the threshold potentially leading to apoptosis (Jackson et al., 2011) to old rats (27 months old). The aim of the study was to analyze the oxidative fiber type-specific effects induced in mitochondrial biogenesis, oxidative stress, quality control and dynamics in the oxidative muscle Sol by RSV, administered to old rats for a short period, and to compare the present results with those previously obtained in fast-twitch muscles.

2. Materials and methods

2.1. Animals

A total of twelve 27-month-old male Fischer 344 × Brown Norway Hybrid rats purchased from the National Institute on Aging were used in this study. All experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of Florida (Study#: 200902992) and performed in accordance with the National Institutes of Health guidelines for the care and use of laboratory animals. Experimental protocols were designed to minimize suffering and the number of animals used in the study. The animals were acclimatized for 4 weeks before the start of intervention and singly housed in separate cages in a temperature (18–22 °C) and light-controlled environment with a 12 h light/dark cycle. The choice of single housing for each rat in separate cages was due to the need to verify daily, by visual inspection, that each animal completely consumed its feeding portion containing, respectively, control or RSV-supplemented diet. The stress originated by social Isolation of each rat and its possible consequences at mitochondrial functional and structural levels (Picard and McEwen, 2018) were considered, but in order to perform a study mainly intended to be a dietary one, the choice of single housing was obligatory. Furthermore, literature about this issue describes effects of isolation by single cage housing only on mitochondria from different areas of brain, whereas we focused on Soleus skeletal muscle. Animals were randomly assigned to one of two treatment groups: ad-libitum (AL) and resveratrol RSV (50 mg/kg/day; 6 weeks). All animals received a daily diet of food pellets (Custom Animal Diets, Bangor, PA). Animals in the RSV-treated groups received a daily dose of one bacon flavored RSV fortified tablet (50 mg/kg/body weight), mixed in with their normal food pellets (Sigma Chemical Co., St. Louis, MO). At the end of the 6 weeks of treatment, all 6 animals from each group were sacrificed, soleus muscle was isolated and weighed, frozen in liquid nitrogen, and stored at −80 °C until further analysis.

2.2. Western blot analysis

Frozen soleus muscle was used for the western blot analysis. Shortly, about 50 mg of tissue were homogenized with a lysis buffer and centrifuged at 13,000 ×*g* for 25 min at 4 °C, from which supernatant was obtained. Total protein content was determined using the Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Then, 30 μg of proteins were separated on a 4–12 % sodium dodecyl sulphate-polyacrylamide gel for electrophoretic separation

(SDS-PAGE) and transferred to a polyvinylidene difluoride membrane (GE10600023 Amersham™ Hybond® P Western blotting membranes, PVDF; Merck KGaA, Darmstadt, Germany). Blots were blocked with TBS-Tween-20 containing 5 % bovine serum albumin (BSA) for 1 h and incubated with primary antibodies overnight at 4 °C. The primary antibodies used were anti-DRP1 (Abnova, Taipei City, Taiwan, #H00010059-M01), anti-MFN2 (Cell Signaling Technology Inc., Danvers, MA, USA #9482S), anti-SIRT1 (Cell Signaling Technology Inc., Danvers, MA, USA #8469S), anti-PGC-1α (Novus Biologicals, Minneapolis, MN, #NBP1-04676PCP), anti-TFAM (Cell Signaling Technology Inc., Danvers, MA, USA #7495S), anti-SOD2 (MnSOD, Cell Signaling Technology Inc., Danvers, MA, USA #13194S), anti-Peroxiredoxin 3, PRXIII (Ab Frontier, Seoul, Korea, #LFPA0030), anti-β-actin (Sigma-Aldrich, St.Louis, MO, USA #A2066). The next day, membranes were incubated with a secondary antibody (Cell Signaling Technology Inc., Danvers, MA, USA anti-mouse #7076; anti-rabbit #7074) conjugated with HRP (dilution 1:10,000) for 1 h and developed using the ECL detection with the ChemiDoc System. Precision Plus Protein Dual Color Standards (Bio-Rad, #1610374) was the marker used for molecular weight estimation. The immunoreactive bands' densitometric analysis was performed by the Bio-Rad Image Lab SoftwareTM 6.0.1 and results were normalized to β-actin and then showed as normalized to AL group set to 1.

2.3. Determination of mtDNA relative content

Total DNA was extracted using commercially available kits. The content of mtDNA relative to nuclear DNA was determined using 3 ng total DNA as template in quantitative real time polymerase chain reaction (qPCR) experiments. SYBR Green chemistry was used. A 83 bp long mtDNA amplicon was obtained using the following primers: mtDNA For 5'GGTTCTTACTTCAGGGCCATCA3' and mtDNA Rev. 5'TGATTAGACCCGTTACCATCGA3' (nucleotide positions 15,785–15,806 and 15,868–15,847, respectively. Numbering is according to GenBankTM accession number AY172581); a 85 bp long β-actin amplicon was obtained using the following primers: β-actin For 5'CCCAGCCATGTACGTAGCCA3' and β-actin Rev. 5'CGTCTCCGGAGTCCATCAC3' (nucleotide positions 2181–2200 and 2266–2248, respectively. Numbering is according to GenBankTM accession number V01217.1). qPCR conditions were as in Chimienti et al., 2019.

2.4. Analysis of modified purines

The incidence of oxidized purines, mainly 8-oxo-deoxyguanosine (OH8dG), in the D-loop region of mtDNA was determined using formamidopyrimidine DNA glycosylase (Fpg) (New England Biolabs, Beverly, MA, USA) digestion, as previously reported in Chimienti et al., 2019. Briefly, 5 ng of total Fpg-treated and untreated total DNA were used as template to obtain a 1000 bp amplicon from the mtDNA D-loop region (primers: D-loop For 5'TCTGGTCTTGTAAACCAAAAATGA3' and D-loop Rev. 5'TGGAATTTTCTGAGGGTAGGC3'; nucleotide positions 15,302–15,325 and 16,302–16,282, respectively. Numbering according to GenBankTM accession number AY172581). Amplicons were visualized by agarose gel electrophoresis. The intensity of the bands was analysed using Image Lab Software (BioRad Laboratories Inc., Hercules, CA, USA). The ratio between Fpg-treated and untreated samples was calculated and expressed as % of the complement to 100.

2.5. Determination of GSH/GSSG

The GSH/GSSG ratio was measured by the method of Rahman et al., 2006.

2.6. Statistical analysis

GraphPad Prism software v8.0.1 was used for statistical analyses. Due to the non-normal distribution of the data, nonparametric tests were used, except for the parametric GSH/GSSH ratio that was analysed using the unpaired *t*-test. Mann-Whitney test was used for comparisons between the two study groups and the Spearman r for the analysis of correlations between variables under investigation. Statistical significance was set at $p < 0.05$. All data represent the results of at least two independent experiments and are expressed as mean ± SEM (Standard Error of Mean).

3. Results

3.1. Animal characteristics

Animals were given a daily RSV dose of 50 mg/kg body weight/day. This dose has been previously shown to be within the range that induces changes in mitochondrial protein expression pathways with long-term treatment but well below the threshold that can potentially lead to apoptosis (Jackson et al., 2011). Body weights indicated in Table 1 were taken prior to and following 6 weeks of intervention, immediately before the animals were sacrificed, whereas soleus weights were taken after the sacrifice of the animals and also reported as a ratio to body weight.

No significant differences were found between body weights pre- and post-treatment in AL and RSV rats neither in Sol muscle weights post-treatment.

3.2. Effects of RSV on mitochondrial biogenesis

According to previous studies, RSV triggers mitochondrial biogenesis through activation of SIRT1 and PGC-1α (Lagouge et al., 2006). Therefore, at the end of the RSV treatment the protein expression of SIRT1 and PGC-1α between the AL group of animals and the RSV-treated counterpart were compared by western blot experiments. While there was no change in the SIRT1 protein amount (Fig. 1A), a highly significant (−60 %) decrease in the PGC-1α protein in the RSV rats with respects to the AL controls was detected ($p < 0.001$) (Fig. 1B), suggestive of a marked reduction in mitochondrial biogenesis, associated to the administration of RSV.

