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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

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ABSTRACT

Aging is associated with a loss in muscle known as sarcopenia that is partially attributed to apoptosis. In aging rodents, caloric restriction (CR) increases health and longevity by improving mitochondrial function and the polyphenol resveratrol (RSV) has been reported to have similar benefits. In the present study, we investigated the potential efficacy of using short-term (6 weeks) CR (20%), RSV (50 mg/kg/day), or combined CR + RSV (20% CR and 50 mg/kg/day RSV), initiated at late-life (27 months) to protect muscle against sarcopenia by altering mitochondrial function, biogenesis, content, and apoptotic signaling in both glycolytic white and oxidative red gastrocnemius muscle (WG and RG, respectively) of male Fischer 344 × Brown Norway rats. CR but not RSV attenuated the age-associated loss of muscle mass in both mixed gastrocnemius and soleus muscle, while combined treatment (CR + RSV) paradigms showed a protective effect in the soleus and plantaris muscle ($P < 0.05$). Sirt1 protein content was increased by 2.6-fold ($P < 0.05$) in WG but not RG muscle with RSV treatment, while CR or CR + RSV had no effect. PGC-1α levels were higher (2-fold) in the WG from CR-treated animals ($P < 0.05$) when compared to ad-libitum (AL) animals but no differences were observed in the RG with any treatment. Levels of the anti-apoptotic protein Bcl-2 were significantly higher (1.6-fold) in the WG muscle of RSV and CR + RSV groups compared to AL ($P < 0.05$) but tended to occur coincident with elevations in the pro-apoptotic protein Bax so that the apoptotic susceptibility as indicated by the Bax to Bcl-2 ratio was unchanged. There were no alterations in DNA fragmentation with any treatment in muscle from older animals. Additionally, mitochondrial respiration measured in permeabilized muscle fibers was unchanged in any treatment group and this paralleled the lack of change in cytochrome *c* oxidase (COX) activity. These data suggest that short-term moderate CR, RSV, or CR + RSV tended to modestly alter key mitochondrial regulatory and apoptotic signaling pathways in glycolytic muscle and this might contribute to the moderate protective effects against aging-induced muscle loss observed in this study.

1. Introduction

Aging is a complex physiological process that is due, in part, to the accumulation of damage at the molecular, cellular, and organ levels that eventually manifests as impairments in whole body function. One of the hallmark features of aging in mammals is the progressive loss of muscle mass and strength known as sarcopenia (Rosenberg, 1997). This condition is characterized by a progressive loss of skeletal muscle and shown to be partially attributed to changes in mitochondrial ultrastructure and decrements in organelle function, biogenesis, and content (Calvani et al., 2012). While the specific molecular underpinnings of these changes continue to be enigmatic, much of the research has focused on the role of life-long exposure to oxidative stress and the steady accretion of intracellular damage due to the reactive oxygen species (ROS) produced largely by mitochondria (Siu et al., 2008). Chronic lifetime exposure of muscle to elevated levels of ROS has been postulated to result in a steady accumulation of damage to macromolecules such as DNA, proteins and lipids (Harman, 2003). This can subsequently lead to reduced bioenergetics and the activation of key mitochondrially-mediated apoptosis signaling pathways resulting in myonuclear loss. Since skeletal muscle is uniquely multi-nucleated, a reduced myonuclear number resulting from apoptosis reduces the myonuclear domain (nuclear

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to cytoplasmic ratio) and ultimately contributes to the sarcopenic phenotype observed in aging (Chabi et al., 2008; Dirks and Leeuwenburgh, 2002; Whitman et al., 2005). Interestingly, age-related muscle abnormalities and susceptibility to fiber loss appear to occur in a fiber-type specific manner with fast-twitch glycolytic fibers being more affected than slow-twitch oxidative fibers (Aspnes et al., 1997; Bua et al., 2002; McKenzie et al., 2002; Phillips and Leeuwenburgh, 2005; Pistilli et al., 2006; Rice and Blough, 2006).

Mitochondrial content in skeletal muscle is dynamic and unique since it can be altered in response to a wide variety of physiological stimuli which includes but is not limited to exercise, chronic contractile activity, chronic muscle disuse, and caloric restriction (Hood, 2001). To date, life-long caloric restriction (consumption of 20–40% fewer calories) remains the only recognized intervention capable of delaying age-related diseases and improving health and longevity in numerous species ranging from yeast to rodents (reviewed in Speakman and Mitchell, 2011). In muscle, caloric restriction delays the age-associated loss of muscle fibers, in part, by improving mitochondrial function, preventing the induction of apoptotic signaling pathways, and reducing the release of pro-apoptotic factors from the mitochondria that lead to myonuclear DNA fragmentation (Aspnes et al., 1997; Dirks and Leeuwenburgh, 2004; Lee et al., 1998). At a molecular level, caloric restriction exerts its beneficial effects by evoking a cellular state of energy deprivation which activates key signaling molecules such as the NAD^+-dependent deacetylase sirtuin 1 (Sirt1), one of the seven mammalian sirtuin homologs of the yeast *Sir2* gene (Cohen et al., 2004; Frye, 1999). In fact, proof-of-concept genetic experiments have shown that Sirt1-overexpressing mice display similar beneficial phenotypes as caloric restricted mice (Bordone et al., 2007), while knockout animals have a shorter lifespan compared to their wild-type counterparts (Guarente and Picard, 2005; Koubova and Guarente, 2003; McBurney et al., 2003). At a biochemical level, Sirt1 functions as a deacetylase and one of its prominent targets is the mitochondrial regulator peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1α) (Rodgers et al., 2005). Upregulation of PGC-1α in muscle activates a number of genes involved in substrate metabolism leading to elevated mitochondrial biogenesis, improved mitochondrial function, as well as a fiber type transition towards muscle with a more oxidative metabolic profile (Lin et al., 2002; Wu et al., 1999). Moreover, increased PGC-1α levels attenuate the muscle mass loss observed in aging animals (Wenz et al., 2009). The effects of PGC-1α on muscle mitochondrial biogenesis are also largely mediated by 5′ AMP-activated protein kinase (AMPK), a key metabolic sensor that regulates PGC-1α by increasing its expression levels, as well as directly phosphorylating the protein (Irrcher et al., 2008; Jager et al., 2007; Suwa et al., 2003). While a number of observations have linked these mitochondrial metabolism and biogenesis regulatory proteins to the caloric restriction-mediated protection observed in aging muscle, the molecular details of their involvement remain elusive.

