The effect of physical stimuli on the expression level of key elements in mitochondrial biogenesis

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Abstract

Proper mitochondrial function is crucial for intact cellular homeostasis. Mitochondrial dysfunction is clearly involved in the pathogenesis of most neurodegenerative- and age-related chronic disorders. The aim of this study is to stimulate ~~probablythe most~~ important compounds of mitochondrial biogenesis, namely the peroxisome proliferator-activated receptor-gamma coactivator (PGC)- and Sirtuin (SIRT)-systems.

We studied the effect of cold challenge and training on the expression of mRNA levels of some compounds of these systems in different brain areas of mice. ~~From the numerous PGC-1α and Sirtuin isoforms, PGC-1α mRNA levels of full-length, the N-truncated isoforms and the reference and brain-specific promoters, and 4 Sirtuin isoforms (SIRT1, SIRT3-M1/M2/M3) were measured.~~With regard to the PGC-system, the mRNA levels of the full- and N-truncated isoforms, and those of the two promoters (brain-specific, reference) were measured. In case of Sirtuins, the mRNA levels of SIRT1 and SIRT3-M1/M2/M3 were assessed.~~For the PGC-system the full- and N-truncated isoforms, and two promoters (brain-specific, reference) mRNA levels were measured, while SIRT1 and SIRT3-M1/M2/M3 for the Sirtuins.~~

We found the following expression level alterations: cooling resulted in the elevation of cortical SIRT3-M1 levels and the decrease of cerebellar SIRT3-M3 levels after 200 min. 900 min cold exposure resulted in the reduction of cortical SIRT1 and striatal SIRT3-M1 levels. A prominent elevation could be observed after 12 days training in the level of all PGC-1α isoforms in the cerebellum. The 12-day-long exercise resulted in increases in cerebellar SIRT3-M1 and SIRT3-M2 mRNA levels as well.

Although the effectivity of cooling in the decrease of core body and brain temperature is questionable, we hypothesize that training exerted a clear effect. The reason behind the prominent cerebellar elevation of PGC-, and Sirtuin isoforms could be the increase of

synaptic plasticity between Purkinje cells, which facilitate better motoric- and non-motoric coordination and more precise movement integration. We propose that these systems may serve as promising targets for future therapeutic studies in neurodegenerative diseases.

Keywords: PGC-1α; Sirtuin; Training; Cold.

1. Introduction

Constant energy supply is crucial for proper tissue function. Mitochondria provide the synthesis of adenosine triphosphate (ATP), and have an importantplays a role in adaptive thermogenesis, intracellular Ca^{2+} homeostasis, aging and cell death. Mitochondrial dysfunction, cellular energy imbalance and increased production of reactive oxygen species are implicated in the pathogenesis of several disorders, including neurodegenerative diseases.

Peroxisome proliferator-activated receptor-gamma (PPARγ) coactivator-1 alpha (PGC-1α) is a transcriptional coactivator that regulates mitochondrial biogenesis, energy homeostasis and adaptive thermogenesis [1, 2]. The PGC-1α protein has a complex structure with multiple domains, which enable the interaction with diverse transcriptional regulatory factors [36]. The N-terminal domain of the PGC-1α protein mediates interactions with nuclear receptors (NRs) and regulates the transcriptional activity, whereas the central and C-terminal domains mediate interactions with nuclear respiratory factors (NRFs), PPARγ, MEF2C and FOXO1 [7]. Besides the full-length protein (FL-PGC-1α, 797 amino acid), multiple PGC-1α isoforms have been presented to date [3, 84]. Alternative promoter usage and alternative splicing increase the complexity of the transcripts. Alternative splicing between exons 6 and 7 of the *Pgc-1α* gene produces the N-truncated, shorter PGC-1α (NT-PGC-1α) isoform which is shorter (267 amino acids) than FL-PGC-1α, but functionally active. This protein retains the N-terminal transcriptional activation domains, but lacks all domains within 268-797 amino acids of the

