Pflügers Archiv - European Journal of Physiology

Mitochondrial biogenesis-associated factors underlie the magnitude of response to aerobic endurance training in rats

--Manuscript Draft--

Manuscript Number:	PAEJ-D-13-00400R3
Full Title:	Mitochondrial biogenesis-associated factors underlie the magnitude of response to aerobic endurance training in rats
Article Type:	Original Paper
Corresponding Author:	Zsolt Radak Semmelweis University Budapest, HUNGARY
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Semmelweis University
Corresponding Author's Secondary Institution:	
First Author:	Orsolya Marton
First Author Secondary Information:	
Order of Authors:	Orsolya Marton
	Erika Koltai
	Takeda Masaki
	Lauren Koch
	Steven Britton
	Kelvin Davies
	Istvan Boldogh
	Zsolt Radak
Order of Authors Secondary Information:	
Abstract:	Trainability is important in elite sport and in recreational physical activity and the wide range for response to training is largely dependent on genotype. In this study, we compare a newly developed rat model system selectively bred for low and high gain in running distance from aerobic training to test whether genetic segregation for trainability associates with differences in factors associated with mitochondrial biogenesis. Low response trainer (LRT) and high response trainer (HRT) rats from generation 11 of artificial selection were trained five times a week, 30 min per day for three months at 70% VO2max to study the mitochondrial molecular background of trainability. As expected, we found significant differential for the gain in running distance between LRT and HRT groups as a result of training. However, the changes in VO2max, COX 4,redox homeostasis associated markers (ROS), silent mating-type information regulation 2 homolog (SIRT1), NAD+/NADH ratio, proteasome (R2 subunit), and mitochondrial network related proteins such as mitochondrial fission protein 1 (Fis1), and mitochondrial fusion protein (Mfn1) suggest that these markers are not strongly involved in the differences in trainability between LRT and HRT. On the other hand, according to our results, we discovered that differences in basal activity of AMP-activated protein kinase alpha (AMPK), and differential changes in aerobic exercise-induced responses of citrate synthase, carbonylated protein, peroxisome proliferator-activated receptor gamma coactivator-1α (PGC1-α), nuclear respiratory factor 1 (NRF1), mitochondrial transcription factor A (TFAM), and Lon protease limits trainability between these selected lines. From this we conclude that mitochondrial biogenesis associated factors adapt differently to aerobic exercise training in training sensitive and training resistant rats.

Response to Reviewers:	Dear Editor in Chief, Dear Prof. Mori Here we resubmit our manuscript after correction. First of all thank you very much for your evaluation. We have corrected the manuscript again, and indicated that we measured AMPKmRNA and protein content. We indicated the subunit of AMPK at the Method, Result, Discussion and Figure Legends sections. We also corrected the layout Figure 4. Dear Editor and Reviewers, Thank you very much for your supporting and helpful comments to improve the quality of our revised manuscript. Sincerely yours, Zsolt RADAK, PhD

Mitochondrial biogenesis-associated factors underlie the magnitude of response to aerobic endurance training in rats

Orsolya Marton[1], Erika Koltai[1], Masaki Takeda[1], Lauren Gerard Koch[2], Steven L. Britton[2], Kelvin J.A. Davies[3], Istvan Boldogh[4], Zsolt Radak[1]

[1]Research Institute of Sport Science, Semmelweis University, Budapest, Hungary, [2]Department of Anesthesiology, University of Michigan Medical School, Ann Arbor, Michigan, USA, [3]Ethel Percy Andrus Gerontology Center of the Davis School of Gerontology; and Division of Molecular & Computational Biology, Department of Biological Sciences of the College of Letters, Arts & Sciences: the University of Southern California, Los Angeles, California 90089-0191, USA, [4]Department of Microbiology and Immunology, University of Texas Medical Branch at Galveston, Galveston, Texas 77555, USA

Running head: Mitochondria, biogenesis and trainability

Correspondence: Zsolt Radak, Ph.D.
Institute of Sport Science
Faculty of Physical Education and Sport Science
Semmelweis University
Alkotas u. 44.TF Budapest, Hungary
Tel: +36 1 3565764, Fax: +36 1 356 6337
Email: radak@tf.hu

Abstract

Trainability is important in elite sport and in recreational physical activity and the wide range for response to training is largely dependent on genotype. In this study, we compare a newly developed rat model system selectively bred for low and high gain in running distance from aerobic training to test whether genetic segregation for trainability associates with differences in factors associated with mitochondrial biogenesis. Low response trainer (LRT) and high response trainer (HRT) rats from generation 11 of artificial selection were trained five times a week, 30 min per day for three months at 70% VO_2max to study the mitochondrial molecular background of trainability. As expected, we found significant differential for the gain in running distance between LRT and HRT groups as a result of training. However, the changes in VO_2max, COX 4,redox homeostasis associated markers (ROS), silent mating-type information regulation 2 homolog (SIRT1), NAD^+/NADH ratio, proteasome (R2 subunit), and mitochondrial network related proteins such as mitochondrial fission protein 1 (Fis1), and mitochondrial fusion protein (Mfn1) suggest that these markers are not strongly involved in the differences in trainability between LRT and HRT. On the other hand, according to our results, we discovered that differences in basal activity of AMP-activated protein kinase alpha (AMPKα), and differential changes in aerobic exercise-induced responses of citrate synthase, carbonylated protein, peroxisome proliferator-activated receptor gamma coactivator-1α (PGC1-α), nuclear respiratory factor 1 (NRF1), mitochondrial transcription factor A (TFAM), and Lon protease limits trainability between these selected lines. From this we conclude that mitochondrial biogenesis associated factors adapt differently to aerobic exercise training in training sensitive and training resistant rats.

Introduction

Clinically, exercise capacity, measured by either maximal oxygen uptake (VO_2max) or a treadmill running test to exhaustion is a strong predictor of morbidity and survivability. Indeed, studies show that regular aerobic exercise leads to enhanced VO_2max and increases the mean lifespan of laboratory animals [11,14,41] and humans [26,38,42]. However, VO_2max is not the only indicator of increased aerobic performance [39]; the adaptive capacity of skeletal muscle to endurance exercise appears to be crucial [16]. Indeed, the quality and quantity of the skeletal muscle mitochondrial network, mitochondrial biogenesis, and the activity of oxidative enzymes are also highly recognized as limiting factors of aerobic endurance capacity [2,15].

Studies within twins and families demonstrate that aerobic trainability is a highly heritable trait [3,5,10,24,39]. Recently an animal model system was developed via artificial selective breeding, which permits researchers to study the inherited components of low and high trainability in rats [19]. This model was set up using a genetically heterogeneous rat population (N/NIH stock) to develop lines named low response trainers (LRT) and high response trainers (HRT) [19]. Selection was based on the change in maximal running distance evaluated by a treadmill-running test to exhaustion. In the untrained condition, LRT and HRT rats are similar for exercise capacity. However after receiving 8 weeks of a standard amount of endurance training, HRT rats improve on average by 200 meters for distance run whereas those bred as LRT failed to improve and on average, declined in running capacity by -65 meters [19]. A recent study by Lessard et al. [23] showed that skeletal muscle mitochondrial capacity was similar between LRT and HRT in the sedentary state and that LRT produced normal increases in mitochondrial density and function in response to moderate intensity endurance training. Nonetheless, significant differences were noted for exercise-induced

angiogenesis and transforming growth factor β signaling in skeletal muscle. Moreover, this study assessed skeletal muscle gene expression and showed that the LRT and HRT differ in their transcriptional responses to the same acute bout of exercise. The differentially expressed genes belonged mostly to biologically functional categories of gene expression, development, cell-cycle regulation, cellular growth, proliferation, and movement.