To further analyze the effects of RSV on mitochondrial biogenesis, the protein amount of TFAM and superoxide dismutase 2 (SOD2) were determined, by western blot experiments, in the two animal groups. There were no significant changes for both the examined proteins (Fig. 2) and these data confirm the absence of stimulation of mitochondrial biogenesis by RSV in the Sol muscle.

3.3. Effects of RSV on mitochondrial oxidative stress

RSV is a very effective scavenger of a variety of oxidants as well as it was shown to increase GSH content in several different conditions of oxidative stress (Truong et al., 2018). Therefore, we evaluated the effects induced by RSV supplementation on the age-related oxidative stress (Chimienti et al., 2021) through the determination of the GSH/GSSG ratio that clearly indicated cell redox homeostasis.

The GSH/GSSG ratio presented a statistically significant increase

Table 1
Weights of animals and soleus muscle.

	AL (*n* = 6)	RSV (n = 6)
Body weight (pre) (g)	571 ± 10.2	568 ± 8.6
Body weight (post) (g)	586 ± 15.3	581 ± 14.4
Soleus muscle wet weight (g)	0.16 ± 0.004	0.16 ± 0.006
Soleus muscle wet weight/BW (mg/g)	0.27 ± 0.005	0.28 ± 0.008

AL, ad-libitum; RSV, Resveratrol (50 mg/kg/day; 6 weeks). Data are means ± SEM.

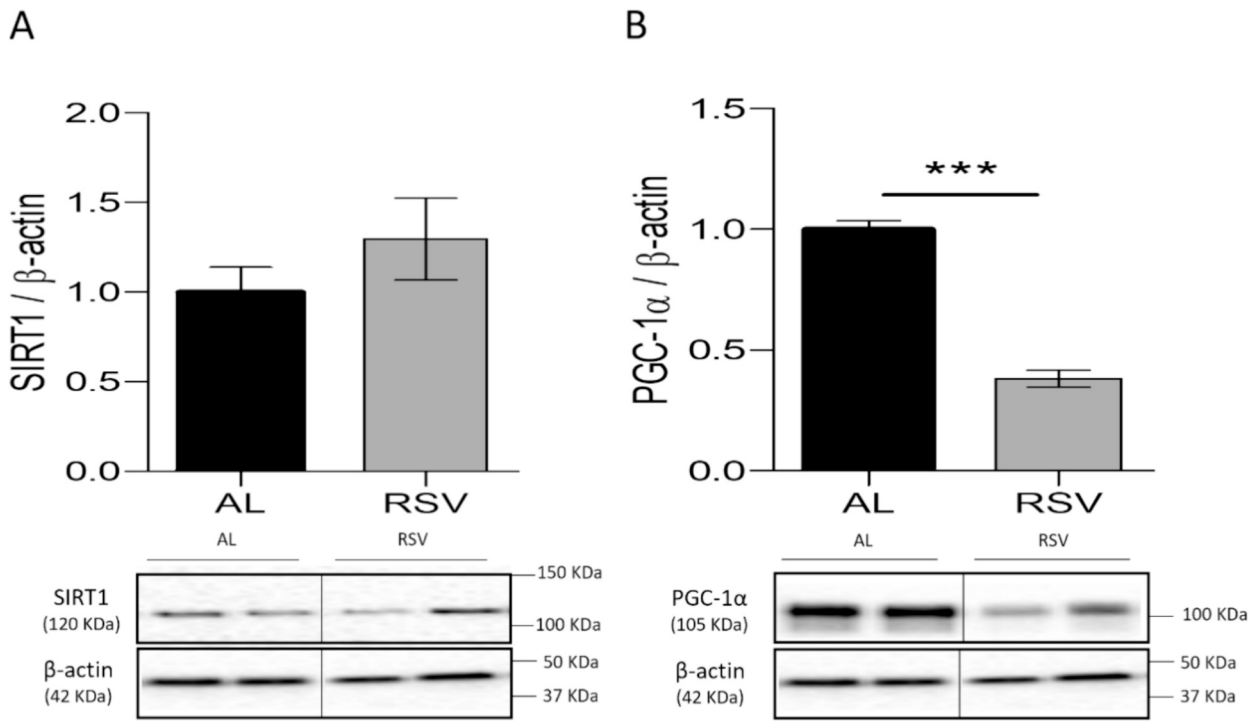

Fig. 1. Western blot analysis of SIRT1 (**A**) and PGC-1α (**B**) proteins in soleus muscle samples from AL and RSV rats. Histograms indicate the quantification of the intensity of the bands of SIRT1 (A) and of PGC-1α (B) normalized to β-actin intensity. The insets show immunobands representative of two rats from each of the analysed groups. Data represent the results from triplicate western blot experiments and were analysed using the Mann-Whitney test. Bars represent the mean values ± SEM ($n = 6$ per group) for the two experimental groups. Data were normalized against the value of the AL rat group, fixed as 1. Statistical significance: ***$p < 0.001$.

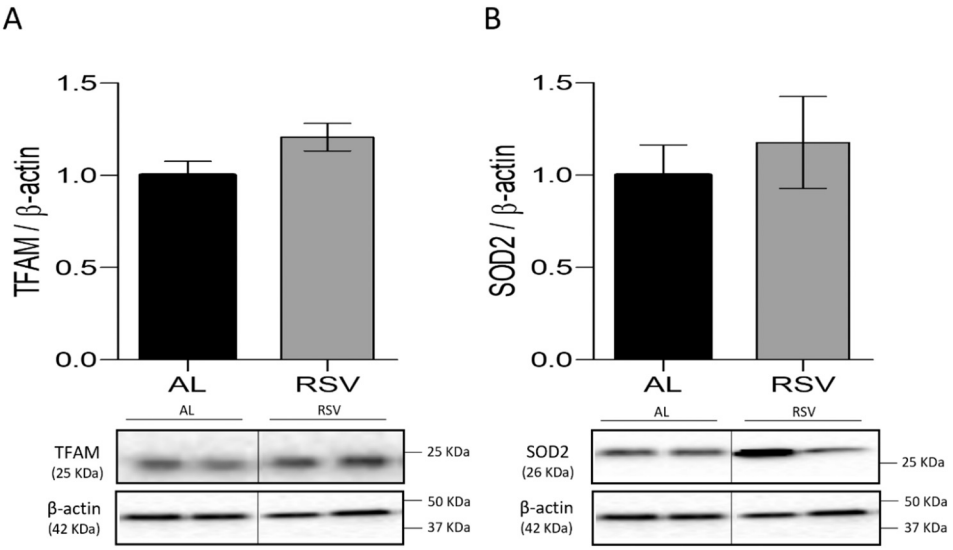

Fig. 2. Western blot analysis of TFAM (**A**) and SOD2 (**B**) proteins in soleus muscle samples from AL and RSV rats. Histograms indicate the quantification of the intensity of the bands of TFAM (A) and of SOD2 (B) normalized to β-actin intensity. The insets show immunobands representative of two rats from each of the analysed groups. Data represent the results from triplicate western blot experiments and were analysed using the Mann-Whitney test. Bars represent the mean values ± SEM (n = 6 per group) for the two experimental groups. Data were normalized against the value of the AL rat group, fixed as 1.

following RSV supplementation to old rats ($p = 0.0075$) (Fig. 3) and this demonstrated the antioxidant action by RSV. The next step was the assessment of the presence of an oxidative stress inside mitochondria and of its possible susceptibility to RSV, through the determination of a mitochondrial marker sensitive to this condition. The protein amount of peroxiredoxin III (PRXIII) was determined in both rat groups, by western blot experiments. This protein is a mitochondrial scavenger of ROS, whose expression is regulated by both mitochondrial biogenesis and ROS presence (Picca et al., 2013). The results showed a significant decrease in the protein amount in the RSV rats (−50 %) in comparison to the AL counterpart ($p < 0.001$) (Fig. 4A).

