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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).

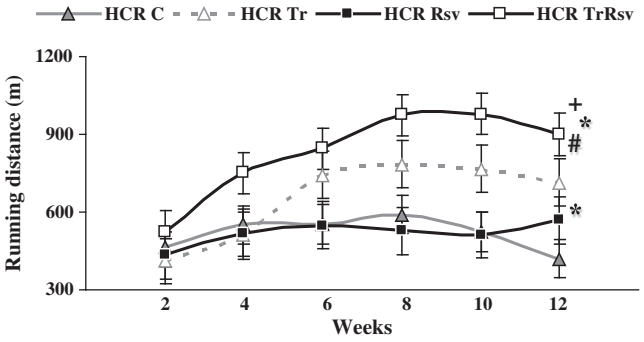

Fig. 1. Running distance of HCR rats. Running distance (m) for high capacity runner, HCR rats was measured every second week across a 12 week exercise training period and during the two weeks of treadmill habituation. Control HCR (HCR-C), trained HCR (HCR-Tr), resveratrol treated control HCR (HCR-Rsv), 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, $p < 0.05$.

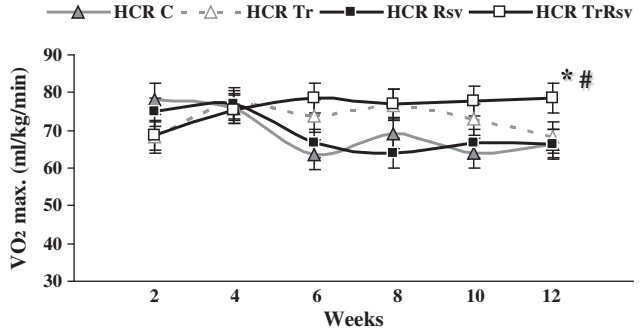

Fig. 2. Relative maximal oxygen uptake (VO_2max) of LCR. Maximal oxygen uptake (VO_2max; ml/kg/min) rats was measured every second week across a 12 week exercise training period. Before training (week 1). Control HCR (HCR-C), trained HCR (HCR-Tr), resveratrol treated control HCR (HCR-Rsv), trained resveratrol 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-

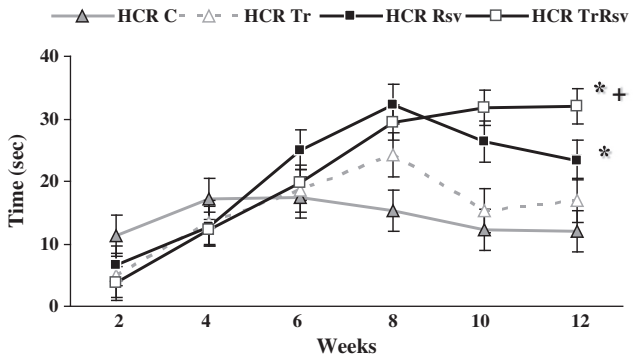

Fig. 3. Forearm strength of LCR rats measured by a gripping test. Upper limb strength, assessed by gripping time (sec), in control HCR (HCR-C), trained HCR (HCR-Tr), resveratrol treated control HCR (HCR-Rsv), trained resveratrol treated HCR (HCR-TrRsv) groups. Values are means ± SD for six animals per group, * Significantly different from control group, + Significantly different from trained group, # Significantly different from resveratrol treated group, $p < 0.05$.

ma–Aldrich UCP3, 1:5000 #ab87253 Abcam CLPB/HSP78/, 1:15,000, #T6199 Sigma alpha-tubulin). The antibody for Lon protease was generated in our laboratory, as described previously (Merrill et al., 1997). After incubation with primary antibodies, membranes were washed in TBS-Tween-20 and incubated with HRP-conjugated secondary antibodies. After incubation with the secondary antibody, membranes were repeatedly washed. Membranes were incubated with an ECL Plus reagent (RPN 2132, Amersham) and protein bands were visualized on X-ray films. The bands were quantified by ImageJ software, and normalized to tubulin, which served as an internal control.