Based on the above evidence that skeletal muscle remodeling response may be partly responsible for differences in the adaptive exercise response, the purpose of this study was to test the hypothesis that impaired mitochondrial biogenesis contributes to the differential in training response between these two selectively bred lines. Here, we trained LRT and HRT rats for 3 months using a relative training protocol where each LRT and HRT rat trained five days a week at 70% VO2max.–We find HRT rats set against LRT rats demonstrated a significantly greater gain in aerobic running performance (distance) compared to the accompanying changes in VO2max. We measured a panel of 14 different molecular factors crucial for skeletal muscle mitochondrial biogenesis including: reactive oxygen species (ROS), NAD^+/NADH ratio, mitochondrial proteins (COX-4, citrate synthase, Lon proteasome), transcription co-factors (PGC1-α, TFAM, NRF1), energy sensing proteins (AMPK, SIRT1), and the regulators of mitochondrial fission (Fis1) and fusion (Mfs1), to determine whether mitochondrial biogenesis interferes with trainability.

Methods

Animals and exercise protocol

Low response trainers (LRT) and high response trainers (HRT) were developed by selective breeding and are maintained as a contrasting animal model system at the University of Michigan by Koch and Britton [19]. Twenty-seven male rats from the 11^{th} generation of

selection, 13 LRT and 14 HRT, were studied. Animals were cared for according to the guiding Principles for the Care and Use of Animals based upon the Helsinki Declaration, 1964. Animals, 12 months of age at the beginning of the study, were divided into control LRT (LRTC) (n=6), exercised LRT (LRTE) (n=7), control HRT (HRTC) (n=6), and exercised HRT (HRTE) (n=8) groups.

Both control and exercised groups were introduced to running on a motor driven treadmill (Columbus Inst. Columbus, Ohio) for five days for ten min per day. For each introduction session, the treadmill incline was set at 5% and speed was gradually increased from 8 m/min to 23 m/min. The exercised groups then trained five times a week, 30 min per day for three months at 70% of their VO_2max, as described previously [12]. During the testing, running distance and body weight were also measured.

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 70% VO_2max was determined and used for daily training. VO_2max was measured every second week and running speed was adjusted accordingly. The total training period lasted 12 weeks.

The animals were sacrificed two days after the last exercise session to avoid the metabolic effects of the final exercise session. The gastrocnemius muscle was quickly excised, weighed, frozen in liquid nitrogen, and stored at -80 °C degree. A section of tissue samples was homogenized in a 10 vol homogenization buffer containing: 137mM NaCl, 20 mM Tris-HCl

pH8.0, 2% NP 40, 10% glycerol and protease inhibitors (PMSF, aprotinin, leupeptin, orthovanadate). The protein levels were measured using BCA methods.

Assays

Measurements of reactive oxygen species (ROS)

The overall ROS generation was determined using modifications of the dichlorodihydrofluoresceindiacetate (H_2DCFDA) staining method [34]. In brief, the H_2DCFDA (Invitrogen-Molecular Probes #D399) was dissolved at 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. In the fluorescence reaction 152 µM/well potassium phosphate buffer (pH 7.4) was filled to the 96-well black microplate, than 8 µl diluted tissue homogenate and 40 µl 125 µM dye were added to achieve a final concentration of 25 µM. The change in fluorescence intensity was monitored every five minutes for 30 minutes with excitation and emission wavelengths set at 485 nm and 538 nm (FluoroskanAscent FL). The fluorescence intensity unit was normalized with the protein content and expressed in relative unit production per minute.

Detection of carbonylated proteins and citrate synthase activity

Changes in oxidized protein levels were determined using an Oxyblot Kit (Chemicon/Millipore, S7150) according to the manufacturer's recommendations. Briefly, proteins were derivatized with 4-dinitrophenylhydrazine (DNPH) for 15 min followed by incubation at room temperature with a neutralization buffer (Chemicon/Millipore). Derivatized proteins were electrophoresed on a 10% SDS-PAGE and blotted on PVDF membranes. Blots were blocked with 5% non-fat dry milk (blocking buffer) in Dulbecco's

PBS containing 0.05% Tween 20 (PBS-T) for three h and incubated with anti-DNP primary antibody (1:150) (Chemicon/Millipore) overnight at 4 °C. After three washes with PBS-T, membranes were incubated for oneh at room temperature with HRP-conjugated secondary antibodies (1:300)(Chemicon/Millipore). Immuno complexes were visualized by an HRP plus reagent (Super Signal West Pico Chemiluminescent Substrate, Thermo Scientific #34080). Activity of citrate synthase (CS) was measured as described previously [35].

Measurement of NAD^+/NADH levels

Proteins were filtered through a 10 kD Microcon filter and applied to a NAD^+/NADH Quantification kit (Bio Vision, K337-100) according to the given protocol. First, total NAD^+ level was measured, then NAD^+ was decomposed by heating to 60 °C for 30 min, then cooled on ice and transferred to the microplate. Next, a 10 µl NADH developer was added to each well, mixed, and the optical density read at 450 nm every 30 min for five h. The NAD^+ levels were calculated according to the manufacturer's directions.

Western blots

Proteins were electrophoresed on 8-12% v/v polyacrylamide SDS-PAGE gels. Proteins were electrotransferred onto PVDF membranes. The membranes were subsequently washed, and after blocking, PVDF membranes were incubated at 4°C with antibodies (1:1000 #sc-69359 Santa Cruz COX-4, 1:150 S7150 Chemicon anti-DNPH, 1:1000 #2459 Cell Signaling PSMA6, 1:500 LonP1, 1:5000 #ab87253 Abcam HSP78, 1:1400 #2532S Cell Signaling AMPKα, 1:500 #2535S Cell Signaling pAMPKα, 1:500 #07-131 Millipore, Upstate SIRT1, 1:500, sc-13067 Santa Cruz PGC1-α, 1:1000 #sc-30963 Santa Cruz TFAM, 1:1000 #sc-33771 Santa Cruz NRF-1, 1:500 #sc-98900 Santa Cruz Fis1, 1:3000 #50330 Santa Cruz Mfn1, 1:15000 #T6199 Sigma α-tubulin). After incubation with primary antibodies, membranes

were washed 3x10 minutes in TBS-Tween-20 (TBS-T) and incubated with horseradish peroxidase (HRP) – conjugated secondary antibodies at 4°C in one hour. After incubation with primary antibodies, membranes were washed in TBS-T and incubated with HRP-conjugated secondary antibodies. After incubation with a secondary antibody, membranes were repeatedly washed. Membranes were incubated with an HRP plus reagent (Super Signal West Pico Chemiluminescent Substrate, Thermo Scientific #34080) and protein bands were visualized on X-ray films. The bands were quantified by ImageJ software, and normalized to α-tubulin, which served as an internal control.

Real time quantitative RT-PCR

The mRNA levels of AMPKα (*PRKAA1*) were measured as described earlier [32]. In brief, total RNA from skeletal muscle samples (~30 mg) was extracted with NucleoSpin® RNA/Protein (Macherey-Nagel, Düren, Germany) according to the manufacturer's protocol. Analyses of the real-time quantitative PCR data were performed using the comparative threshold cycle [Ct] method as suggested by Applied Biosystems (User Bulletin #2). The following primers were used for AMPKα:

Forward: 5'-GACTGGACATAAAGTTGCTGTGA-3' 23,

Reverse: 5-'GGATTTTCCCGACCACGTC-3' 19

The expression of mRNA of AMPKα was normalised to beta-actin [32].