A correlation analysis between PGC-1α and PRXIII protein amounts in all assessed rats was performed to explore the relationships between PRXIII expression and mitochondrial biogenesis. A significant

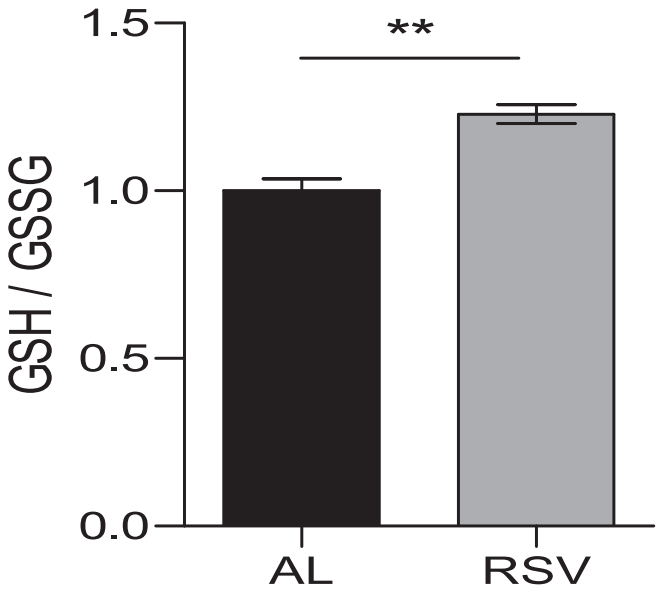

Fig. 3. Analysis of glutathione redox status in soleus muscle samples from AL and RSV rats through the determination of the redox ratio (GSH/GSSG). Bars represent the value of the GSH/GSSG ratio. Data were normalized against the value of the AL rat group, fixed as 1. Statistical significance: $^{**}p < 0.01$.

correlation ($p = 0.0055$, correlation coefficient r: 0.7622) between the tested variables was found (Fig. 4B).

It is evident that the points corresponding to the AL rats were characterized by high values of both PGC-1α and PRXIII protein amounts, whereas those representing the RSV animals were all featuring small values, suggestive of a different outcome of the coordinated regulation of the assessed proteins, depending on the animal group. The mtDNA relative content, whose amount has been shown to vary across age and oxidative stress (Chimienti et al., 2019; Orlando et al., 2019), has also been measured. The results of q-PCR analysis, in both groups of rats, are reported in Fig. 5A.

The frequency of oxidatively modified bases, mainly 8-hydroxydeoxyguanosine (OH8dG), in a specific region of mtDNA, namely the control region, spanning over the D-loop of mtDNA and crucial for replication and transcription of mtDNA (Picca et al., 2013) was also detected. The unexpected results of such analysis, carried out through digestion with the OH8dG-sensitive enzyme Fpg of the D-loop amplified region are shown in Fig. 5B.

Although no change was found in the mtDNA relative content between the AL and the RSV groups (Fig. 5A), the frequency of the oxidized base OH8dG in the analysed mtDNA specific region from the RSV group showed a significant (+50 %) increase in comparison to the AL group counterpart ($p = 0.0152$) (Fig. 5B). This indicated the existence of a heavy oxidative stress inside the organelles, resulting in a marked damage to mtDNA molecules, which might affect mitochondrial biogenesis through the extent of retrograde communication to the nucleus.

A correlation analysis was performed in all assessed rats to explore the relationships between mitochondrial biogenesis proteins and extent of oxidative damage, finding a significant correlation ($p = 0.0489$, correlation coefficient r: 0.5874) between PGC-1α protein and Fpg-sensitive damage (Fig. 6A) as well as a significant correlation ($p = 0.0390$, correlation coefficient r: 0.6084) between PRXIII protein and Fpg-sensitive damage (Fig. 6B).

The results show that AL rats featured high amounts of both proteins involved in biogenesis and low levels of mtDNA oxidative damage, while rats from the RSV group presented a reciprocal situation with low amounts of PGC-1α as well as PRXIII protein and high levels of oxidative damage. The last mitochondrial activity analysed in both groups of rats

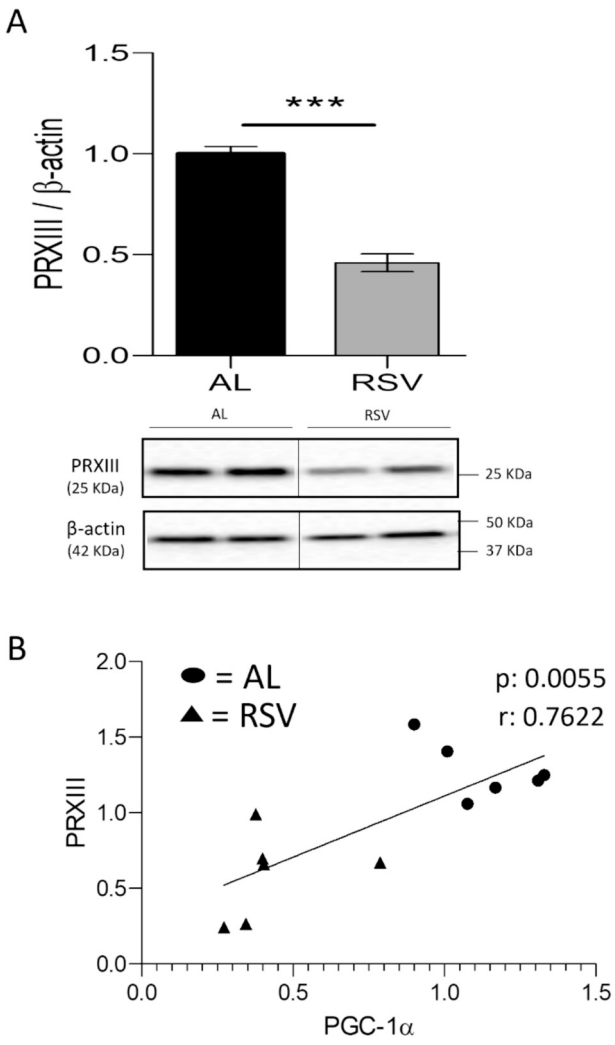

Fig. 4. Western blot analysis of PRXIII protein in soleus muscle samples from AL and RSV rats (**A**) and analysis of the correlation between PGC-1α and PRXIII proteins amounts in all assessed animals (**B**). (A) The histogram above indicates the quantification of the intensity of the bands of PRXIII normalized to β-actin intensity. The inset shows immunobands representative of two rats from each of the analysed groups. Data represent the results from triplicate western blot experiments and were analysed using the Mann-Whitney test. Bars represent the mean values ± SEM ($n = 6$ per group) for the two experimental groups. Data were normalized against the value of the AL rat group, fixed as 1. Statistical significance: $^{***}p < 0.001$. (B) r, p = Spearman r correlation test.

was dynamics.

3.4. *Effects of RSV on mitochondrial dynamics*

The effects induced by RSV on mitochondrial dynamics were verified through the determination of the protein amount of dynamin-related protein 1 (DRP1) and mitofusin 2 (MFN2), by western blot experiments, and the sequential calculation of the Fusion Index (F.I.) as the MFN2/DRP1 ratio in both rat groups under analysis. The DRP1 protein amount, indicative of fission activities, was significantly increased (+50 %) in the RSV group ($p = 0.0249$) (Fig. 7A), whereas the MFN2 protein amount, indicative of fusion activities, did not show any change between groups (Fig. 7B), as fusion was not sensitive at all to RSV's administration.