2.5. Assessment of SIRT1 activity

To measure SIRT1 deacetylase activity, a Cyclex SIRT1/Sir2 Deacetylsase Fluorometric Assay Kit (Cyclex, CY-1151) was used according to the established protocol including the separation of nuclear extract (Koltai et al., 2012). To prepare nuclear fractions, the homogenate was centrifuged at 1000g for 10 min at 4 °C, and the pellet was suspended in HB and re-centrifuged. The pellet was then re-suspended in HB with 0.5% NP40 and again centrifuged. Next, the pellet was washed twice in HB. After centrifugation, the final nuclear pellet was rocked for 30 min after the addition of a 1/10 (vol/vol) of 2.5 M KCl and centrifuged at 14,000 rpm for 30 min. For the measurement of SIRT1, 5 μl of nuclear extracts of rat gastrocnemius muscle were mixed with a reaction mixture (40 μl) containing 50 mM Tris–HCl pH 8.8, 4 mM $MgCl_2$, 0.5 mM DTT, 0.25 mAU/ml Lysyl endopeptidase, 1 μM Trichostatin A, 20 μM Fluoro-Substrate Peptide, and 200 μM NAD^+ on a microplate. The samples were mixed and incubated for 10 min at room temperature and the fluorescence intensity (ex. 355 nm, em. 460 nm) was read every 10 min for 2 h and normalized by the protein content (Koltai et al., 2010).

2.6. Measurement of mtDNA by PCR

The mtDNA content was quantified as the mtDNA to nuclear DNA (nDNA) ratio (mtDNA/nDNA). Total DNA was extracted (Fast DNA kit #6540-400 BIO 101 Systems Qbiogene) and quantified spectrophotometrically. The mtDNA content was measured by PCR (Rotor–Gene 6000, Corbett Research, Australia) using the following conditions: 94 °C for 2 min (initial denaturation), 94 °C for 30 s, 60 °C for 30 s, 72 °C for 45 s (25 cycles), 72 °C for 10 min (final extension) and corrected by the simultaneous measurement of a single copy nuclear BDNF gene. Primers used for the analysis of mtDNA were R-CYTB-F (5′-CCC CAG AGG ATT AAA CTC CAA CGC A-3′), and R-CYTB-R (5′-GGG TGG GGT CAG GGG GT-3′). Primers used for the analysis of nDNA were R-BDNF-genom-exon-IV-F (5′-TTG GGA TGG GAA AGA TGG G-3′), and R-BDNF-genom-exon-IV-R (5′-CAG AGT AGG AGG GAA CAA GTG TGA C-3′). The mtDNA content was normalized to nDNA. Data are expressed as the mean of three measurements.