Statistical analysis

The results were compared with a Kruskal-Wallis analysis of variance (ANOVA) followed by Tukey's post hoc test. Significance levels are reported for $p < 0.05$ and $p < 0.01$.

Results

The body mass of the LRTE group decreased after the training period ($p<0.05$) compared to LRTC group (422.14±15.19g vs. 474.00±14.10g), and a similar change was observed between HRTE vs. HRTC groups (410.63±9.52g vs. 471.00±12.88g).Before exercise training, maximal oxygen consumption (VO_2max) was similar between all four experimental groups – LRTC, LRTE, HRTC, and HRTE,– and was on average ~ 65 ± 7.5 ml/kg/min. Aerobic exercise training significantly increased VO_2max in both LRTE and HRTE groups ($p<0.05$). However, the increase was more enhanced in HRTE animals ($p<0.01$) compared to LRTE ($p<0.05$) during the final weeks of training (FIG. 1A). The running distance measured during the VO_2max test was similar between the four experimental groups before training and increased with significantly in the trained groups. However, there was a significant differential for the change in running distance between LRTE and HRTE groups. HRTE groups ran more than 20% longer than LRTE animals during the final treadmill running test (FIG. 1B).

The mitochondrial content, evaluated by COX-4 levels increased significantly between the control and exercised groups for both the LRT and HRT rats (FIG. 1C).However, the activity of citrate synthase (CS) increased only in the HRTE group resulting in significant differences between the HRTC vs. HRTE and LRTE vs. HRTE groups ($p<0.05$, FIG. 1D). Interestingly, the activity of CS was significantly lower in exercised LRT rats compared to control LRT rats.

The levels of reactive oxygen species (ROS) were evaluated and indicated significantly lower levels in HRT rats compared to the LRT rats in the control condition (14% difference between HRTC vs. LRTC) (FIG. 2A). Exercise training tended to increase the levels of

ROS for both LRT and HRT. Consistent with ROS levels, the NAD^+/NADH ratio was higher in the HRTC group than in the LRTC group (FIG. 2B). Protein carbonyls were measured to assess the modifications of proteins due to exercise-induced oxidative stress and the data showed a significant increase in the HRTE group whereas the LRTE group was unchanged (FIG. 2C). Content of the main protein degrading enzymes were evaluated and showed that exercise training induced the R2 subunit of proteasome in both LRTE and HRTE groups (FIG. 3A), while the induction of mitochondria located Lon proteasome occurred only in the HRTE group (FIG. 3B). The mitochondrial chaperone HSP78 content did not change significantly (FIG. 3C) between any of the four groups tested.

The activity of AMPK, a critical mediator of skeletal muscle adaptations with training, was measured by the ratio pAMPKα/AMPKα, and was found to be significantly elevated in the HRTC group only (FIG. 4A-B). SIRT1 is a sensitive marker of metabolic stress and the data revealed that although not different in control conditions (LRTC vs. HRTC), exercise training increased SIRT1 content in both LRTE and HRTE groups (FIG. 4C).

The content of PGC1-α, known to be elevated in response to exercise training was increased only in the HRTE group (FIG. 5A). PGC1-α content was unchanged between LRTC and LRTE groups. PGC1-α can regulate both TFAM and NRF1 levels. Similar to the pattern for PGC1-α, NRF1 increased only in HRTE group (FIG. 5B) but there was no difference in content between LRTC and LRTE groups. The TFAM levels were significantly lower in the LRTE group compared to LRTC group (FIG. 5C). In case of HRT animals, exercise training increased the levels of TFAM (FIG. 5C). The quality control of the mitochondria is partly regulated by fission and fusion. The mitochondrial fission protein(Fis1) levels of HRTC group was lower than LRTC group (FIG. 5D) and was

induced by exercise training in both LRT and HRT rats (p=0.045). Alternately, the Mitofusin-1 (Mfn1) contents were not different in control conditions but decreased significantly with exercise training in both experimental groups (FIG. 5E).

Discussion

Trainability is a critical issue in high level sport, but it could be also important for the health benefits of daily physical activity. A complex mixture of gene-environment interactions contributes to the large range in training-induced adaptations and creates a considerable challenge for identifying the mechanistic connection between exercise capacity and human health. Here we used a contrasting rat model system, which was developed by artificial selective breeding to segregate animals into lines of low and high training response, thus allowing us to study trainability in an unbiased mechanistic way.

Before training, there is no significant differences for VO_2max or running distance in rats selected for low versus high response to training, which suggests trainability is not strongly dependent upon baseline VO_2max [4]. The differences in response to aerobic exercise training between the LRT and HRT was greater for running distance compared to VO_2max, indicating the limited trainability of VO_2max reported previously in human studies [40,39,17]. Further it supports data from humans demonstrating that training adaptations for improvement in aerobic performance and aerobic capacity can be uncoupled [36].

The mitochondrial network is crucial for coping with the metabolic challenge provided by physical exercise. The pioneering study of Holloszy showed that exercise training increases the activity of a number of mitochondrial enzymes and the content of mitochondria [13]. Davies and coworkers proposed that ROS could play a role in the biogenesis of mitochondria as a response to exercise training [8]. Indeed ROS are involved in a wide

range of signaling processes and redox homeostasis is closely linked to cellular metabolism [37]. In this study, we found an inverse relationship between ROS levels and NAD^+/NADH ratio in control versus trained rats selectively bred for high response to training (i.e., HRTC and HRTE groups), suggesting controlled redox homeostasis. This expected relationship was missing in LRT groups. On the other hand, significant differences were not found for COX-4 levels suggesting that the possible differences in redox balance did not significantly affect the rate of mitochondrial biogenesis.

We have observed a significant increase in the levels of carbonylated proteins in HRTC rats compared to LRTC animals. Significant degree of protein carbonylation used as a marker of oxidative damage of proteins, however moderate degree of carbonylation could be associated with the degree of protein turnover [36]. The ROS levels were apprised by H_2DCFDA staining which showed no difference between HRTC and LRTC groups. The degradation of proteins was evaluated by the contents of R2 subunit of proteasome and Lon protease, indeed regular exercise can elevate the levels of these housekeeping proteins. This is an important process because oxidative modification of proteins results in loss of function [33,30]. The lack of differences between LRT and HRT groups on proteasome induction could indicate that the housekeeping of aberrant proteins in the cytosol maybe independent from trainability. However, this was not the case for mitochondrial degradation of oxidized proteins, since Lon was induced only in HRTE groups. The Lon levels tend to be lower in LRTC than in HRTC rats ($p=0.22$), which might explain the differences in carbonylated proteins in these groups. However, based on our earlier finding that aging down-regulates Lon in skeletal muscle, and exercise can attenuate this effect and in turn, increase endurance performance [20]. Therefore, it cannot be ruled out that the housekeeping role of Lon protease influences trainability.