~~FL-PGC-1α~~ [5]. Beside classical proximal promoter ~~R~~recently, novel tissue-specific PGC-1α isoforms have been described, including muscle-~~ specific~~, liver- and central nervous system-specific (CNS-PGC-1α) isoforms [3, ~~8,~~4, 6, 7].~~]. Beside the classical proximal promoter (PP), three different promoters were discovered. The liver-specific promoter (LP) is localized in intron 2 and plays an important role in the regulation of the expression in the liver. The alternative promoter, which is active in skeletal muscle and adipose tissue, is located 14 kb upstream of the previously characterised proximal promoter. Átfogalmazni úgy, hogy az előző mondat kapcsolódjon az előzőekhez.Whereas the novel CNS-specific isoforms originated from a new promoter located 587 kb upstream of exon 2 [9].].~~

Sir2-like proteins (Sirtuins) are mainly NAD^+-dependent deacetylases which play a prominent role in mitochondrial biogenesis~~, genomic stability, apoptosis, cell survival and different metabolic processes~~ [8, 9]. Seven mammalian Sirtuin subtypes (SIRT1-7) were identified that are present in different subcellular localizations~~, which depends on isoform specific localization sequences~~ [10]. ~~The Sirtuin family has numerous molecular targets. The best-known member of the family, SIRT1, is a mammalian orthologue of yeast Sir2, which deacetylates histones, p53, MEF2C, FOXOs and PGC-1α [16]. It is highly expressed in several brain areas, heart, skeletal muscle and white adipose tissue. SIRT3 is found inside of mitochondria in skeletal muscle, heart, brown adipose and brain. A hétből a 3. nagyon fontos lehet (releváns) a sirtuinok agyi funkciója szempontjából …Furthermore, T~~t~~The alternative promoter usage and the splicing variability results in a wide range of Sirtuin isoforms. ~~F~~From the perspective of our research the ~~three~~ four most important subtypes were SIRT1 ~~of~~and SIRT3~~ were~~ -M1, -M2 and -M3~~, which are generated by alternative exon translation starting site~~ [11-14]. ~~The expression of these genes is strongly influenced by changes in the environment, diet, and lifestyle [21, 22].~~

It is well known that tissue-specific PGC-1α and Sirtuin alterations develop by challenging the energy homeostasis, e.g., with cold exposure and physical exercise [1, 6, 15-27]. Previous studies described that the full length PGC-1α mRNA expression was elevated in mouse brown adipose tissue (BAT) and skeletal muscle following cold exposure [1]. ~~Recently published studies demonstrated that there is a shorter, but functionally active isoform of NT-PGC-1α (NT-PGC-1α254) which is able to initiate the thermogenic program without the full-length PGC-1α [11, 47].~~ It is also known that cold exposure shifts the transcription from PP to an alternative promoter in BAT [6]. ~~In addition, PGC-1α expression was determined in mouse brain after 3 h or 12 h at 4°C in parallel with UCP-1 mRNA as well, finding that PGC-1α and UCP-1 mRNA expression was not induced by short-term cold exposure in brain [25].~~ Recent studies demonstrated that the mRNA levels of SIRT2 and SIRT3 in mice BAT were increased by cold exposure (3, 6, 12 h periods on 5°C) and decreased if the environmental temperature was higher than thermoneutral (16 h period on 27.5°C) [22]. SIRT6 mRNA and protein levels were also elevated in the brown and inguinal white adipose tissue of 8-week-old mice following 4°C overnight cold exposure [26]. Although data are increasing about the changes of these 2 systems in BAT following cooling, but there are only a few data with regard to the related alterations in the brain. A study demonstrated that the brain mRNA level of PGC-1α did not change after 3 h or 12 h at 4°C [25].