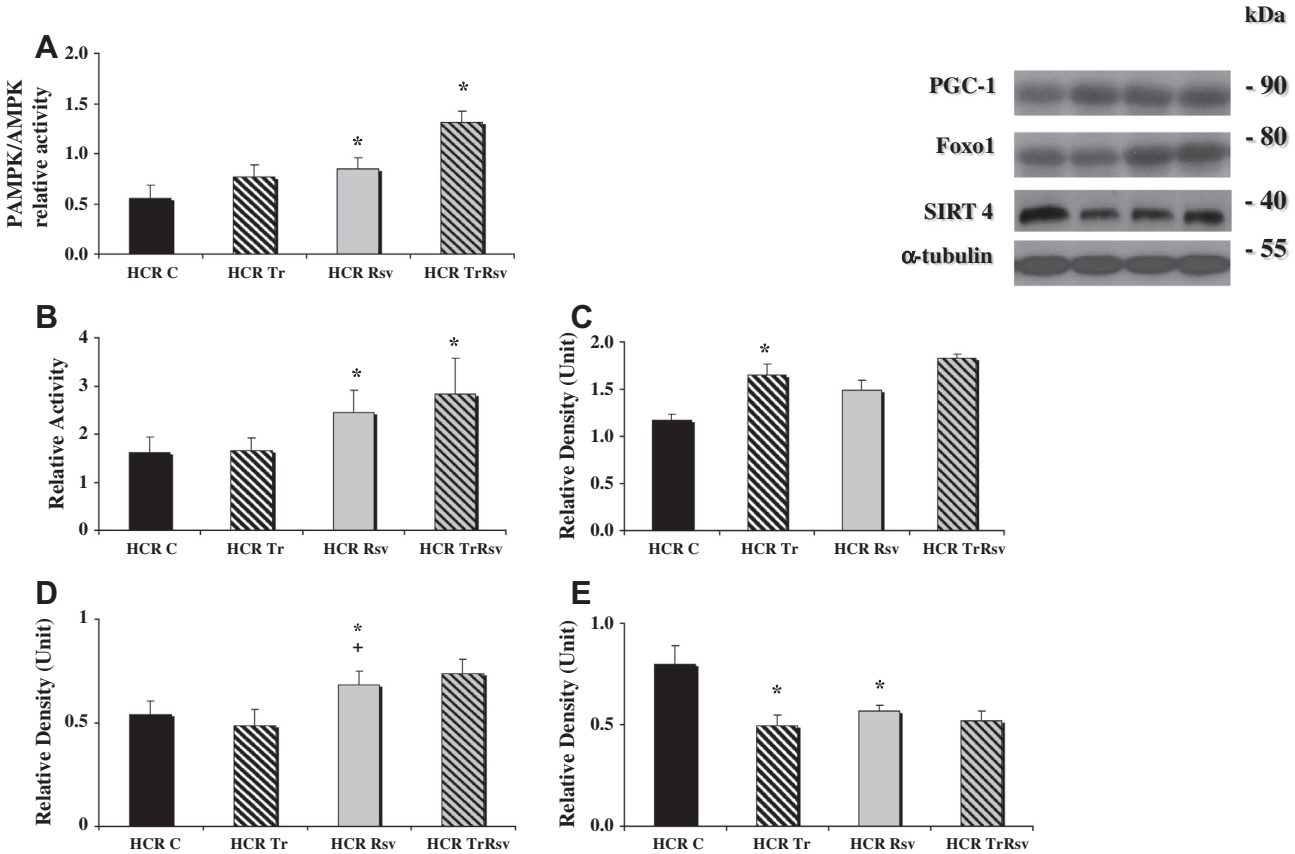

Fig. 4. The effect of exercise and resveratrol on mitochondrial metabolic factors. The activity of AMPK, assessed by the pAMPK/AMPK ratio increased in HCR rats with training plus resveratrol (A). The SIRT1 deacetylase activity (B) and the contents of PGC-1α (D), were induced by resveratrol treatments. FOXO1 (E) was induced by exercise, while the SIRT4 (F) levels were attenuated by exercise and reveratrol. 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, $p < 0.05$.

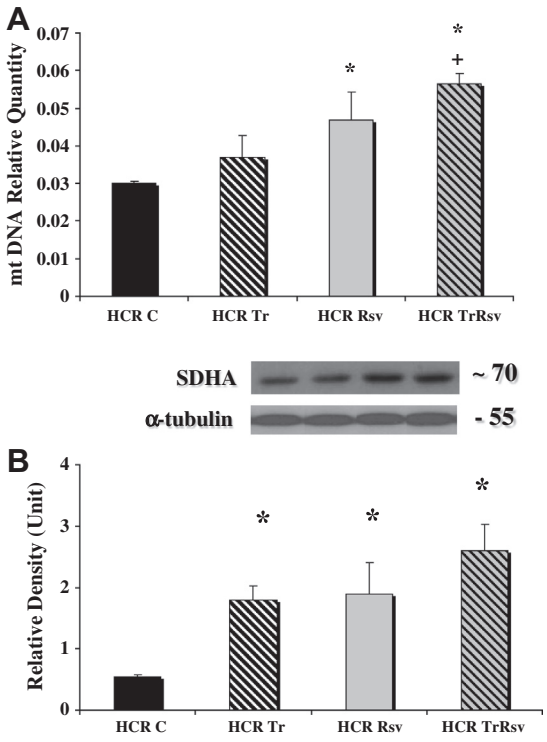

Fig. 5. The levels of mtDNA and SDH in gastrocnemius muscle of HCR rats. Moderate increase in mtDNA levels was observed in the study (A) but the levels of SDH (B) increased in larger degree. Control HCR (HCR-C), trained HCR (HCR-Tr), resveratrol treated control HCR (HCR-Rsv), trained resveratrol treated 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, $p < 0.05$.

2.7. Statistical analyses

Because of the limited sample size and for the purpose of finding the appropriate statistical procedure, normality was tested on all dependent variables (Shapiro Wilk's W-test). It was found that few of the dependent variables demonstrated a normal distribution, namely: NRF1, Fis1, NAMPT, LonP, Mfn1, and VO_2max. Therefore, non-parametric Kruskal-Wallis ANOVA analysis was used to test for differences among the dependent variables. The Mann–Whitney U-test was applied for post hoc analyses. Significance level was set at $p < 0.05$.