The activity of AMP-dependent protein kinase activity (AMPK) is an important signaling molecule for endurance exercise activity, and stimulating AMPK via AICAR administration has been shown to enhance aerobic performance [27]. Interestingly enough, we found in non-trained control conditions that the HRT rats have significantly higher activity of AMPK than LRT rats suggesting a greater potential for metabolic-responsiveness. Therefore, it cannot be ruled out that high inherent levels of AMPKα activity provide a favorable metabolic base for high trainability to aerobic exercise. We did not find significant differences at the mRNA levels of AMPKα among the groups, but the variation between mRNA and protein levels is not very surprising, due to the degradation of mRNA, impaired transcription, regulation by miRNA, or altered degradation of proteins. A study by Lessard and co-workers [23], reported that AMPK was normally activated by an acute bout of exercise in both LRT and HRT and unlikely to be related to the differential for exercise-induced adaptation response [1]. The decreased AMPK activities in LRT along with a reduction in citrate synthase in response to training suggest that, mitochondria-associated factors could be important for trainability. Indeed, a reduction of the beta subunits of AMPK in skeletal muscle can results in impaired exercise tolerance without significant alteration of mitochondrial contents or sugar metabolism [22].

We also found that exercise training induced SIRT1 in both low and high response trainers suggesting that the differential in trainability between LRT and HRT is independent from SIRT1 activity. Of interest is the finding that the PGC1-α content increased only in HRTE group. Currently there are conflicting data on the involvement of SIRT1 on the activation of PGC1-α. Some reports [28,31] including a recent paper from Holloszy's group [11]suggest that deacetylation of PGC1-α inhibits the activity of SIRT1 and mitochondrial biogenesis, while other papers indicate that SIRT1 mediated deacetylation activates PGC1-α [9,21,6].

The aim of the present investigation was to study the idea that mitochondrial biogenesis may contribute to the differential in response to training. PGC1-α, NRF1 and TFAM regulate mitochondrial biogenesis. As described above, PGC1-α was increased in the high responsive HRT group but not the low responsive LRT group in response to exercise training. TFAM levels decreased with exercise training in LRT animals and increased in the HRT group. TFAM has been linked to higher aerobic endurance [29] which we also measured to be greater in HRTE groups. The adverse training response of TFAM in LRT compared to HRT, therefore, puts this protein on the list of potentially limiting factors for exercise resistance. Moreover, the change of Lon content paralleled TFAM levels. Lon protease is involved in the stability, replication, transcription and translation of proteins, and targets TFAM, steroidogenic acute regulatory protein (StAR), and aconitase for degradation [25]. In addition, NRF1 pattern was also similar to the pattern for PGC1-α; NRF1was induced with training in HRT rats but not in LRT rats. Hence, downstream response elements of PGC1-α increased only in those animals that showed sensitivity to aerobic training.

In conclusion, these rat models of low response trainers (LRT) and high response trainers (HRT) represent the first heterogeneous substrate that can serve as reagents towards understanding the molecular networks responsible for variation in trainability [19]. Our data suggest: 1) Baseline levels of VO_2max do not strongly affect the adaptive response to aerobic exercise training. 2) Exercise induced changes in redox balance are not strong limiting factors of differential for trainability, and 3) AMPK activity, citrate synthase, carbonylated protein, NRF1, PGC1-α, TFAM and Lon protease response to aerobic exercise training are associated with trainability. Compared to inbred animal models, in which essentially all loci are fixed, outbred selected lines maintain genetic complexity, thus permitting unique combinations of

allelic variants at multiple interacting loci to be enriched differentially by selection pressure [7]. For translational purposes, the production of low and high selected lines seemingly segregate response elements for mitochondrial biogenesis and thus provides unbiased evidence in support of a hypothesis that growth and remodeling features are responsible for differential gains from training [18,39] but does not provide proof at the level of cause and effect [18].

References:

1. Ai H, Ihlemann J, Hellsten Y, Lauritzen HP, Hardie DG, Galbo H, Ploug T (2002) Effect of fiber type and nutritional state on AICAR- and contraction-stimulated glucose transport in rat muscle. Am J Physiol Endocrinol Metab 282 (6):E1291-1300. doi:10.1152/ajpendo.00167.2001

2. Baar K (2004) Involvement of PPAR gamma co-activator-1, nuclear respiratory factors 1 and 2, and PPAR alpha in the adaptive response to endurance exercise. Proc Nutr Soc 63 (2):269-273. doi:10.1079/pns2004334

3. Bouchard C (2012) Genomic predictors of trainability. Exp Physiol 97 (3):347-352. doi:10.1113/expphysiol.2011.058735

4. Bouchard C, An P, Rice T, Skinner JS, Wilmore JH, Gagnon J, Perusse L, Leon AS, Rao DC (1999) Familial aggregation of VO(2max) response to exercise training: results from the HERITAGE Family Study. J Appl Physiol (1985) 87 (3):1003-1008

5. Bouchard C, Sarzynski MA, Rice TK, Kraus WE, Church TS, Sung YJ, Rao DC, Rankinen T (2011) Genomic predictors of the maximal O(2) uptake response to standardized exercise training programs. J Appl Physiol 110 (5):1160-1170. doi:10.1152/japplphysiol.00973.2010

6. Canto C, Gerhart-Hines Z, Feige JN, Lagouge M, Noriega L, Milne JC, Elliott PJ, Puigserver P, Auwerx J (2009) AMPK regulates energy expenditure by modulating NAD+ metabolism and SIRT1 activity. Nature 458 (7241):1056-1060. doi:10.1038/nature07813

7. Carlborg O, Jacobsson L, Ahgren P, Siegel P, Andersson L (2006) Epistasis and the release of genetic variation during long-term selection. Nat Genet 38 (4):418-420. doi:10.1038/ng1761

8. Davies KJ, Quintanilha AT, Brooks GA, Packer L (1982) Free radicals and tissue damage produced by exercise. Biochem Biophys Res Commun 107 (4):1198-1205

9. Feige JN, Lagouge M, Canto C, Strehle A, Houten SM, Milne JC, Lambert PD, Mataki C, Elliott PJ, Auwerx J (2008) Specific SIRT1 activation mimics low energy levels and protects against diet-induced metabolic disorders by enhancing fat oxidation. Cell Metab 8 (5):347-358. doi:10.1016/j.cmet.2008.08.017

10. Gagnon J, Ho-Kim MA, Chagnon YC, Perusse L, Dionne T, Leon AS, Rao DC, Skinner JS, Wilmore JH, Bouchard C (1997) Absence of linkage between VO2max and its response to training with markers spanning chromosome 22. Med Sci Sports Exerc 29 (11):1448-1453

11. Goodrick CL (1980) Effects of long-term voluntary wheel exercise on male and female Wistar rats. I. Longevity, body weight, and metabolic rate. Gerontology 26 (1):22-33

12. Hart N, Sarga L, Csende Z, Koltai E, Koch LG, Britton SL, Davies KJ, Kouretas D, Wessner B, Radak Z (2013) Resveratrol enhances exercise training responses in rats selectively bred for high running performance. Food Chem Toxicol. doi:10.1016/j.fct.2013.01.051

13. Holloszy JO (1967) Biochemical adaptations in muscle. Effects of exercise on mitochondrial oxygen uptake and respiratory enzyme activity in skeletal muscle. J Biol Chem 242 (9):2278-2282

14. Holloszy JO (1993) Exercise increases average longevity of female rats despite increased food intake and no growth retardation. J Gerontol 48 (3):B97-100

15. Holloszy JO (2008) Regulation by exercise of skeletal muscle content of mitochondria and GLUT4. J Physiol Pharmacol 59 Suppl 7:5-18