Increased PGC-1α expression was observed in the skeletal muscles by physical exercise [24, 27]. An isoform-specific expression pattern during different intensity exercises was demonstrated as well, which is caused by promoter shift [22, 24]. ~~The mRNA expression derived from PP could be increased by high-intensity exercise, whereas the alternative promoter was activated by low-, medium, and high-intensity exercise [42, 46]. It was demonstrated as well~~It was proved that PGC-1 mRNA level increased in rats already in the first day following 1-week training on alternate days [20]. However, following a similar

increase in the first day, an opposite, decreasing trend was observed during consecutive training for 4 days [20]. Although muscle is the primary tissue used during exercise, the brain function is involved and modified as well. There is evidence that physical activity evokes mitochondrial biogenesis in the nervous system [29, 40]. Studies reported that long-term intensive exercise training induced FL-PGC-1α expression and mitochondrial biogenesis in the whole brain, particularly in some brain areas of mice [23]. The 17-day-long training did not change PGC-1α protein levels in the examined brain regions (cortex, striatum) neither in young (4 weeks old) nor in old (24 months old) mice [18].

Although exercise failed to extend life span in animal experiments, but seemingly had an influence in the Sirtuin-system [15-23]. Several studies have been carried out to investigate the correlation between training intensity, the age of animals and Sirtuin expression in the skeletal muscle, liver and heart [15, 21]. The results of these studies can be summarized as an increase in SIRT1, SIRT6 levels after exercise with different protocols [39]. However, the clarification of the effect of training on the Sirtuin-system in the mouse brain needs further studies. Steiner and Bayod found an elevation of SIRT1 protein level in specific brain regions regions (frontal lobe, hypothalamus, hippocampus, cortex, midbrain, but not in the cerebellum and brainstem) of mice after exercise [15, 23], whereas Lezi E et al. found no difference in the expression level of SIRT1 in brains of mice following exercise [40]. With regard to another subtype of the Sirtuin-system, SIRT3, its level was found to be elevated in the brain of exercised mice [16].

It has been already demonstrated that there is a direct relationship between the Sirtuin- and PGC-system [28, 29]. PGC-1α is one of the most important downstream target of Sirtuins [48]. This system is implicated in neurodegenerative diseases, such as ALS, Huntington's, Parkinson's and Alzheimer's disease [30].

Although the expression of PGC-1α and Sirtuins have been already investigated in rodent brain following the above-mentioned environmental stimuli, the results are controversial [1, 6, 15-27]. From the perspective of the PGC-system, only the FL-PGC-1α and NT-PGC-1α were examined previously, whereas it is well established that in the brain, the short-term cold exposure could not alter the level of the examined isoforms. The training elevated the levels of PGC-1α isoforms with intensity-, age- and duration specific manners. However, there is no available data about the recently identified novel isoforms of PGC- and Sirtuin-system in different brain regions. Accordingly, the aim of the current study was to assess the effect of cold challenge and training on the expression levels of FL-PGC-1α, NT-PGC-1α, CNS-PGC-1α, PP-PGC-1α, SIRT1, SIRT3-M1, SIRT3-, -M2 and SIRT3-M3 in the striatum, cortex and cerebellum of wild-type C57Bl/6J mice.

2. Experimental Procedure

2.1 Animals

20-week-old female C57Bl/6J mice were involved in this study. The rationale for of the application of female mice was that the PGC-system has a gender-specific expression pattern [31, 32]. Weydt et al. detected an SNP (rs3736265, *PPARGC1A*) in patients with Huntington's disease which caused earlier disease onset only in men [32]. They also showed that there is an earlier disease onset and age of death in ALS transgenic, FL-PGC-1α deficient male mice [31]. Accordingly, these data indicate that the PGC-system mediated protective effects may be more active in females. With regard to the Sirtuin-system, it can be said that overexpression of SIRT6 increases the lifespan in transgenic male mice, but not in females. As a conclusion, it is likely to exist consist a more activethe Sirtuin-system seems to be more active in female mice as well [33]. The animals were originally obtained from Jackson Laboratory (Bar Harbor, ME, USA). The

mice were housed in cages under standard conditions with 12-12 h light-dark cycle and free access to food and water. The experiments were carried out in accordance with the European Communities Council Directive (86/609/EEC) and were approved by the local animal care committee. All animals were euthanized via isoflurane overdose (Forane; Abott Laboratories Hungary Ltd., Budapest, Hungary).

