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Resveratrol enhances exercise training responses in rats selectively bred for high running performance

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ABSTRACT

High Capacity Runner (HCR) rats have been developed by divergent artificial selection for treadmill endurance running capacity to explore an aerobic biology-disease connection. The beneficial effects of resveratrol supplementation have been demonstrated in endurance running and the antioxidant capacity of resveratrol is also demonstrated. In this study we examine whether 12 weeks of treadmill exercise training and/or resveratrol can enhance performance in HCR. Indeed, resveratrol increased aerobic performance and strength of upper limbs of these rats. Moreover, we have found that resveratrol activated the AMP-activated protein kinase, SIRT1, and mitochondrial transcription factor A ($p < 0.05$). The changes in mitochondrial fission/fusion and Lon protease/HSP78 levels suggest that exercise training does not significantly induce damage of proteins. Moreover, neither exercise training nor resveratrol supplementation altered the content of protein carbonyls. Changes in the levels of forkhead transcription factor 1 and SIRT4 could suggest increased fat utilization and improved insulin sensitivity. These data indicate, that resveratrol supplementation enhances aerobic performance due to the activation of the AMPK-SIRT1-PGC-1α pathway.

1. Introduction

In 1996 Koch and Britton (2001) initiated a prospective test of the linkage between aerobic capacity and disease risk by applying large-scale artificial selective breeding in rats with widely varying genetic backgrounds to produce low and high strains that differ for intrinsic (i.e., untrained) aerobic endurance treadmill running capacity (Koch and Britton, 2001). The hypothesis was that rats selectively bred as Low Capacity Runners (LCRs) would display disease risks and the rats bred as High Capacity Runners (HCRs) would have positive health effects. HCR demonstrate greater maximal oxygen consumption, insulin sensitivity, lower level of oxidative damage, and longer life-span (Koch et al., 2011; Swallow et al., 2010; Tweedie et al., 2011). There are a number of reports that exercise training and nutritional intervention have beneficial effects on groups suffering from metabolic disorders (Cameron et al., 2012), those fed on high fat diet (Farias et al., 2012), the aged (Koltai et al., 2010) and those genetically selected for low running capacity (Lessard et al., 2011). Much less information is available on how to upgrade endurance capacity in those animals which already have high VO_2max.

Other metabolic characteristics of skeletal muscle, such as the concentration or activities of the enzymes involved in oxidative metabolism, mitochondrial number, and respiratory capacity strongly affect aerobic endurance capacity (Flueck, 2009; Gnaiger, 2009). Therefore, impaired mitochondrial biogenesis could be a limiting factor of aerobic endurance. We were interested in how exercise training could further induce aerobic endurance capacity of HCR rats. It was suggested that the activity of AMP-activated protein kinase (AMPK), peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α), nuclear respiratory factor 1 (NRF1) mitochondrial transcription factor A (TFAM), and sirtuins could play an important role in the exercise-induced adaptive response. The mitochondria-dependent production of reactive oxygen species (ROS) is dependent on the density of mitochondria, since more mitochondria work at lower levels of respiration to

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produce the same amount of ATP (Radak et al., 2013). Therefore, mitochondrial biogenesis could be a part of the antioxidant system.

It is also not well known how mitochondrial fusion and fission would react to exercise-induced adaptation in animals having high VO_2max. Mitochondrial fusion and fission are important mechanisms for maintenance of the mitochondrial network and for quality control (Westermann, 2010), and thus impact mitochondrial function (Otera and Mihara, 2011). The quality control of mitochondrial proteins is supervised by Lon protease and HSP78, which prevent the accumulation of oxidized and dysfunctional proteins in mitochondria (Bota and Davies, 2002; Ngo and Davies, 2009; Rottgers et al., 2002).

SIRT1 is an important regulator of metabolism by controlling the activity of key transcription factors such as PGC-1α, FOXO1, and p53, which play a key role in the training response. Therefore, activators of SIRT1, such as resveratrol could have potentially beneficial effects which enhance aerobic performance, even in rats having a high endurance capacity.