16. Keller P, Vollaard N, Babraj J, Ball D, Sewell DA, Timmons JA (2007) Using systems biology to define the essential biological networks responsible for adaptation to

endurance exercise training. Biochem Soc Trans 35 (Pt 5):1306-1309. doi:10.1042/bst0351306

17. Keller P, Vollaard NB, Gustafsson T, Gallagher IJ, Sundberg CJ, Rankinen T, Britton SL, Bouchard C, Koch LG, Timmons JA (2011) A transcriptional map of the impact of endurance exercise training on skeletal muscle phenotype. J Appl Physiol 110 (1):46-59. doi:10.1152/japplphysiol.00634.2010
18. Koch LG, Britton SL, Wisloff U (2012) A rat model system to study complex disease risks, fitness, aging, and longevity. Trends Cardiovasc Med 22 (2):29-34. doi:10.1016/j.tcm.2012.06.007
19. Koch LG, Pollott GE, Britton SL (2013) Selectively bred rat model system for low and high response to exercise training. Physiol Genomics 45 (14):606-614. doi:10.1152/physiolgenomics.00021.2013
20. Koltai E, Hart N, Taylor AW, Goto S, Ngo JK, Davies KJ, Radak Z (2012) Age-associated declines in mitochondrial biogenesis and protein quality control factors are minimized by exercise training. Am J Physiol Regul Integr Comp Physiol 303 (2):R127-134. doi:10.1152/ajpregu.00337.2011
21. 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 (6):1109-1122. doi:10.1016/j.cell.2006.11.013
22. Lee HB, Park MS, Chung SH, Lee YB, Kim KS, Hwang SD, Moon C (1990) Peritoneal solute clearances in diabetics. Perit Dial Int 10 (1):85-88
23. Lessard SJ, Rivas DA, Alves-Wagner AB, Hirshman MF, Gallagher IJ, Constantin-Teodosiu D, Atkins R, Greenhaff PL, Qi NR, Gustafsson T, Fielding RA, Timmons

JA, Britton SL, Koch LG, Goodyear LJ (2013) Resistance to aerobic exercise training causes metabolic dysfunction and reveals novel exercise-regulated signaling networks. Diabetes 62 (8):2717-2727. doi:10.2337/db13-0062

24. Lucia A, Martin MA, Esteve-Lanao J, San Juan AF, Rubio JC, Olivan J, Arenas J (2006) C34T mutation of the AMPD1 gene in an elite white runner. Br J Sports Med 40 (3):e7. doi:10.1136/bjsm.2005.019208

25. Matsushima Y, Kaguni LS (2012) Matrix proteases in mitochondrial DNA function. Biochim Biophys Acta 1819 (9-10):1080-1087. doi:10.1016/j.bbagrm.2011.11.008

26. Nakamura E, Moritani T, Kanetaka A (1996) Effects of habitual physical exercise on physiological age in men aged 20-85 years as estimated using principal component analysis. Eur J Appl Physiol Occup Physiol 73 (5):410-418

27. Narkar VA, Downes M, Yu RT, Embler E, Wang YX, Banayo E, Mihaylova MM, Nelson MC, Zou Y, Juguilon H, Kang H, Shaw RJ, Evans RM (2008) AMPK and PPARdelta agonists are exercise mimetics. Cell 134 (3):405-415. doi:10.1016/j.cell.2008.06.051

28. Nemoto S, Fergusson MM, Finkel T (2005) SIRT1 functionally interacts with the metabolic regulator and transcriptional coactivator PGC-1{alpha}. J Biol Chem 280 (16):16456-16460. doi:10.1074/jbc.M501485200

29. Norrbom J, Wallman SE, Gustafsson T, Rundqvist H, Jansson E, Sundberg CJ (2010) Training response of mitochondrial transcription factors in human skeletal muscle. Acta Physiol (Oxf) 198 (1):71-79. doi:10.1111/j.1748-1716.2009.02030.x

30. Poppek D, Grune T (2006) Proteasomal defense of oxidative protein modifications. Antioxid Redox Signal 8 (1-2):173-184. doi:10.1089/ars.2006.8.173

31. Puigserver P, Adelmant G, Wu Z, Fan M, Xu J, O'Malley B, Spiegelman BM (1999) Activation of PPARgamma coactivator-1 through transcription factor docking. Science 286 (5443):1368-1371

32. Radak Z, Bori Z, Koltai E, Fatouros IG, Jamurtas AZ, Douroudos, II, Terzis G, Nikolaidis MG, Chatzinikolaou A, Sovatzidis A, Kumagai S, Naito H, Boldogh I (2011) Age-dependent changes in 8-oxoguanine-DNA glycosylase activity are modulated by adaptive responses to physical exercise in human skeletal muscle. Free Radic Biol Med 51 (2):417-423. doi:10.1016/j.freeradbiomed.2011.04.018

33. Radak Z, Chung HY, Koltai E, Taylor AW, Goto S (2008) Exercise, oxidative stress and hormesis. Ageing Res Rev 7 (1):34-42. doi:10.1016/j.arr.2007.04.004

34. Radak Z, Chung HY, Naito H, Takahashi R, Jung KJ, Kim HJ, Goto S (2004) Age-associated increase in oxidative stress and nuclear factor kappaB activation are attenuated in rat liver by regular exercise. FASEB J 18 (6):749-750. doi:10.1096/fj.03-0509fje

35. Radak Z, Sasvari M, Nyakas C, Pucsok J, Nakamoto H, Goto S (2000) Exercise preconditioning against hydrogen peroxide-induced oxidative damage in proteins of rat myocardium. Arch Biochem Biophys 376 (2):248-251. doi:10.1006/abbi.2000.1719

36. Radak Z, Zhao Z, Goto S, Koltai E (2011) Age-associated neurodegeneration and oxidative damage to lipids, proteins and DNA. Mol Aspects Med 32 (4-6):305-315. doi:10.1016/j.mam.2011.10.010

37. Radak Z, Zhao Z, Koltai E, Ohno H, Atalay M (2013) Oxygen consumption and usage during physical exercise: the balance between oxidative stress and ROS-dependent adaptive signaling. Antioxid Redox Signal 18 (10):1208-1246. doi:10.1089/ars.2011.4498

38. Sarna S, Sahi T, Koskenvuo M, Kaprio J (1993) Increased life expectancy of world class male athletes. Med Sci Sports Exerc 25 (2):237-244

39. Timmons JA, Knudsen S, Rankinen T, Koch LG, Sarzynski M, Jensen T, Keller P, Scheele C, Vollaard NB, Nielsen S, Akerstrom T, MacDougald OA, Jansson E, Greenhaff PL, Tarnopolsky MA, van Loon LJ, Pedersen BK, Sundberg CJ, Wahlestedt C, Britton SL, Bouchard C (2010) Using molecular classification to predict gains in maximal aerobic capacity following endurance exercise training in humans. J Appl Physiol 108 (6):1487-1496. doi:10.1152/japplphysiol.01295.2009

40. Vollaard NB, Constantin-Teodosiu D, Fredriksson K, Rooyackers O, Jansson E, Greenhaff PL, Timmons JA, Sundberg CJ (2009) Systematic analysis of adaptations in aerobic capacity and submaximal energy metabolism provides a unique insight into determinants of human aerobic performance. J Appl Physiol 106 (5):1479-1486. doi:10.1152/japplphysiol.91453.2008