2.2 Treatments and sample handling

2.2.1 Cold exposure

Animals were randomly divided into four groups (n = 7-8 in each group). The first group was kept at 4°C for 40 min/day, for 5 days (200 min), the second one was kept 4°C for 180 min/day for 5 days (900 min). After the cold exposure, mice were placed back under standard conditions (22-24ºC). The third and fourth groups were control groups and were housed at 22-24°C in the same room. Ninety minutes after the last cold exposure the animals were deeply anesthetized with isoflurane and their brains were dissected immediately.

2.2.2 Exercise training

Exercise training was examined via the application of rotarod. The mice were randomly allocated into four groups (n = 5-8 in each group). The first and second groups were the training groups. The mice were placed on the rotarod for a 2-session period (9.00 a.m., 4.00 p.m.) for 5 days (first group) or 12 days (second group). The speed profile was standard 5 RPM for 30 minutes. Prior to the training, the mice were transported to the testing room for an acclimatization period of at least 30 minutes. The third and fourth groups were control groups. Ninety minutes after the last measure, the animals were anesthetized, and the brains were dissected immediately.

2.3 RT-PCR Analysis

Total RNA was isolated from striatum, cortex and cerebellum with Trizol according to the manufacturer's protocol. RNA concentrations were measured by MaestroNano spectrophotometer, and the integrity of RNA was confirmed by gel electrophoresis using 1% agarose gel. cDNA was generated from 1 µg of total RNA with random hexamer primers and reverse transcriptase according to the Revert Aid First Strand cDNA Synthesis Kit protocol (Thermo Scientific, USA). cDNA was kept at -20ºC until further use. Real-time PCR was performed on CFX 96 Real TimeSystem (Bio-Rad, USA) to detect changes in mRNA expression, using various primer pairs at a final volume of 20 µl. We used previously described ~~the following PP~~GC-1α and Sirtuin primers [6, 12, 14~~: ~~]. (See the exact thermal cycling conditions in Supplementary File 1.). ~~**FL-PGC-1α:** 5'-TGCCATTGTTAAGACCGAG-3' **(forward)** and 5'-TTGGGGTCATTTGGTGAC-3' **(reverse)**; **NT-PGC-1α:** 5'-TGCCATTGTTAAGACCGAG-3' **(forward)** and 5'-GGTCACTGGAAGATATGGC-3' **(reverse)**; **CNS-PGC-1α:** 5'-AATTGGAGCCCCATGGATGAAGG-3' **(forward)** and 5'-TCAAATGAGGGCAATCCGTC-3' **(reverse)**; **Ref-PGC-1α** 5'-TGAGTCTGTATGGAGTGACATCGAGTG-3' **(forward)**, and 5'-TCAAATGAGGGCAATCCGTC-3' **(reverse)** [11, 12]. The Sirtuin primers: **SIRT1:** 5'-GCACTAATTCCAAGTTCTATACCC-3' **(forward)** and 5'-GTGGTACAGTTCTTTCAGGTG-3' **(reverse)**; **SIRT3-M1:** 5'-TCAGACTGTGGGGTCCGGGAGTGTTA-3' **(forward)** and 5'-CAACATGAAAAAGGGC-3' **(reverse)**; **SIRT3-M2:** 5'-GACTGTGGGGTCCGGGAGGTGG-3' **(forward)** and 5'-CAACATGAAAAAGGGC-3' **(reverse)**; **SIRT3-M3:** 5'-GGCGTTTGGCGAGGACTA-3' **(forward)** and 5'-CAAC ATGAAAAAGGGC-3' **(reverse)** [20]. Thermal cycling conditions were the following: PGC-1α primers: 95ºC for 2 min, followed by 40 cycles of 95ºC for 10 s, and 60ºC for 30 s; SIRT1:~~