3. Results

3.1. Exercise performance

Exercise training significantly increased the level of running distance of HCR-Tr group ($p < 0.05$). In addition, resveratrol supplementation enhanced the effects of exercise training of these HCR rats ($p < 0.05$) (Fig. 1). Despite the increased running distance of HCR-Tr rats, the VO_2max of these animals remained unchanged. Resveratrol supplementation increased VO_2max of trained rats ($p < 0.05$) but was ineffective in control animals (Fig. 2). When the gripping strength of the upper limbs was tested significant increases in gripping time in HCR-Tr and HCR-TrRsv groups were measured compared to HCR-C rats, and resveratrol enhanced the training effects of this measurement (Fig. 3).

3.2. Effects of exercise and resveratrol on mitochondrial metabolic factors

AMPK activity is an important sensor of cellular metabolism and the pAMPK/AMPK ratio increased significantly in HCR-Rsv and HCR-TrRsv rats (Fig. 4A), indicating that resveratrol is a potent inductor of AMPK. The activity of SIRT1 changed showing a similar pattern to the activity of AMPK and increased in HCR-Rsv and HCR-TrRsv groups (Fig. 4B). The protein content of PGC-1α, on the other hand, was induced only in HCR-Tr rats (Fig. 4C). Moreover, the protein content of SIRT1 regulated FOXO1 was also similarly modulated by exercise training and resveratrol treatments (Fig. 4D).

The mitochondrial sirtuin, SIRT4, is related to fat metabolism and the data revealed that exercise training and resveratrol treatment decreased SIRT4 content in HCR-Tr, HCR-Rsv, HCR-TrRsv groups (Fig 4E).

mtDNA levels were used to evaluate mitochondrial content and the data show that HCR-Rsv and HCR-TrRsv rats have higher mtDNA levels than HCR-C (Fig. 5A). SDH levels, on the on the other hand, were changed by both, training and resveratrol treatments (Fig. 5B).

3.3. The effects of exercise and resveratrol on mitochondrial quantity and quality control

TFAM protein levels were induced by resveratrol in both untrained and trained groups ($p < 0.05$) (Fig. 6A), while the levels of NRF-1 were not significantly affected by exercise training or resveratrol treatments (Fig. 6B). The mitochondrial fission controlling protein, Fis1, was increased by the combined effects of exercise training and resveratrol treatments (Fig 6D). The Mfn1 levels, a mediator of mitochondrial fusion, decreased in HCR-Tr animals (Fig. 6C). Lon protease was measured to assess the level of protein degradation in mitochondria, which could be important for quality control. However, significant differences were not noted in either groups (Fig. 6E). Similar results were present in the levels of HSP78 which function is associated with Lon protease (Fig. 6F).

3.4. Oxidative stress markers

The levels of ROS, as measured by the fluorescent activity of H_2-DCFDA, increased with exercise training HCR rats, and decreased in HCR-Rsv (Fig. 7A). The levels of carbonyl groups were assessed as potential markers of oxidative protein damage and significant alteration was not found (Fig. 7B).

4. Discussion

One of the main findings of the present investigation is that resveratrol supplementation enhanced the effects of exercise on endurance capacity, and this happened in rats which already had a high level of aerobic endurance. This finding suggests that resveratrol could be used as an enhancer of aerobic performance. Our data revealed that resveratrol can increase the activity of AMPK and SIRT1. Moreover, it appears that the resveratrol-associated signaling also includes the transcription factor FOXO1, since SIRT1 is an up-stream regulator of FOXO1.