Histograms indicate the quantification of the intensity of the bands of DRP1 (A) and of MFN2 (B) normalized to β-actin intensity. The insets show immunobands representative of two rats from each of the analysed

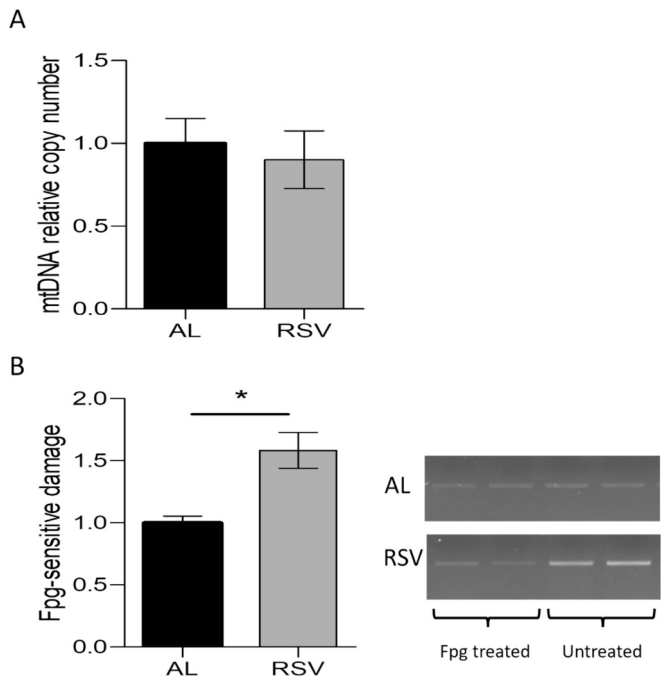

Fig. 5. MtDNA relative copy number (**A**) and oxidative damage to mtDNA (**B**). **(A)** mtDNA relative copy number in soleus muscle samples from AL and RSV rats. Data were normalized against the value of the AL rats, fixed as 1. **(B)** On the left, incidence of oxidatively modified purines at the D-loop in soleus muscle samples from AL and RSV rats. Bars in the graph represent the ratio between treated and untreated band intensities. On the right, a representative agarose gel showing amplicons obtained from Fpg-treated and untreated total DNA. Data are means ± SEM ($n = 6$ per group) and were normalized against the value of the AL rat group, fixed as 1. Statistical significance: $^*p < 0.05$.

groups. Data represent the results from triplicate western blot experiments and were analysed using the Mann-Whitney test. Bars represent the mean values ± SEM (n = 6 per group) for the two experimental groups. Data were normalized against the value of the AL rat group, fixed as 1. Statistical significance: $^*p < 0.05$. (C) Bars represent the value of the fusion index (F.I.) as the ratio between MFN2/DRP1. Data were normalized against the value of the AL rat group, fixed as 1.

Accordingly, a marked decrease in the F.I. in the RSV-treated rats, due to their strong activation of fission, was identified (Fig. 7C).

4. Discussion

Among the multiple responses, elicited by RSV administration and contributing to healthy aging, a special attention has to be dedicated to the compound's effects in skeletal muscle where it has been demonstrated to offset disuse atrophy (Petrocelli and Drummond, 2020) and skeletal muscle atrophy presented by several pathological conditions (Wang et al., 2018; Huang et al., 2019). Also the age-related progressive loss of muscle mass and strength, known as sarcopenia, has been shown to positively resent RSV treatment (Liao et al., 2017). However, a differential sarcopenic effect of aging has been demonstrated, dependent on muscle fiber types (preferentially affecting fast glycolytic fibers versus slow oxidative ones) (McKiernan et al., 2004; Picard et al., 2011; Hosoda et al., 2023). Since data about RSV effects in the slow oxidative Sol muscle are lacking, it was very interesting for us to deepen the eventual induction of fiber type-specific responses by RSV in this muscle. In fact, previous studies have largely reported the ability of RSV, in fast twitch muscles, to cause a shift towards more oxidative fibers, driving to increased mitochondrial content in glycolytic fibers compared to oxidative fibers (Lagouge et al., 2006; Price et al., 2012; Ljubicic et al., 2014). In the present work RSV dosage (50 mg/kg/day) and length of treatment (6 weeks supplementation to aged rats) were the same applied in a previous study that analysed separately the white glycolytic part of gastrocnemius (WG) and the red oxidative part of gastrocnemius (RG) (Joseph et al., 2013). The results of that paper have been used for comparisons with our findings from Sol muscle to detect eventual fiber type-specific differences in the evoked responses. In particular, in the present study the effects, induced at level of mitochondrial biogenesis, oxidative stress, quality control and dynamics, by RSV supplementation, have been thoroughly examined to widen knowledge about the efficacy of RSV administration to aged subjects for short periods. The results of Table 1 indicated that RSV did not affect body weight nor Sol weight in comparison with the AL counterpart, thus ruling out any eventual activity of RSV to increase muscle's protein mass. Furthermore, the first expected response, related to the CR-mimetic activity of RSV (Muhammad and Allam, 2018; Iside et al., 2020) namely the induction of SIRT1 expression, was not found in the RSV group as if RSV supplementation was not activating one of the molecule's beneficial cell pathways in the Sol oxidative fibers. However, in the comparable study on RSV's effects in WG and RG, RSV increased SIRT1 expression in the glycolytic WG, but

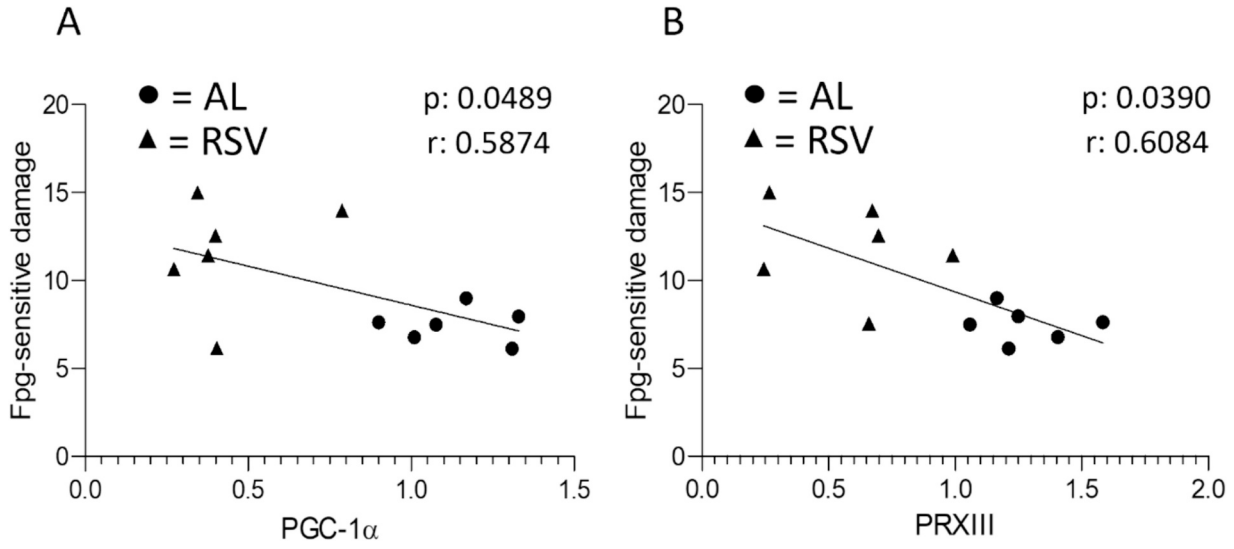

Fig. 6. Analysis of the correlation between mitochondrial biogenesis proteins and Fpg-sensitive damage in the assessed animals ($n = 6$ per group); r, p = Spearman r correlation test. (**A**) Analysis of the correlation between PGC-1α and Fpg-sensitive damage. (**B**) Analysis of the correlation between PRXIII and Fpg-sensitive damage.