~~95°C for 2 min, followed by 40 cycles of 95°C for 10 s, and 62°C for 30 s; SIRT3-M1, SIRT3-M2: 95°C for 2 min, followed by 40 cycles of 95°C for 10 s, and 62.4°C for 30 s; SIRT3-M3: 95°C for 2 min, followed by 40 cycles of 95°C for 10 s, and 56.6°C for 30 s.~~

Target gene expression was normalized to the endogenous control gene 18S rRNA (Applied Biosystems, USA). The relative expression was calculated by the $2^{-\Delta\Delta Ct}$ method [34].

2.4 Statistics

All statistical analyses were performed with the use of the R software (R Development Core Team). Levene test was performed for the analysis of the homogeneity of variances. To assess the differences of PGC-1α- and Sirtuin gene expression levels of all brain areas relative to their respective control groups, approximative (10 000 random permutation) two sample Fisher-Pitman permutation test was applied. We calculated the gene expression level of PGC-transcripts in all brain areas relative to FL-PGC-1α and CNS-PGC-1α control striatum groups. In ~~the~~ case of Sirtuins we compared the SIRT1 expression levels to SIRT1-FL, and all SIRT3 isoforms to SIRT3-M1 control striatum groups. The differences were considered significant when the p values were less than 0.05.

3. Results

3.1 PGC-1α transcript levels

3.1.1 Cold exposure

There were no detectable changes in the levels of PGC-1α transcripts in the different brain areas after the total 200 min or 900 min cold exposure. The expression level of all the investigated transcripts was detected at room temperature and this expression was not altered by cold exposure (Supplementary F~~f~~igure 1 and 2).

3.1.2 Exercise training

The levels of PGC-1α transcripts did not show any change in the investigated brain areas after the 5-day-long rotarod training (Supplementary Ffigure 3). However, 12-day-long exercise training resulted in significant increases in FL-PGC-1α, NT-PGC-1α, CNS-PGC-1α and Ref-PGC-1α mRNA expression in the cerebellum (FL-PGC-1α: ctrl: 1.32 ± 0.20; EX: 1.59 ± 0.19; p = 0.024; NT-PGC-1α: ctrl: 0.29 ± 0.04; EX: 0.38 ± 0.04; p = 0.0002; CNS-PGC-1α: ctrl: 1.35 ± 0.23; EX: 1.80 ± 0.32; p = 0.003, Ref-PGC-1α: ctrl: 0.21 ± 0.03; EX: 0.30 ± 0.02; p = 0.0003;) Figure 1 C). With regard to the striatum and the cortex no other significant differences were detected (Figure 1 A, B). Furthermore,To verify that the CNS specific promoter is only poorly expressed in peripheral tissues, the widely applied quadriceps muscle was utilized demonstrating hardly detectable expression levels. However, the the expression level of FL-PGC-1α (ctrl: 1.01 ± 0.19; EX: 3.19 ± 1.25; p = 0.003), NT-PGC-1α (ctrl: 0.10 ± 0.02; EX: 0.50 ± 0.19; p = 0.001) and Ref-PGC-1α (ctrl: 1.00 ± 0.11; EX: 1.69 ± 0.52; p = 0.016) mRNA was significantly elevated after 5 days training in the quadriceps muscle. The FL-PGC-1α and NT-PGC-1α expression levels showed an approximately 3-fold (ctrl: 1.01 ± 0.19; EX: 3.19 ± 1.25; p = 0.003) and 6-fold (ctrl: 0.10 ± 0.02; EX: 0.50 ± 0.19; p = 0.001) increases, respectively, whereas Ref-PGC-1α mRNA levels increased by ~1.7-fold (ctrl: 1.00 ± 0.11; EX: 1.69 ± 0.52; p = 0.016).