41. von Duvillard SP, Foxall TL, Davis WP, Terpstra AH (2000) Effects of exercise on plasma high-density lipoprotein cholesteryl ester metabolism in male and female miniature swine. Metabolism 49 (7):826-832. doi:10.1053/meta.2000.6746

42. Yates LB, Djousse L, Kurth T, Buring JE, Gaziano JM (2008) Exceptional longevity in men: modifiable factors associated with survival and function to age 90 years. Arch Intern Med 168 (3):284-290. doi:10.1001/archinternmed.2007.77

Figure legends:

Fig. 1: **Maximal oxygen uptake and mitochondrial content**

The VO_2max (**A**) and the running distance (**B**) during VO_2max testing were measured every second week during the training period and the intensity was adjusted to 70% of VO2max. The mitochondrial biogenesis was appraised by the COX-4 content (**C**) and the adaptation to exercise training byCitrate Synthase (CS) activity (D). Low response trainers control LRT (LRTC) (n=6), exercised LRT (LRTE) (n=7), high response trainers control HRT (HRTC) (n=6) and exercised HRT (HRTE) (n=8) groups.* $p<0.05$ HRTC vs HRTE** $p<0.01$ HRTC vs HRTE + $p<0.05$ LRTC vs LRTE++ $p<0.01$ LRTC vs LRTE.Δ $p<0.05$ LRTE vs HRTE

Fig. 2.**Oxidative stress markers**

The measurements of the ROS levels were done by fluorescent detection of H2DCFDA (**A**).The ratio of NAD^+/NADH was used to evaluate the redox balance (**B**), while the carbonyl group levels indicate the oxidative modification of proteins **(C, D).** Low response trainers control LRT (LRTC) (n=6), exercised LRT (LRTE) (n=7), high response trainers control HRT (HRTC) (n=6) and exercised HRT (HRTE) (n=8) groups, * $p<0.05$ HRTC vs HRTE ** $p<0.05$ HRTC vs HRTE + $p<0.05$ LRTC vs LRTE++ $p<0.05$ LRTC vs LRTE.Δ $p<0.05$ LRTE vs HRTE

Fig. 3.**Quality control of proteins**

The R2 subunit of proteasome (A) mitochondrial Lon Protease (B) and the HSP87 (C) protein content were measured by immunoblot method. Low response trainers control LRT (LRTC) (n=6), exercised LRT (LRTE) (n=7), high response trainers control HRT (HRTC) (n=6) and exercised HRT (HRTE) (n=8) groups, * $p<0.05$

Fig. 4.**Metabolic markers**

The mRNA levels of AMPK were assessed by RT-PCR (**A**), while activity of AMPKα was appraised by the pAMPKα/AMPKα ratio (**B**). SIRT1 content was measured by immunoblots (**C**). Low response trainers control LRT (LRTC) (n=6), exercised LRT

(LRTE) (n=7), high response trainers control HRT (HRTC) (n=6) and exercised HRT (HRTE) (n=8) groups, * $p<0.05$

Fig. 5: **Mitochondrial biogenesis associated transcription factors**

Mitochondrial biogenesis-associated transcription factors, such as PGC1-α (**A**), NRF1 (**B**), TFAM (**C**) and mitochondrial fission Fis1 (**D**) and fusion Mfn1 (**E**) proteins were measured. Low response trainers control LRT (LRTC) (n=6), exercised LRT (LRTE) (n=7), high response trainers control HRT (HRTC) (n=6) and exercised HRT (HRTE) (n=8) groups, * $p<0.05$

Figure

Fig.1. Maximal oxygen uptake (a), Running distance (b), COX-4 levels (c) and citrate synthase (d)