It has been shown that mice with deficient subunits of AMPK do not respond to resveratrol treatment, while the wild type express increased metabolic rate and reduced fat mass when exposed to a high fat diet (Um et al., 2010). Therefore, it is possible that the resveratrol mediated activation of PGC-1α is mediated by AMPK, since AMPK can be an upstream regulator of SIRT1 (Canto et al., 2009; Kim et al., 2012). Indeed, it has been shown that resveratrol enhances mitochondrial biogenesis and induces AMPK in skeletal muscle of mice. However, these effects were absent when SIRT1 was knocked outs (Price et al., 2012). These studies also demonstrated that high doses of resveratrol were necessary for AMPK mediated activation of SIRT1, which is very likely the case in our study, as SIRT1 was important for the beneficial effects of resveratrol on mitochondrial biogenesis (Price et al., 2012). Indeed, we

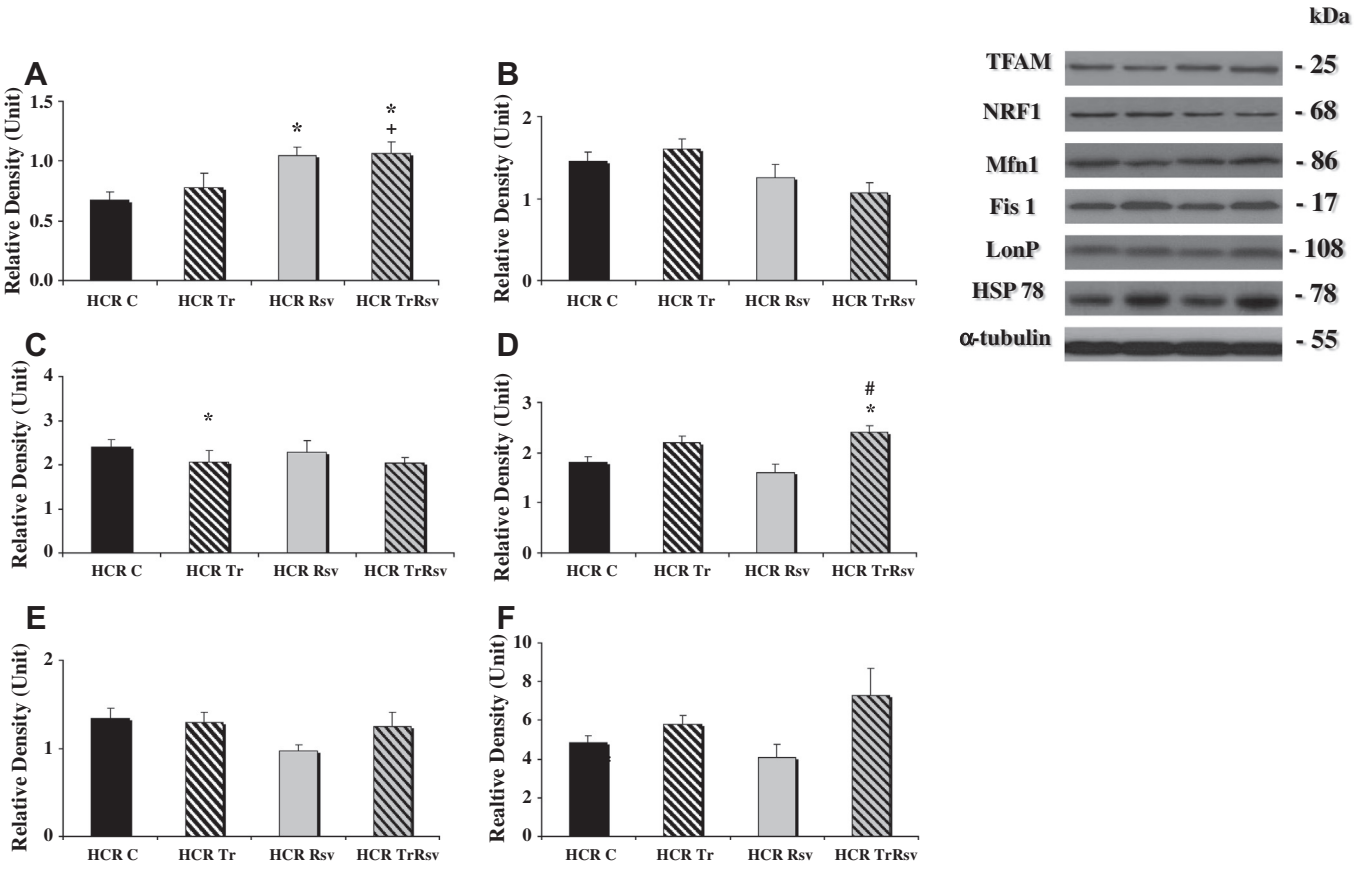

Fig. 6. Markers of quantity and quality control of mitochondria. Resveratol increased the levels of TFAM (A), while the NRF1 was unaltered by exercise or resveratrol (B), Mfn1(C), fission Fis1 (D), Lon protease (E) and HSP78 (F) were measured to assess quality control of the mitochondrial network. Control 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, $p < 0.05$.