Recently, resveratrol (3, 5, 4′-trihydroxystilbene), a natural polyphenol found in grape skins and red wine has gained much attention for its ability to induce Sirt1 activity and has been purported to exert anti-aging effects on various organisms (Howitz et al., 2003). Resveratrol is marketed as, and termed a "caloric restriction mimetic" since it can extend lifespan in lower organisms including yeast, drosophila, and small vertebrates and seems to operate via the same molecular machinery as caloric restriction (Howitz et al., 2003; Valenzano et al., 2006; Wood et al., 2004). The effects of resveratrol appear to be mediated through an AMPK-Sirt1-PGC-1α pathway but the mechanisms of this regulation are currently not well understood (Baur et al., 2006; Canto et al., 2009; Dasgupta and Milbrandt, 2007; Lagouge et al., 2006; Price et al., 2012; Um et al., 2010). Nonetheless, the benefits of resveratrol are not limited only to life extension properties since it has also been shown to improve the health decrements associated with diet-induced obesity. Specifically, in mice fed a high fat diet, resveratrol protected against aging diet-induced obesity, increased overall mitochondrial content, improved muscle strength and enhanced aerobic capacity, while importantly also extending lifespan in these rodents (Baur et al., 2006; Lagouge et al., 2006). Favorable metabolic improvements following resveratrol treatment have also been reported in humans (Brasnyo et al., 2011; Crandall et al., 2012; Timmers et al., 2011). Thus, resveratrol treatment appears to improve general health measures in both humans and animals and this seems to be partially mediated via mitochondrial adaptations. Interestingly, the health benefits derived from resveratrol treatment can be realized with short-term supplementation as opposed to the life-long commitment of caloric restriction which has significant clinical implications from an intervention standpoint. It is currently unclear whether short-term and/or late-life implementation of resveratrol supplementation can provide similar mitochondrial adaptations to improve overall health and suppress muscle wasting conditions such as sarcopenia. Some studies report improved mitochondrial protein expression profile, skeletal muscle function, and an attenuation of muscle mass loss during muscle disuse, cachexia, and aging (Jackson et al., 2010; Wyke et al., 2004), while other studies fail to show similar benefits. However, these contrasting results seem to be mainly attributed to differences in treatment dose and time (Jackson et al., 2011). Despite these inconsistent findings, the general consensus in the field is that due to its ability to modulate mitochondrial gene expression and its potential antioxidant properties, resveratrol may attenuate the oxidative stress burden imposed by aging to potentially protect and/or suppress the onset of sarcopenia (Jackson et al., 2010, 2011; Murase et al., 2009; Ryan et al., 2010). To date however, it is unclear whether the benefits of caloric restriction and resveratrol treatment occur via similar pathways, or whether there may be some independence between the intracellular pathways that activate mitochondrial biogenesis, improve mitochondrial function and/or suppress mitochondrially-mediated apoptotic signaling pathways. Furthermore, given the differential sarcopenic effect of aging on muscle fiber-types (preferentially affecting fast/glycolytic versus slow/oxidative), it is currently unknown whether these treatment paradigms induce fiber-type specific responses.

The purpose of the current study was to evaluate the efficacy of moderate short-term caloric restriction, resveratrol, or combined caloric restriction/resveratrol treatments to modulate mitochondrial function, biogenesis, and apoptotic susceptibility proteins to potentially delay sarcopenia in late-life aged animals. This is the first study to assess whether these short-term treatment paradigms implemented in late-life could provide beneficial mitochondrial and muscle adaptations. We hypothesized that 6 weeks of caloric restriction (20%) or resveratrol (50 mg/kg/day) supplementation would independently improve mitochondrial function and content, and reduce apoptotic susceptibility, and that combined treatment would exert an additive or potentially a synergistic protective effect in aging muscle. Additionally, we hypothesized that the regulation of these pathways would differ between the fast-twitch and slow-twitch gastrocnemius muscle.

2. Materials and methods

2.1. Animals

Muscle tissue from a total of thirty-one 27-month-old male Fischer 344 × Brown Norway Hybrid rats purchased from the National Institute on Aging was used in this study. These animals were part of a larger study (n = 64). 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. The animals were acclimatized for 4 weeks before the start of intervention and housed in separate cages in a temperature (18–22 °C) and light-controlled environment with a 12-hour light/

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