3.2 Sirtuin transcript levels

3.2.1 Cold exposure

After 200 minutes cold exposure there were no detectable changes in the levels of SIRT1 and SIRT3-M2 transcripts in any brain regions (Figure 2), but SIRT3-M1 levels elevated in the cortex (ctrl: 1.26 ± 0.49; EX: 1.97 ± 0.60; p = 0.036; Figure 2 B), whereas SIRT3-M3 levels decreased in the cerebellum (ctrl: 1.16 ± 0.05; EX: 0.10 ± 0.03; p = 0.027;) (Supplementary figureFigure 42 C). Total 900 minutes long cooling resulted in the decrease of cortical SIRT1 (ctrl: 1.14 ± 0.31; EX: 0.66 ± 0.24; p = 0.008; Figure 2 E) and striatal SIRT3-M1 (ctrl: 1.04 ±

0.30; EX: 0.72 ± 0.21; p = 0.029; Figure 2 D) relative expression levels ~~(Supplementary figure~~Figure ~~52)~~.

3.2.2 Exercise training

After 5 days rotarod training the SIRT1 cortical levels found to be elevated (ctrl: 0.78 ± 0.10; EX: 0.97 ± 0.16; p = 0.042), but the other isoforms did not change (Supplementary figure ~~46~~). However, 12 days long exercise training resulted in the increase of both SIRT3-M1 and SIRT3-M2 mRNA expression in the cerebellum (SIRT3-M1: ctrl: 0.79 ± 0.18; EX: 1.28 ± 0.30; p = 0.002; SIRT3-M2: ctrl: 0.33 ± 0.09; EX: 0.50 ± 0.10; p = 0.007; Figure 1 F). We did not find differences in SIRT1 and SIRT3-M3 levels in any other brain area (Figure 1 D, E).

4. Discussion

Maintenance of energy homeostasis is crucial for survival. PGC-1α and Sirtuins ~~coordinate the activity of several transcription factors to~~ modulate ~~in the brain the~~ mitochondrial biogenesis and other cellular mechanisms in the brain in response to ~~environmental stimuli, such as~~ physical exercise and cold exposure ~~in different tissues, including the brain.~~. In this study we ~~comprehensively~~ investigated the isoform- and brain area-specific expression pattern of PGC-1α and Sirtuin following environmental stimuli.

The alteration of PGC-1α has already been examined in the brain, but previous findings suggested that very short-term cold exposure could not influence PGC-1α expression in the brain [25]. Accordingly, we also could not demonstrate any changes in any brain area after cold exposure in the FL-PGC-1α, NT-PGC-1α, CNS-PGC-1α or Ref-PGC-1α levels between control and short- or long-duration cold-exposed animals.

However, currently there is no data about the effect of cooling on the level of Sirtuin isoforms in the brain. We found that short exposure (200 min) elevated the level of SIRT3-M1 isoform in the cortex, and decreased the SIRT3-M3 level in the cerebellum. The long exposure (900

min) revealed a decline in cortical SIRT1, and striatal SIRT3-M1 levels. As an explanation, we suppose that this cold-challenge regime is not effective in decreasing the core body temperature sufficiently and the early compensatory mechanisms in BAT and skeletal muscle protect the brain against cold exposure.

Previous studies reported that physical activity reduce the risk of dementia and Alzheimer's disease [35]. The possible effects of inactivity are the impaired learning and memory functions, dementia and neurodegeneration [36]. It is well-known that exercise increases mitochondrial biogenesis via the up-regulation of PGC-1α and Sirtuin pathways in various tissues.

Previous studies demonstrated that metabolic stress occurring in the brain during exercise is similar to that known to stimulate mitochondrial biogenesis in the muscle. Therefore, the effects of exercise training on PGC-1α have been examined in the brain as well, but the results are inconsistent. Lezi E et al. could not detect any alteration of PGC-1α mRNA level in young and old mice following exercise training. Contrarily, another group reported a considerable elevation of PGC-1α mRNA in different brain areas, but the training protocols were different [23].