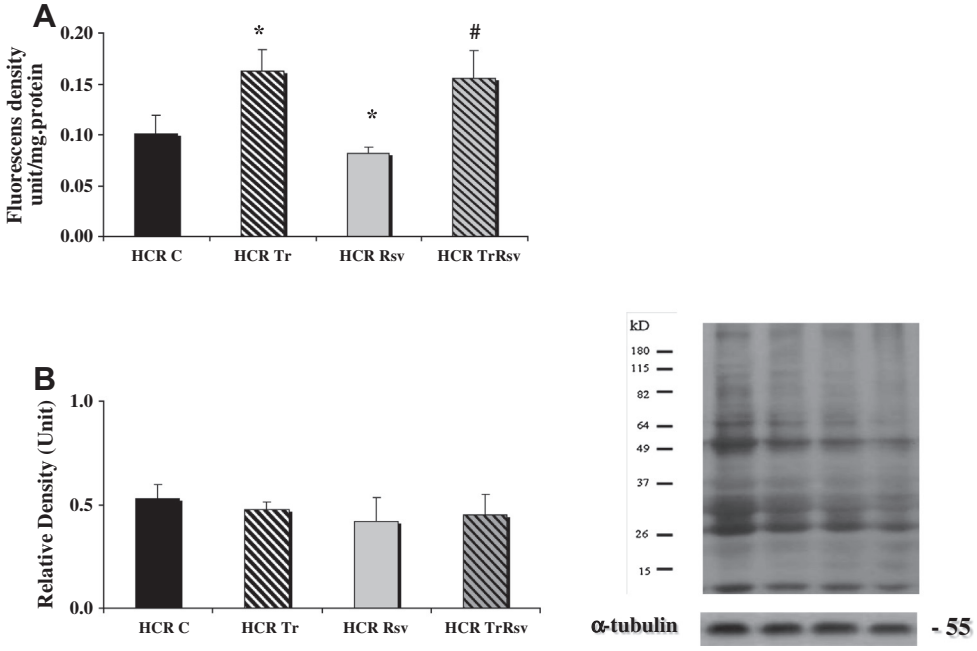

Fig. 7. Oxidative stress markers. Rat gastrocnemius muscle was stained with dichlorodihydrofluorescein diacetate (H_2DCFDA) to measure relative steady-state oxidant levels and redox-active iron release levels (both increase DCF fluorescence) as an estimate of levels of reactive oxygen species (ROS) (A). The oxidative damage of proteins was evaluated by protein carbonyl groups (B). 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, $p < 0.05$.

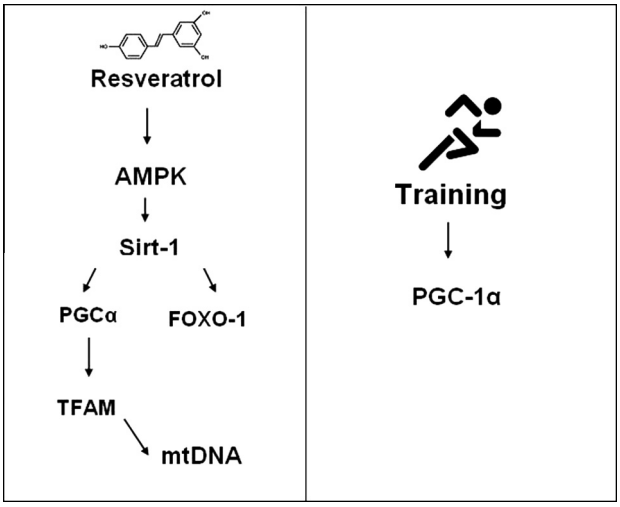

Fig. 8. Suggested mechanism of resveratrol signaling. Resveratrol readily activated the AMPK-SIRT1-PGC-1α pathway in this rat model. In addition, resveratrol play a role in SIRT1-FOXO1 signaling.

checked the relationship between the activity of SIRT1 and enhanced running distance and a strong relationship was found ($r = 0.88$). Therefore, we have confirmed the very recent novel finding of Sinclair's team (Price et al., 2012) that high doses of resveratrol induce mitochondrial biogenesis *via* AMPK mediated activation of PGC-1α with the involvement of SIRT1. We have further shown that this effects results in enhanced endurance capacity.