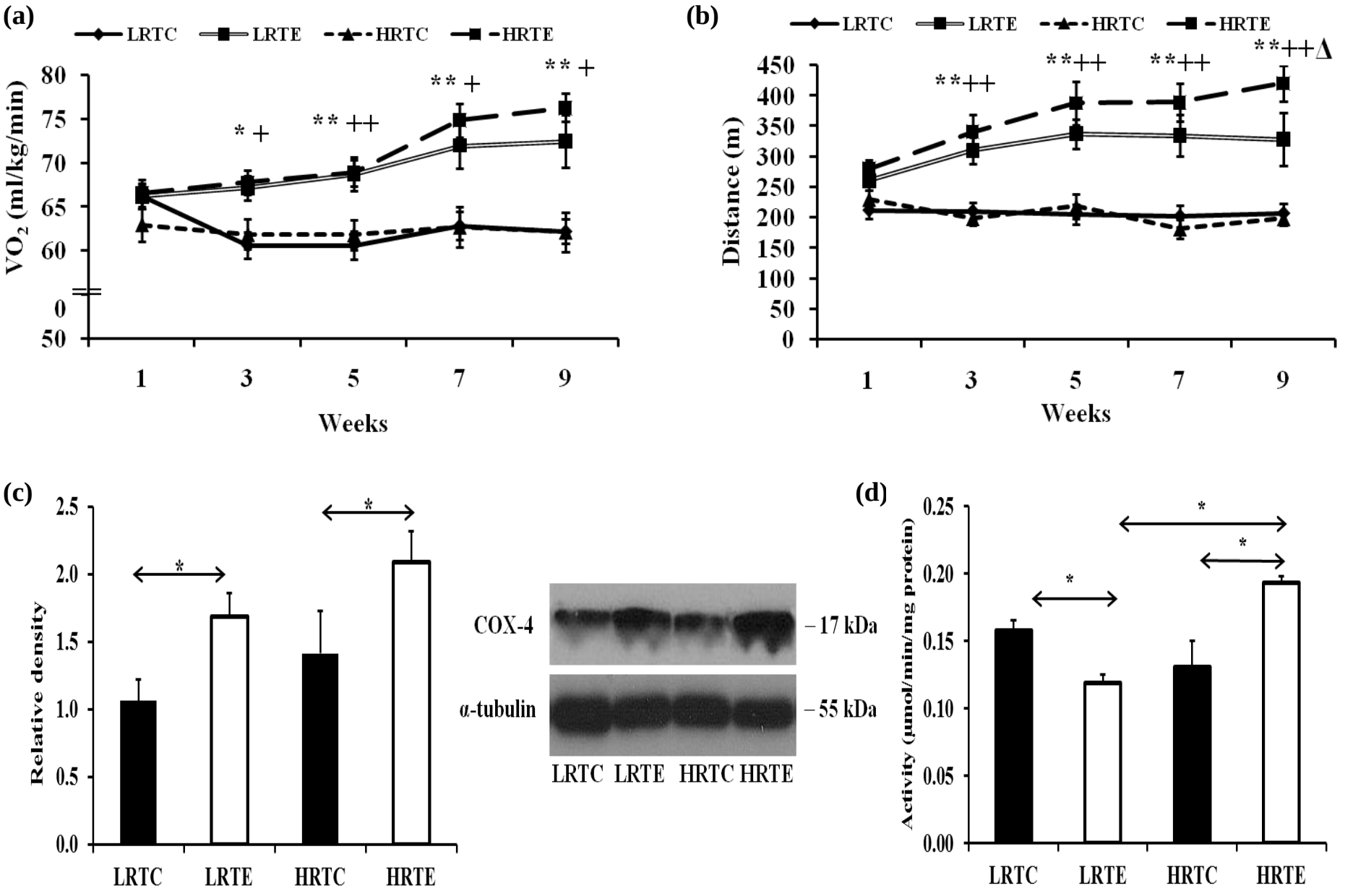
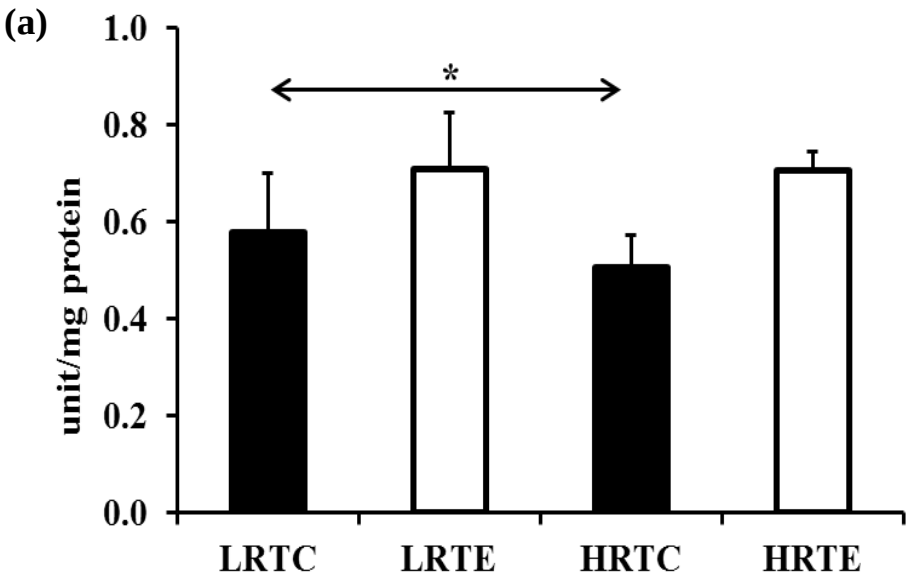
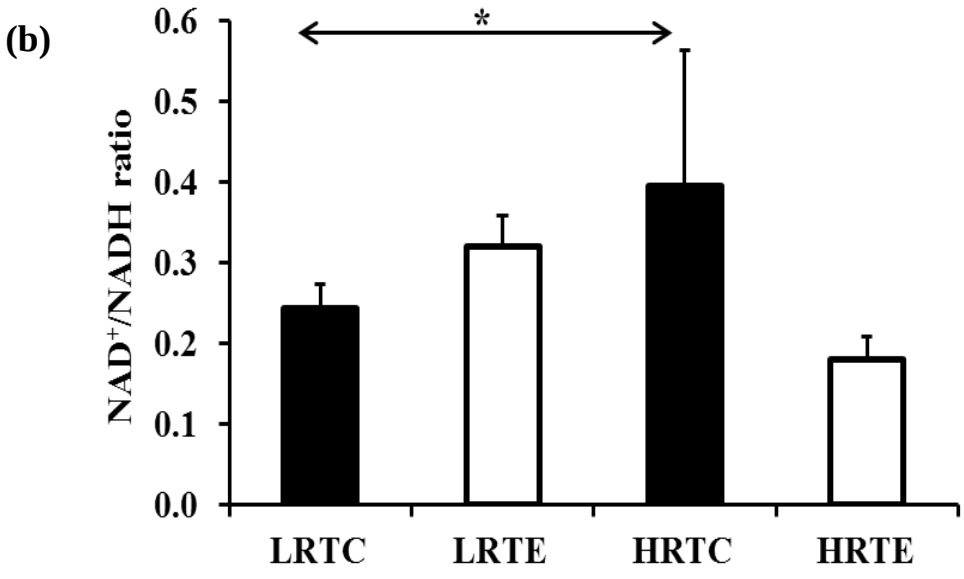
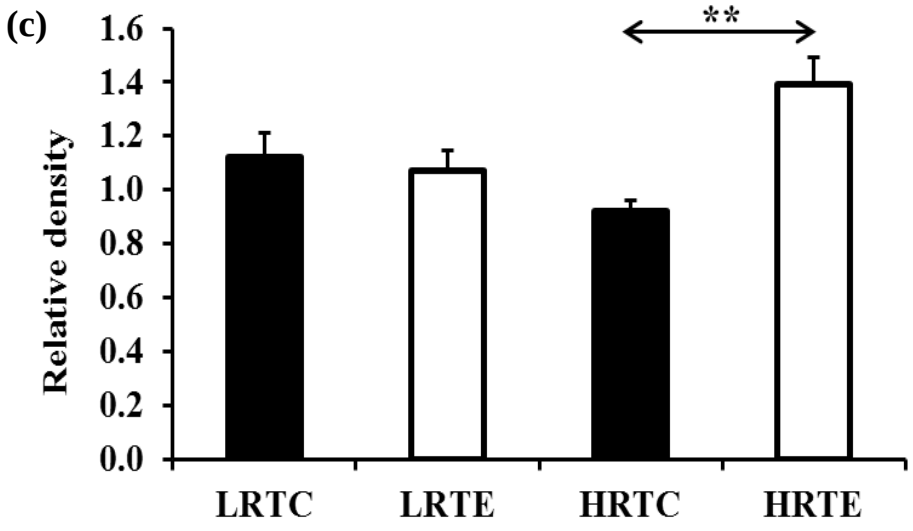


Fig.2. H2DCFDA (with 100 uM H2O2) (a), NAD$^+$/NADH ratio (b), Carbonylated protein (c)







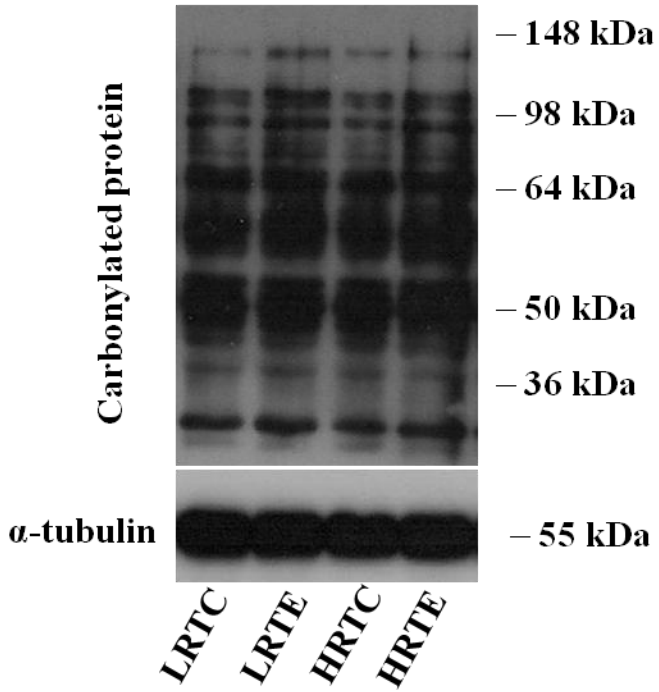


Fig. 3. Proteasome (R2 subunit) (a), LonP (b) and HSP78 (c)