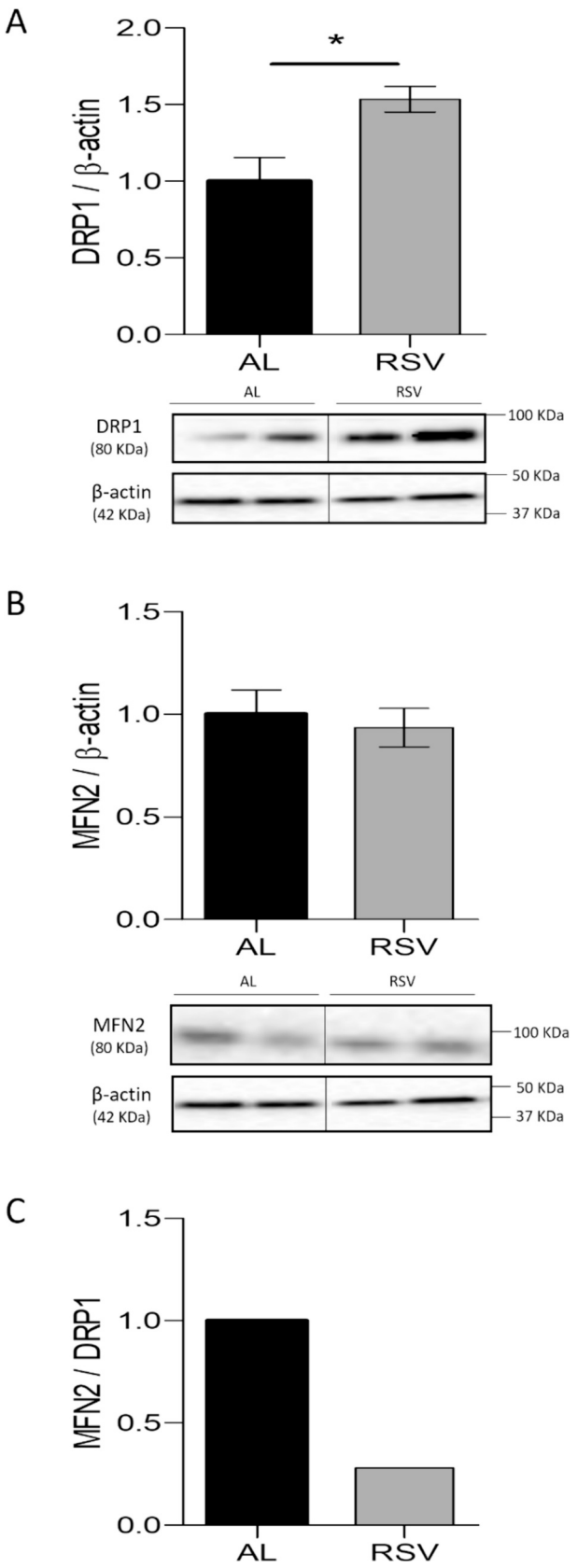

Fig. 7. Western blot analysis of DRP1 (**A**) and MFN2 (**B**) proteins and Fusion Index (F.I.) (**C**) in soleus muscle samples from AL and RSV rats.

not in the mixed glycolytic-oxidative RG as if RSV was inducing a progressive negative response for SIRT1 expression, in comparison to the AL rat counterpart, passing from the glycolytic WG, to the mixed glycolytic-oxidative RG (Joseph et al., 2013) to the oxidative Sol. Furthermore, it is well proven that some beneficial effects of RSV on metabolism are due to an increase in mitochondrial biogenesis, obtained through SIRT1 activity (Price et al., 2012) and the SIRT1-dependent increase in expression and activity of PGC-1α (Nemoto et al., 2005; Lagouge et al., 2006; Muhammad and Allam, 2018; Niu et al., 2021). In the present study a statistically significant, relevant (−60 %) decrease in PGC-1α amount in RSV rats Sol muscle was found, whereas in the comparable work RSV did not affect PGC-1α amount in WG as well as in RG (Joseph et al., 2013). A reasonable explanation of such results is that RSV was inducing a growing inhibitory effect on mitochondrial biogenesis in SM passing from the glycolytic WG, to the mixed glycolytic-oxidative RG to the oxidative Sol, in comparison to the AL rats. To deepen this issue, we measured the protein expression of two genes regulated by mitochondrial biogenesis namely TFAM and SOD2 (Picca et al., 2013; Kitada et al., 2020), finding no significant change in their respective protein amounts in the RSV rat group. Also, in the comparable paper there was no change of SOD2 expression in both WG and RG (Joseph et al., 2013), whereas a recent report demonstrated that RSV treatment led to the upregulation of SOD2 in the heart through mediation by SIRT1 (Li et al., 2020). These data supported the absence of stimulation of mitochondrial biogenesis by RSV in the oxidative fibers of Sol. Expression of SOD2 has been reported to be induced also by release of mitochondrial ROS (Storz et al., 2005) and, therefore, the cell redox homeostasis was assessed in both groups of rats through the determination of the GSH/GSSG ratio. RSV was shown to increase GSH content in several different conditions of oxidative stress (Truong et al., 2018) and in the present study RSV supplementation increased GSH/GSSG ratio in Sol as whether RSV very effective scavenging activity could counteract the age-related oxidative stress (Chimienti et al., 2021). The determination of the protein amount of PRXIII in both rat groups verified also the antioxidant RSV action at mitochondrial level. In fact, the expression of the PRXIII mitochondrial scavenger of Reactive Oxygen Species (ROS) is regulated by both mitochondrial biogenesis (Huh et al., 2012; Picca et al., 2013) and ROS (Hwang et al., 2010; Wang et al., 2020). The results showed a very relevant, statistically significant decrease in the protein amount in the RSV rats (−50 %) in comparison to the AL counterpart, which was consistent with the PGC-1α reduction in the same group of rats. The correlation analysis between PGC-1α and PRXIII protein amounts, carried out in all assessed rats, demonstrated a highly significant correlation between the tested variables. This was a novel result of the present study because it demonstrated the coordinated modulation of PGC-1α and PRXIII expression in different conditions, while, previously, expression of PRXIII has been shown to be physiologically regulated by FoxO3a through the involvement of the latter in a complex with PGC-1α, controlled by SIRT1 (Olmos et al., 2013). As for the effect of RSV supplementation on PRXIII expression in SM, no previous data are available, whereas a recent work focused on PRXIII acetylation level, regulated by the RSV-sensitive mitochondrial sirtuin SIRT3, activated by SIRT1 in the porcine intestinal epithelial cell line IPEC-J2 (Chen et al., 2021). This report also deepened the other crucial activity of RSV that is the compound's antioxidant effect on cell and mitochondrial redox homeostasis. In fact, RSV supplementation has been shown to inhibit, via a SIRT1-directed pathway, a H_2O_2-induced mitochondrial ROS accumulation leading to increased OH8dG level and decreased content of mtDNA in the same IPEC-J2 cells (Chen et al., 2021). According to this study, SIRT1 should be crucial in RSV's ability to counteract mitochondrial oxidative stress and its functional consequences through promotion of mitochondrial biogenesis and antioxidant capacity. In the present study the expected RSV ability to support mitochondrial biogenesis and antioxidant activity, through SIRT1 increased expression and activity, was totally lacking in the oxidative fibers of Sol. A very intriguing result of this work is the 50 %-increased frequency of oxidatively modified bases,