Since FOXO1 activation is insulin dependent, absence leads to transactivating of gluconeogenic genes, while in the presence of insulin, FOXO1 translocates to cytoplasm reducing its translocational activity (Marinho et al., 2012). We have found that resveratrol increased the contents of FOXO1, which is in accordance with a previous finding, where artificially made resveratrol mimics, SRT170 (Feige et al., 2008).

The exact function of mitochondrial located SIRT4 is still in debate. However, a recent study revealed that knockdown of SIRT4 by shRNA significantly increased fat metabolism in myotubes (Nasrin et al., 2010), which could indicate that lower levels of SIRT4 result in increased free fatty acid utilization, a key element for increased endurance capacity (Holloszy and Coyle, 1984; Spina et al., 1996). Indeed, the present data agree with these findings. Moreover, it is also known that SIRT4 is involved in the development of insulin resistance (Chen et al., 2010). Thus, the down-regulating effects of exercise training on SIRT4 content could have beneficial effects on glucose handling.

The TFAM levels and the activity of SIRT1 changed in similar patterns to resveratrol and exercise interventions, suggesting a functional relationship between these proteins, which could indicate enhanced mitochondrial biogenesis. TFAM is reported to increase the half life of and stabilize mtDNA, and thus increased levels of TFAM could have beneficial effects (Ikeuchi et al., 2005). In the present study similar changes in TFAM and mtDNA contents were noted, supporting the well known fact that regular endurance training causes increased mitochondrial biogenesis.

On the other hand, the exercise mediated plasticity of the mitochondrial network is not well known. Mitochondrial fission and fusion interact with each other. Fusion enables mitochondria to mix their contents within interconnected mitochondrial reticulum in order to minimize abnormalities. Fusion pathways have been shown to play beneficial roles in muscular atrophy (Romanello et al., 2010), endurance (Garnier et al., 2005), lifespan (Scheckhuber et al., 2007) and ROS production (Yu et al., 2006). Fission segregates mitochondria from the network, especially abnormal and damaged ones, to facilitate their removal by autophagy (Benard and Karbowski, 2009). Together, well coordinated fusion and fission minimize the formation of giant and dysfunctional mitochondria. Indeed, cells which are unable to remove damaged mitochondria, due to the ablation of Fis1, show senescence-associated phenotypic changes (Lee et al., 2007). The data from the present study demonstrate an increased fission with the combined effects of resveratrol and exercise training. This finding was evident in the group with the highest content of mtDNA which could reflect the obvious results of enhanced mitochondrial division. Fusion was lowest in the HCR-Tr group, which could be an indication of a lack of significant oxidative stress as a result of exercise training (Youle and van der Bliek, 2012).

Lon protease plays an important role in the quality control of mitochondrial proteins by degrading oxidatively modified proteins, and a recent paper suggests that Lon protease is involved in TFAM metabolism (Matsushima et al., 2010). However, we could not detect any alteration in Lon protease levels using the exercise model with young rats. In general, the available information on LonP in exercise models is very limited. However, it has recently been shown that LonP decreases with aging in skeletal muscle of rats, which could be normalized by endurance training (Koltai et al., 2012). It should be noted that exercise did not change the levels of Lon in young animals, which is similar to our current data. HSP78, an important mitochondria chaperone involved in LonP associated degradation and quality control (Rottgers et al., 2002), was also unchanged, suggesting that significant induction of protein degradation was not necessary in the present experimental model. This is supported by the level of protein carbonylation, which was also similar in all groups.

In summary, our data show that resveratrol enhances aerobic endurance capacity and performance in high running capacity rats. This beneficial effect is mediated by enhanced mitochondrial biogenesis with the activation of the AMPK-SIRT1-PGC-1α pathway. The changes in mitochondrial fission/fusion and LonP/HSP78 do not support the presence of exercise related oxidative stress in the mitochondria of skeletal muscle in these animals. These findings raise the possibility that resveratrol could be a potent enhancer of aerobic endurance even in subjects with high endurance capacity (see Fig. 8).

Conflict of Interest

The authors declare that there are no conflicts of interest.

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