Therefore, in the present study, we investigated the mitochondrial adaptive response to exercise training and resveratrol supplementation on rats selectively bred for high running capacity.

2. Methods

2.1. Animals

Artificial selective breeding, starting with a founder population of 186 genetically heterogeneous rats (N:NIH stock), was used to develop rat strains differing in inherent aerobic capacity. The procedure has been described in detail by Koch and Britton (2001). Briefly, endurance running capacity was assessed on a treadmill and the total distance run during a speed-ramped exercise test was used as a measure of maximal aerobic capacity. Rats with the highest running capacity from each generation were bred to produce the HCR strain. A subgroup of 48 male rats from generation 22 was phenotyped for intrinsic treadmill running capacity when 11 weeks old, at the University of Michigan (Ann Arbor, USA) and then shipped via air freight to Semmelweis University (Budapest, Hungary) for further study. Investigations were carried out according to the requirements of The Guiding Principles for Care and Use of Animals, EU, and approved by the ethics committee of Semmelweis University.

2.2. Exercise protocol and resveratrol treatment

Twenty four HCR male rats, aged 13 months, were assigned to control HCR (HCR-C), trained HCR (HCR-Tr), resveratrol treated control HCR (HCR-Rsv), trained resveratrol treated HCR (HCR-TrRsv) groups ($n = 6$ rats per group). Control rats had access to the treadmill three times a week for 10 min with an electrical stimulator in place. Trained rats were introduced to treadmill running for 3 days, then for the next 2 weeks the running speed was set to 10 m/min, on a 5% incline for 30 min. The treadmill was equipped with a high pressure air pipe and electric grid to stimulate running.

In the following week, maximal oxygen uptake (VO_2max) was measured on a motor driven treadmill (Columbus Inst. Columbus, Ohio) with a gradually increasing intensity. VO_2max was measured for each animal, using three criteria: (i) no change in VO_2 when speed was increased, (ii) rats no longer kept their position on the treadmill, and (iii) respiratory quotient ($RQ = VCO_2/VO_2$) > 1. Based on the level of VO_2max, a treadmill speed corresponding to 60% VO_2max was determined and used for daily training for one hr, five times per week. VO_2max was measured every second week and running speed was adjusted accordingly. The total training period lasted 12 weeks. In addition, the forelimb strength of the animals was assessed weekly by using a gripping test as described by Marton et al. (Marton et al., 2010). Resveratrol supplementation (100 mg/kg, oral dosing) (Smith et al., 2009) was started 2 weeks before habitual treadmill running was introduced to the animals, and 4 weeks before the actual training started, therefore lasting 16 weeks. A dose response study on the toxicity of resveratrol revealed no toxic effects up to 1000 mg/kg in rats (Johnson et al., 2011).

The animals were sacrificed 2 days after the last exercise session to avoid the acute metabolic effects of the final run. The skeletal muscle gastrocnemius was dissected and homogenized in buffer (HB) containing 137 mM NaCl, 20 mM Tris–HCl (pH 8.0), 2% NP 40, 10% glycerol and protease inhibitors.

2.3. ROS, protein carbonyl and antioxidant enzyme activities

Intracellular oxidant and redox-active iron levels (Kalyanaraman et al., 2012) were estimated using modifications of the dichlorodihydrofluorescein diacetate (H_2DCFDA) staining method (Radak et al., 2004). The oxidative conversion of stable, nonfluorometric, DCF-DA, to highly fluorescent 2′7′-dichlorofluorescein (DCF) was measured in the presence of esterases, as previously reported (Radak et al., 2004). This assay approximates levels of reactive species, such as superoxide radical, hydroxyl radical, and hydrogen peroxide. The method has been widely used in the literature but does have the problem of not being particularly specific, and results can be strongly affected by release of labile iron or copper (Kalyanaraman et al., 2012). Briefly, the H_2DCFDA (Invitrogen-Molecular Probes #D399) was dissolved to a concentration of 12.5 mM in ethanol and kept at −80 °C in the dark. The solution was freshly diluted with potassium phosphate buffer to 125 μM before use. For fluorescence reactions, 96-well, black microplates were loaded with potassium phosphate buffer (pH 7.4) to a final concentration of 152 μM/well. Then 8 μl diluted tissue homogenate and 40 μl 125 μM dye were added to achieve a final dye concentration of 25 μM. The change in fluorescence intensity was monitored every 5 min for 30 min with excitation and emission wavelengths set at 485 nm and 538 nm (Fluoroskan Ascent FL) respectively. Data obtained after 15 min were used. The fluorescence intensity unit was normalized with the protein content and expressed in relative unit production per minute.