mainly OH8dG, in the assayed mtDNA region only from the RSV group. A possible explanation for such unexpected result might be the presence of a severe age-related oxidative stress inside mitochondria of both groups, which in organelles from the RSV group Sol led to the mtDNA oxidative damage accumulation, but not to a loss of mtDNA content, suggestive of a SM-specific "tolerance" towards mtDNA oxidative damage as proposed in a very recent study (Aimaretti et al., 2023). The present RSV results support the idea of a high threshold of oxidative damage to mtDNA in the oxidative Sol, which could be tolerated without marked consequences for mtDNA content, but was associated to a decrease in both mitochondrial biogenesis and antioxidant activity, revealed by the inverse correlation, respectively, between PGC-1α amount and Fpg-sensitive damage and between PRXIII amount and Fpg-sensitive damage. It can be envisioned that, in the RSV rats, RSV antioxidant activity prevented the retrograde communication of the existing age-related mitochondrial oxidative stress to the nucleus and the induction of some compensatory mechanisms promoting mitochondrial biogenesis and antioxidant activities as well as counteracting the accumulation of oxidized mtDNA. The likely suppression/alteration of retrograde communication passed an erroneous message, not leading to the repair of oxidized mtDNA molecules, but preserving the overall quantity of heavily damaged mtDNA. Contrary to what found in RSV rats, the above-mentioned correlations between PGC-1α or PRXIII protein amounts and Fpg-sensitive damage demonstrated in AL rats the presence of reduced mtDNA damage associated to induced mitochondrial biogenesis and antioxidant activity, likely obtained through an efficient retrograde communication. To appraise the hypothesis of RSV's influence on retrograde communication, the balance of mitochondrial dynamics was also analysed, through the determination of the protein amount of DRP1 and MFN2 and the sequential calculation of the F.I. in both rat groups. A significant and relevant increase (+50 %) in DRP1 amount, indicative of fission activities, was found in the RSV group, whereas the MFN2 amount, indicative of fusion activities, did not show any change between both groups, as fusion was not sensitive to RSV's administration. Such changes led to a marked decrease in the F.I. in the RSV-treated rats due to their strong activation of fission. As for the effects of aging on SM mitochondrial dynamics, literature reported different results dependent on the predominant fiber type of the assayed muscle. In fact, an increase in DRP1 protein (pro-fission) was described in the oxidative Sol versus a concomitant increase in the expression of both pro-fusion and pro-fission proteins in the glycolytic WG of old AL rats (Faitg et al., 2019). As for RSV effects on mitochondrial dynamics, previous reports described a positive action by the molecule through promotion of fusion and reduction of fission leading to an improvement in mitochondrial function in tissues different from SM (Yu et al., 2023) and RSV was also shown to inhibit the overexpression of DRP1, induced by an oxidative stress condition (Lei et al., 2022). In the present results from Sol of RSV-treated rats, the age-related overexpression of DRP1 (Faitg et al., 2019) was even enhanced by RSV supplementation in comparison with the value from AL rats. Therefore, RSV was not able to decrease DRP1 overexpression in the Sol because the compound did not cause SIRT1 and PGC-1α induction/activation. On overall, the present experimental results clearly demonstrate the lack of positive effects on mitochondrial functionality, after RSV supplementation to old rats, in the oxidative muscle Sol and this can be explained by a novel hypothesis about RSV impingement on retrograde communication from mitochondria to nucleus. The retrograde communication may be induced by different stimuli, including ROS, in a dose-dependent manner (Walker and Moraes, 2022). It might be suggested that RSV in old Sol, neutralizing all ROS directed to the nucleus, might have prevented their signaling activity, required to regulate skeletal muscle function and adaptation, resulting deleterious. In fact, it is fully acknowledged the dual function of mitochondrial ROS to both promote cell damage and promote cell adaptation. Therefore, being mitochondrial ROS needed for normal physiological processes, an increase in antioxidant capacity, as occurring with RSV supplementation to old rats, likely prevented normal adaptation to stress and turned out to be detrimental rather than beneficial. In particular, inhibition of mitochondrial ROS by RSV and other antioxidants has an unpredictable outcome on cell function because mitochondrial ROS effects change according to different environmental conditions (Sena and Chandel, 2012). Present results suggest that in the oxidative fibers of Sol from old rats the age-related oxidative stress might have induced compensatory responses through a ROS-mediated retrograde communication. The increased antioxidant activity, obtained by RSV supplementation, should have prevented an efficient signaling to the nucleus and led to a decrease in mitochondrial biogenesis in spite of the accumulated oxidative damage to mtDNA. This defective response is fiber-type specific and pinpoints the need that all analyses about the effects of nutritional or lifestyle interventions, genetic manipulations or treatments on skeletal muscle, focused on mitochondria, have to be carried out in both glycolytic and oxidative muscles to reach, through comparison of the respective results, complete and reliable conclusions because the impact of aging and anti-aging strategies appears fiber type-specific.

5. Limitations

Our study has some limitations, in fact, due to the very small size of Sol samples, we had to limit our present analysis to a little number of markers especially as for mitochondrial dynamics, assessed only through the determination of DRP1 and MFN2, without having the possibility of testing other relevant markers involved in fusion as MARCH5, OMA1, PARL and in fission as CDK1, Calcineurin, MAPL (Li et al., 2021). Furthermore, for the same limitation of samples size, we could not analyze at all mitophagy, which is deeply involved in the mitochondrial quality control processes (Li et al., 2021) and that will certainly be investigated in a future study about the other effects induced by RSV supplementation to old rats Soleus muscle. The present study, indeed, focused on some mitochondrial molecular markers, which were analysed aiming to verify if RSV supplementation to old rats was able to promote mitochondrial biogenesis in the oxidative Sol muscle as already demonstrated in the glycolytic muscles TA (Hosoda et al., 2023) and G (Muhammad and Allam, 2018). Another limitation of the present study is the absence of a functional analysis evaluating the eventual RSV-related changes in physical performance by means of grip strength or rotarod tests, which will be certainly included in future studies on this subject.

6. Conclusions

The take-home message that can be derived from the present study is that the administration of RSV to aged rats did not induce in the oxidative Sol SM responses able to counteract the age-related decline of mitochondrial functions, whereas likely impinged on retrograde communication affecting expression of SIRT1, PGC-1α and PRXIII in comparison with AL animals. Our interpretation of present data is that RSV suppressed ROS important for cell signaling and thus abolished the retrograde communication, which would have otherwise led to changes in expression of proteins involved in mitochondrial biogenesis (SIRT1, PGC-1α, TFAM), antioxidant induction (SOD2, PRXIII) and dynamics (DRP1, MFN2), effective in counteracting the mitochondrial decline. The comparison with a previous parallel paper on glycolytic SM, treated with the same RSV protocol, revealed different fiber type-specific responses. Therefore, future studies should test the effects of RSV in both oxidative and glycolytic SM to evaluate in a very comprehensive way the potential differential responses induced by the compound and to allow a more reliable foresight as for translatable applications to humans.

Funding

The University of Bari Aldo Moro has supported and funded this work through Fondi di Ateneo 2020 and 2021 (University of Bari) to

Angela Maria Serena Lezza and Vito Pesce. This research was funded also by grant MIUR-FFABR 2018 to Angela Maria Serena Lezza, grant MIUR-FFABR 2018 to Vito Pesce. We are grateful for the support of the Consorzio Interuniversitario per le Biotecnologie (CIB).

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Declaration of competing interest

The authors declare that there is no potential conflict of interest.

References

Aimaretti, E., Chimienti, G., Rubeo, C., Di Lorenzo, R., Trisolini, L., Dal Bello, F., Moradi, A., Collino, M., Lezza, A.M.S., Aragno, M., Pesce, V., 2023. Different effects of high-fat/high-sucrose and high-fructose diets on advanced glycation end-product accumulation and on mitochondrial involvement in heart and skeletal muscle in mice. Nutrients 15, 4874. https://doi.org/10.3390/nu15234874.

Brooke, M.H., Kaiser, K.K., 1970. Muscle fiber types: how many and what kind? Arch. Neurol. 23, 369–379. https://doi.org/10.1001/archneur.1970.00480280083010.

Camins, A., Junyent, F., Verdaguer, E., Beas-Zarate, C., Rojas-Mayorquín, A.E., Ortuño-Sahagún, D., Pallàs, M., 2009. Resveratrol: an antiaging drug with potential therapeutic applications in treating diseases. Pharmaceuticals (Basel) 2, 194–205. https://doi.org/10.3390/ph2030194.

Carter, E.E., Thomas, M.M., Murynka, T., Rowan, S.L., Wright, K.J., Huba, E., Hepple, R. T., 2010. Exp. Gerontol. 45, 662–670. https://doi.org/10.1016/j.exger.2010.04.001.

Chen, Y., Zhang, H., Ji, S., Jia, P., Chen, Y., Li, Y., Wang, T., 2021. Resveratrol and its derivative pterostilbene attenuate oxidative stress-induced intestinal injury by improving mitochondrial redox homeostasis and function via SIRT1 signaling. Free Radic. Biol. Med. 177, 1–14. https://doi.org/10.1016/j.freeradbiomed.2021.10.011.

Chimienti, G., Picca, A., Fracasso, F., Marzetti, E., Calvani, R., Leeuwenburgh, C., Russo, F., Lezza, A.M.S., Pesce, V., 2019. Differences in liver TFAM binding to mtDNA and mtDNA damage between aged and extremely aged rats. Int. J. Mol. Sci. 20, 2601. https://doi.org/10.3390/ijms20102601.

Chimienti, G., Picca, A., Fracasso, F., Russo, F., Orlando, A., Riezzo, G., Leeuwenburgh, C., Pesce, V., Lezza, A.M.S., 2021. The age-sensitive efficacy of calorie restriction on mitochondrial biogenesis and mtDNA damage in rat liver. Int. J. Mol. Sci. 22, 1665. https://doi.org/10.3390/ijms22041665.