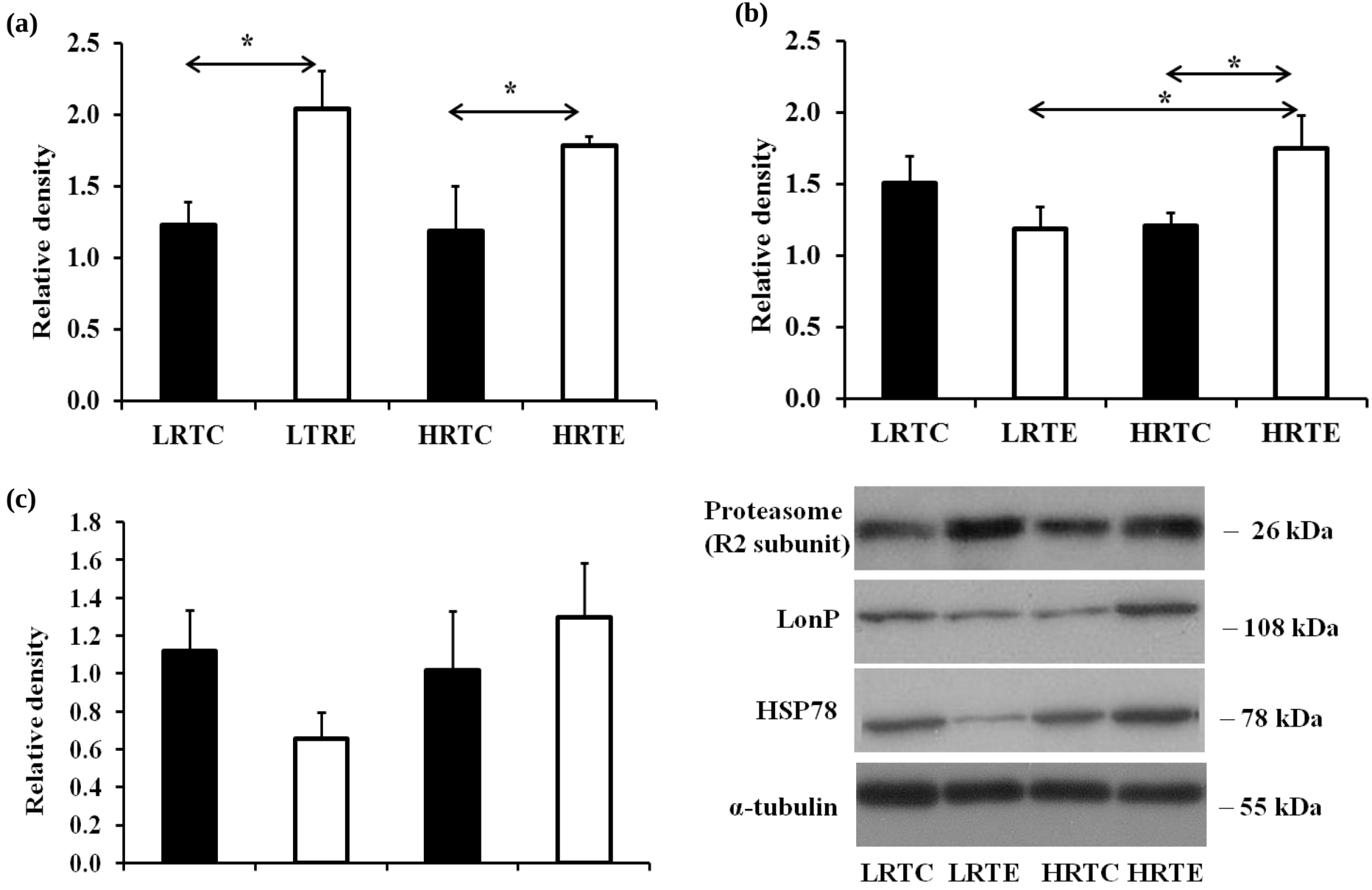


Fig. 4. mRNA levels of AMPK (a) pAMPK/AMPK ratio (b) and SIRT1 (c) contents

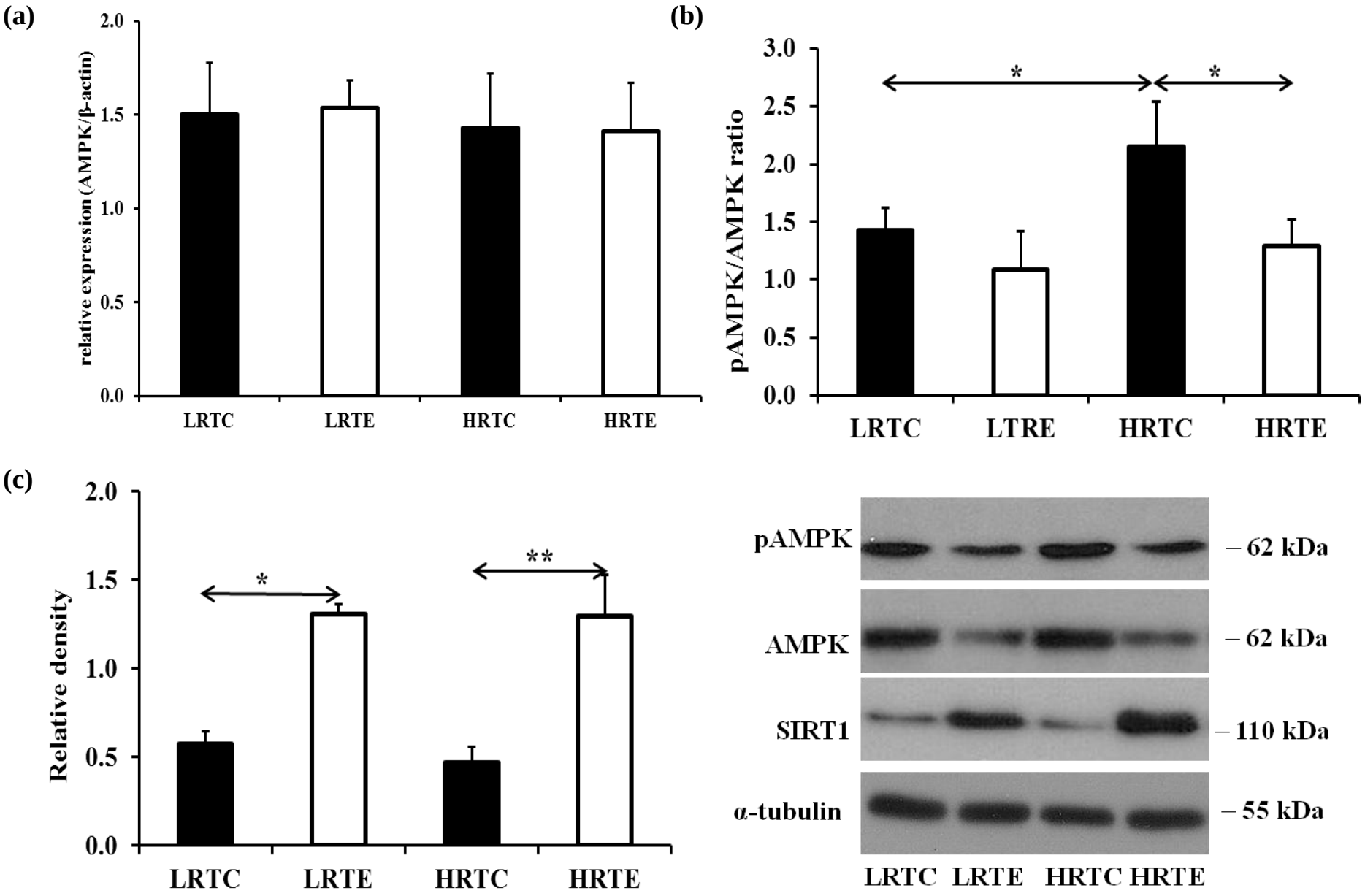


Fig. 5. PGC1-α (a), NRF-1 (b), TFAM (c), Fis1 (d), Mfn1 (e)