The protein carbonyl measurement was done as described earlier (Koltai et al., 2012).

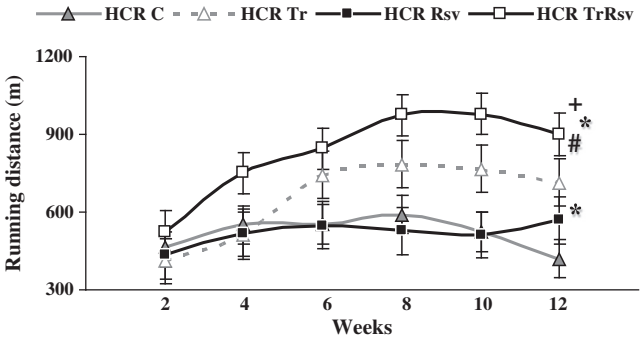

Fig. 1. Running distance of HCR rats. Running distance (m) for high capacity runner, HCR rats was measured every second week across a 12 week exercise training period and during the two weeks of treadmill habituation. Control HCR (HCR-C), trained HCR (HCR-Tr), resveratrol treated control HCR (HCR-Rsv), trained resveratrol treated HCR (HCR-TrRsv) groups. Values are means ± SD for six animals per group, * Significantly different from control group, + Significantly different from trained group, # Significantly different from resveratrol treated group, $p < 0.05$.

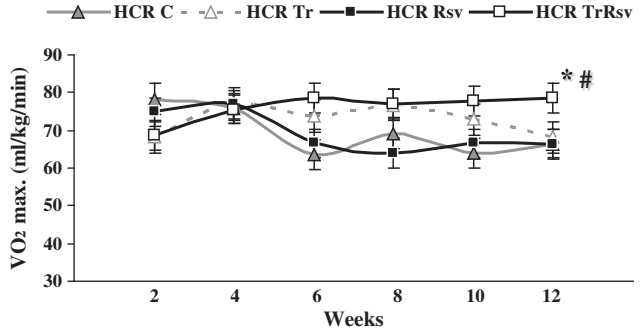

Fig. 2. Relative maximal oxygen uptake (VO_2max) of LCR. Maximal oxygen uptake (VO_2max; ml/kg/min) rats was measured every second week across a 12 week exercise training period. Before training (week 1). Control HCR (HCR-C), trained HCR (HCR-Tr), resveratrol treated control HCR (HCR-Rsv), trained resveratrol treated HCR (HCR-TrRsv) groups. Values are means ± SD for six animals per group, * Significantly different from control group, + Significantly different from trained group, # Significantly different from resveratrol treated group, $p < 0.05$.

2.4. Western blots

Ten to fifty micrograms of protein were electrophoresed on 8–12% v/v polyacrylamide SDS–PAGE gels. Proteins were electrotransfered onto PVDF membranes. The membranes were subsequently blocked and incubated at room temperature with antibodies (1:500 #sc-13067 Santa Cruz PGC-1 (H-300), 1:1400 #2532 Cell Signaling AMPKα, 1:500 #2535 Cell Signaling p-AMPKα (Thr172) (40H9), 1:1000 #sc-33771 Santa Cruz NRF-1 (H-300), 1:500 #sc-30963 Santa Cruz mtTFA (E-16)/TFAM/, 1:500 #sc-98900 Santa Cruz Fis1 (Fl-152), 1:10,000 #sc-50330 Santa Cruz Mfn1 (H-65), 1:1000 #sc-99006 Santa Cruz PNPase (H-124), 1:200 #U7757 Sig-