Faitg, J., Leduc-Gaudet, J.P., Reynaud, O., Ferland, G., Gaudreau, P., Gouspillou, G., 2019. Effects of aging and caloric restriction on fiber type composition, mitochondrial morphology and dynamics in rat oxidative and glycolytic muscles. Front. Physiol. 10, 420. https://doi.org/10.3389/fphys.2019.00420.

Gadaleta, M.N., Cormio, A., Pesce, V., Lezza, A.M.S., Cantatore, P., 1998. Aging and mitochondria. Biochimie 80, 863–879. https://doi.org/10.1016/s0300-9084(00)88881-1.

Handy, R.M., DesOrmeaux, G.J., Barbeau, P.A., Frangos, S.M., Holloway, G.P., 2023. Independent, but not co-supplementation, with nitrate and resveratrol improves glucose tolerance and reduces markers of cellular stress in high-fat-fed male mice. Am. J. Physiol. Regul. Integr. Comp. Physiol. 324, R317–R328. https://doi.org/10.1152/ajpregu.00196.2022.

Holloszy, J.O., Coyle, E.F., 1984. Adaptations of skeletal muscle to endurance exercise and their metabolic consequences. J. Appl. Physiol. Respir. Environ. Exerc. Physiol. 56, 831–838. https://doi.org/10.1152/jappl.1984.56.4.831.

Hosoda, R., Nakashima, R., Yano, M., Iwahara, N., Asakura, S., Nojima, I., Saga, Y., Kunimoto, R., Horio, Y., Kuno, A., 2023. Resveratrol, a SIRT1 activator, attenuates aging-associated alterations in skeletal muscle and heart in mice. J. Pharmacol. Sci. 152, 112–122. https://doi.org/10.1016/j.jphs.2023.04.001.

Huang, Y., Zhu, X., Chen, K., Lang, H., Zhang, Y., Hou, P., Ran, L., Zhou, M., Zheng, J., Yi, L., Mi, M., Zhang, Q., 2019. Resveratrol prevents sarcopenic obesity by reversing mitochondrial dysfunction and oxidative stress via the PKA/LKB1/AMPK pathway. Aging (Albany NY) 11, 2217–2240. https://doi.org/10.18632/aging.101910.

Huh, J.Y., Kim, Y., Jeong, J., Park, J., Kim, I., Huh, K.H., Kim, Y.S., Woo, H.A., Rhee, S. G., Lee, K.J., 2012. Peroxiredoxin 3 is a key molecule regulating adipocyte oxidative stress, mitochondrial biogenesis, and adipokine expression. Antioxid. Redox Signal. 16, 229–243. https://doi.org/10.1089/ars.2010.3766.

Hwang, I.K., Yoo, K.Y., Kim, D.W., Lee, C.H., Choi, J.H., Kwon, Y.G., Kim, Y.M., Choi, S. Y., Won, M.H., 2010. Changes in the expression of mitochondrial peroxiredoxin and thioredoxin in neurons and glia and their protective effects in experimental cerebral ischemic damage. Free Radic. Biol. Med. 48, 1242–1251. https://doi.org/10.1016/j.freeradbiomed.2010.02.007.

Iside, C., Scafuro, M., Nebbioso, A., Altucci, L., 2020. SIRT1 activation by natural phytochemicals: an overview. Front. Pharmacol. 11, 1225. https://doi.org/10.3389/fphar.2020.01225.

Jackson, J.R., Ryan, M.J., Always, S.E., 2011. Long-term supplementation with resveratrol alleviates oxidative stress but does not attenuate sarcopenia in aged mice. J. Gerontol. A Biol. Sci. Med. Sci. 66, 751–764. https://doi.org/10.1093/gerona/glr047.

Joseph, A.M., Malamo, A.G., Silvestre, J., Wawrzyniak, N., Carey-Love, S., Nguyen, L.M. D., Dutta, D., Xu, J., Leeuwenburgh, C., Adhihetty, P.J., 2013. Short-term caloric restriction, resveratrol, or combined treatment regimens initiated in late-life alter mitochondrial protein expression profiles in a fiber-type specific manner in aged animals. Exp. Gerontol. 48, 858–868. https://doi.org/10.1016/j.exger.2013.05.061.

Kitada, M., Xu, J., Ogura, Y., Monno, I., Koya, D., 2020. Manganese superoxide dismutase dysfunction and the pathogenesis of kidney disease. Front. Physiol. 11, 755. https://doi.org/10.3389/fphys.2020.00755.

Lagouge, M., Argmann, C., Gerhart-Hines, Z., Meziane, H., Lerin, C., Daussin, F., Messadeq, N., Milne, J., Lambert, P., Elliott, P., Geny, B., Laakso, M., Puigserver, P., Auwerx, J., 2006. Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1alpha. Cell 127, 1109–1122. https://doi.org/10.1016/j.cell.2006.11.013.

Lam, Y.Y., Peterson, C.M., Ravussin, E., 2013. Resveratrol vs. calorie restriction: data from rodents to humans. Exp. Gerontol. 48, 1018–1024. https://doi.org/10.1016/j.exger.2013.04.005.

Larsson, L., Edström, L., 1986. Effects of age on enzyme-histochemical fibre spectra and contractile properties of fast- and slow-twitch skeletal muscles in the rat. J. Neurol. Sci. 76, 69–89. https://doi.org/10.1016/0022-510x(86)90143-7.

Lei, M.Y., Cong, L., Liu, Z.Q., Liu, Z.F., Ma, Z., Liu, K., Li, J., Deng, Y., Liu, W., Xu, B., 2022. Resveratrol reduces DRP1-mediated mitochondrial dysfunction via the SIRT1-PGC1α signaling pathway in manganese-induced nerve damage in mice. Environ. Toxicol. 37, 282–298. https://doi.org/10.1002/tox.23397.

Lexell, J., Taylor, C.C., Sjöström, M., 1988. What is the cause of the ageing atrophy? Total number, size and proportion of different fiber types studied in whole vastus lateralis muscle from 15- to 83-year-old men. J. Neurol. Sci. 84, 275–294. https://doi.org/10.1016/0022-510x(88)90132-3.

Li, P., Song, X., Zhang, D., Guo, N., Wu, C., Chen, K., Liu, Y., Yuan, L., Chen, X., Huang, X., 2020. Resveratrol improves left ventricular remodeling in chronic kidney disease via Sirt1-mediated regulation of FoxO1 activity and MnSOD expression. Biofactors 46, 168–179. https://doi.org/10.1002/biof.1584.

Li, L., Qi, R., Zhang, L., Yu, Y., Hou, J., Gu, Y., Song, D., Wang, X., 2021. Potential biomarkers and targets of mitochondrial dynamics. Clin. Transl. Med. 11, e529 https://doi.org/10.1002/ctm2.529.

Liao, Z.Y., Chen, J.L., Xiao, M.H., Sun, Y., Zhao, Y.X., Pu, D., Lv, A.K., Wang, M.L., Zhou, J., Zhu, S.Y., Zhao, K.-X., Xiao, Q., 2017. The effect of exercise, resveratrol or their combination on sarcopenia in aged rats via regulation of AMPK/Sirt1 pathway. Exp. Gerontol. 98, 177–183. https://doi.org/10.1016/j.exger.2017.08.032.

Liuzzi, G.M., Latronico, T., Branà, M.T., Gramegna, P., Coniglio, M.G., Rossano, R., Larocca, M., Riccio, P., 2011. Structure-dependent inhibition of gelatinases by dietary antioxidants in rat astrocytes and sera of multiple sclerosis patients. Neurochem. Res. 36, 518–527. https://doi.org/10.1007/s11064-010-0373-2.

Ljubicic, V., Burt, M., Lunde, J.A., Jasmin, B.J., 2014. Resveratrol induces expression of the slow, oxidative phenotype in mdx mouse muscle together with enhanced activity of the SIRT1-PGC-1α axis. Am. J. Physiol. Cell Physiol. 307, C66–C82. https://doi.org/10.1152/ajpcell.00357.2013.

Madeo, F., Carmona-Gutierrez, D., Hofer, S.J., Kroemer, G., 2019. Caloric restriction mimetics against age-associated disease: targets, mechanisms, and therapeutic potential. Cell Metab. 29, 592–610. https://doi.org/10.1016/j.cmet.2019.01.018.

McKiernan, S.H., Bua, E., McGorray, J., Aiken, J., 2004. Early-onset calorie restriction conserves fiber number in aging rat skeletal muscle. FASEB J. 18, 580–581. https://doi.org/10.1096/fj.03-0667fje.

Muhammad, M.H., Allam, M.M., 2018. Resveratrol and/or exercise training counteract aging-associated decline of physical endurance in aged mice; targeting mitochondrial biogenesis and function. J. Physiol. Sci. 68, 681–688. https://doi.org/10.1007/s12576-017-0582-4.

Musillo, C., Borgi, M., Saul, N., Möller, S., Luyten, W., Berry, A., Cirulli, F., 2021. Natural products improve healthspan in aged mice and rats: a systematic review and meta-analysis. Neurosci. Biobehav. Rev. 121, 89–105. https://doi.org/10.1016/j.neubiorev.2020.12.001.

Nemoto, S., Fergusson, M.M., Finkel, T., 2005. SIRT1 functionally interacts with the metabolic regulator and transcriptional coactivator PGC-1alpha. J. Biol. Chem. 280, 16456–16460. https://doi.org/10.1074/jbc.m501485200.

Niu, W., Wang, H., Wang, B., Mao, X., Du, M., 2021. Resveratrol improves muscle regeneration in obese mice through enhancing mitochondrial biogenesis. J. Nutr. Biochem. 98, 108804 https://doi.org/10.1016/j.jnutbio.2021.108804.

Olmos, Y., Sanchez-Gomez, F.J., Wild, B., Garcia-Quintans, N., Cabezudo, S., Lamas, S., Monsalve, M., 2013. SirT1 regulation of antioxidant genes is dependent on the formation of a FoxO3a/PGC-1alpha complex. Antioxid. Redox Signal. 19, 1507–1521. https://doi.org/10.1089/ars.2012.4713.

Orlando, A., Chimienti, G., Pesce, V., Fracasso, F., Lezza, A.M.S., Russo, F., 2019. An in vitro study on mitochondrial compensatory response induced by gliadin peptides in Caco-2 cells. Int. J. Mol. Sci. 20, 1862. https://doi.org/10.3390/ijms20081862.

Pesce, V., Fracasso, F., Cassano, P., Lezza, A.M.S., Cantatore, P., Gadaleta, M.N., 2010. Acetyl-L-carnitine supplementation to old rats partially reverts the age-related mitochondrial decay of soleus muscle by activating peroxisome proliferator-

activated receptor gamma coactivator-1alpha-dependent mitochondrial biogenesis. Rejuvenation Res. 13, 148–151. https://doi.org/10.1089/rej.2009.0955.
Petrocelli, J.J., Drummond, M.J., 2020. PGC-1α-targeted therapeutic approaches to enhance muscle recovery in aging. Int. J. Environ. Res. Public Health 17, 8650. https://doi.org/10.3390/ijerph17228650.
Picard, M., McEwen, B.S., 2018. Psychological stress and mitochondria: a systematic review. Psychosom. Med. 80, 141–153. https://doi.org/10.1097/PSY.0000000000000545.
Picard, M., Ritchie, D., Thomas, M.M., 2011. Wright KJ, Hepple RT. Alterations in intrinsic mitochondrial function with aging are fiber type-specific and do not explain differential atrophy between muscles. Aging Cell 10, 1047–1055. https://doi.org/10.1111/j.1474-9726.2011.00745.x.
Picca, A., Pesce, V., Fracasso, F., Joseph, A.M., Leeuwenburgh, C., Lezza, A.M.S., 2013. Aging and calorie restriction oppositely affect mitochondrial biogenesis through TFAM binding at both origins of mitochondrial DNA replication in rat liver. PloS One 8, e74644. https://doi.org/10.1371/journal.pone.0074644.
Picca, A., Pesce, V., Fracasso, F., Joseph, A.M., Leeuwenburgh, C., Lezza, A.M.S., 2014. A comparison among the tissue-specific effects of aging and calorie restriction on TFAM amount and TFAM-binding activity to mtDNA in rat. Biochim. Biophys. Acta 1840, 2184–2191. https://doi.org/10.1016/j.bbagen.2014.03.004.
Price, N.L., Gomes, A.P., Ling, A.J.Y., Duarte, F.V., Martin-Montalvo, A., North, B.J., Agarwal, B., Ye, L., Ramadori, G., Teodoro, J.S., Hubbard, B.P., Varela, A.T., Davis, J.G., Varamini, B., Hafner, A., Moaddel, R., Rolo, A.P., Coppari, R., Palmeira, C.M., de Cabo, R., Baur, J.A., Sinclair, D.A., 2012. SIRT1 is required for AMPK activation and the beneficial effects of resveratrol on mitochondrial function. Cell Metab. 15, 675–690. https://doi.org/10.1016/j.cmet.2012.04.003.
Qu, Z., Zhou, S., Li, P., Liu, C., Yuan, B., Zhang, S., Liu, A., 2021. Natural products and skeletal muscle health. J. Nutr. Biochem. 93, 108619 https://doi.org/10.1016/j.jnutbio.2021.108619.
Rahman, I., Kode, A., Biswas, S.K., 2006. Assay for quantitative determination of glutathione and glutathione disulfide levels using enzymatic recycling method. Nat. Protoc. 1, 3159–3165. https://doi.org/10.1038/nprot.2006.378.
Schrauwen, P., Timmers, S., 2014. Can resveratrol help to maintain metabolic health? Proc. Nutr. Soc. 73, 271–277. https://doi.org/10.1017/s0029665113003856.
Sena, L.A., Chandel, N.S., 2012. Physiological roles of mitochondrial reactive oxygen species. Mol. Cell 48, 158–167. https://doi.org/10.1016/j.molcel.2012.09.025.
Storz, P., Döppler, H., Toker, A., 2005. Protein kinase D mediates mitochondrion-to-nucleus signaling and detoxification from mitochondrial reactive oxygen species. Mol. Cell. Biol. 25, 8520–8530. https://doi.org/10.1128/MCB.25.19.8520-8530.2005.
Thompson, L.V., 2022. Skeletal muscle adaptations with age, inactivity, and therapeutic exercise. J. Orthop. Sports Phys. Ther. 32, 44–57. https://doi.org/10.2519/jospt.2002.32.2.44.
Truong, V.L., Jun, M., Jeong, W.S., 2018. Role of resveratrol in regulation of cellular defense systems against oxidative stress. Biofactors 44, 36–49. https://doi.org/10.1002/biof.1399.
Walker, B.R., Moraes, C.T., 2022. Nuclear-Mitochondrial Interactions. Biomolecules 12, 427. https://doi.org/10.3390/biom12030427.
Wang, D., Sun, H., Song, G., Yang, Y., Zou, X., Han, P., Li, S., 2018. Resveratrol improves muscle atrophy by modulating mitochondrial quality control in STZ-induced diabetic mice. Mol. Nutr. Food Res. 62, e1700941 https://doi.org/10.1002/mnfr.201700941.
Wang, Z., Sun, R., Wang, G., Chen, Z., Li, Y., Zhao, Y., Liu, D., Zhao, H., Zhang, F., Yao, J., Tian, X., 2020. SIRT3-mediated deacetylation of PRDX3 alleviates mitochondrial oxidative damage and apoptosis induced by intestinal ischemia/reperfusion injury. Redox Biol. 28, 101343 https://doi.org/10.1016/j.redox.2019.101343.
Yu, T., Wang, L., Zhang, L., Deuster, P.A., 2023. Mitochondrial fission as a therapeutic target for metabolic diseases: insights into antioxidant strategies. Antioxidants (Basel) 12, 1163. https://doi.org/10.3390/antiox12061163.
Zhang, L.X., Li, C.X., Kakar, M.U., Khan, M.S., Wu, P.F., Amir, R.M., Dai, D.F., Naveed, M., Li, Q.Y., Saeed, M., Shen, J.-Q., Rajput, S.A., Li, J.-H., 2021. Show more resveratrol (RV): a pharmacological review and call for further research. Biomed. Pharmacother. 143, 112164 https://doi.org/10.1016/j.biopha.2021.112164.