From the perspective of Sirtuins it seems that in the brain there could be an elevation in the expression level of SIRT1 and SIRT6, but the available data are controversial [15, 16, 23, 40]. In this study, we investigated FL-PGC-1α, NT-PGC-1α, CNS-PGC-1α, Ref-PGC-1α mRNA levels in two different training protocols. The 5-day-long training period did not cause alterations in PGC-1α transcripts in any brain regions. Contrarily, the 12-day-long training period induced changes in all isoforms of the PGC-system in cerebellum. In the Sirtuin-system the 5-day-long training also did not cause mRNA level alteration, but the long-term exercise resulted cerebellar elevation in SIRT3-M1 and SIRT3-M2 mRNA levels. These results suggest that the very short-term exercise was unable to induce the PGC-1α and SIRT

systems. Contrarily, the 12-day-long training period induced changes in the cerebellum, which seems to be consistent with our previously findings [37]. We hypothesize that the reason behind the prominent cerebellar elevation of PGC-, and Sirtuin isoforms could be the increase of synaptic plasticity between Purkinje cells, which facilitate better motoric coordination and more precise movement integration. Previous studies demonstrated, that in the cerebellum of *Pgc* knockout mice there is a decrease of cell number and firing rate between Purkinje cells [18, 38].

In conclusion we suggest that all PGC isoforms and SIRT3-M1,-M2 (i.e., the mitochondrial Sirtuins, except SIRT3-M3, which seems does not play important role in the cerebellum) take part in mitochondrial energy production, enhancing synaptic functioning. However, additional studies are needed to understand better the interaction between mitochondria and each PGC- and Sirtuin isoform in the cerebellum.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Figure legends

Figure 1. – Striatal~~,~~ (A, D), cortical (B, E) and cerebellar (C, F) relative mRNA expression level of PGC1-1α (A, B, C) and Sirtuin (D, E, F) isoforms of mice after 12 days rotarod training (5 RPM~~rpm~~). The FL-PGC-1α, NT-PGC-1α, CNS-PGC-1a (B4~~ and~~), Reference promoter (REF~~) levels were significantly increased in the cerebellum of exercised mice. Values are plotted as medians and interquartile range; *p < 0.05, **p < 0.01, ***p < 0.005; *12D* – 12 days rotarod training; *str* – striatum; *ctx* – cortex; *crb* - cerebellum.~~

~~**Figure 2.** – Striatal, cortical and cerebellar relative mRNA expression level of Sirtuin isoforms of mice after 12 days rotarod training (5 rpm). The~~), SIRT3-M1 and –M2 levels were significantly increased in the cerebellum of exercised mice. Values are plotted as medians and interquartile range; *p < 0.05, **p < 0.01, ***p < 0.005; *12D* – 12 days rotarod training; *str* – striatum; *ctx* – cortex; *crb* - cerebellum.

~~**Supplementary f**~~**Figure 42.** – Striatal, (A, D), cortical (B, E) and cerebellar (C, F) relative mRNA expression level of Sirtuin isoforms of mice after 200 (A, B, C) and 900 (D, E, F) minutes cold exposure (4°C). After 200 minutes the SIRT3-M1 isoform was significantly upregulated in mice cortex and the cerebellar SIRT3-M3 was also significantly decreased. After 900 minutes the SIRT1-FL isoform was significantly downregulated in mice cortex and the striatal SIRT3-M1 was also significantly decreased. Values are plotted as medians and

interquartile range; *p < 0.05, **p < 0.01, ***p < 0.005; *200* – 200 minutes cold exposure; *str* – striatum; *ctx* – cortex; *crb* - cerebellum.

Supplementary Ffigure 1. – Striatal, cortical and cerebellar relative mRNA expression level of PGC1-1α isoforms of mice after 200 minutes cold exposure (4°C). Expression level of the examined isoforms was not changed. Values are plotted as medians and interquartile range; *p < 0.05, **p < 0.01, ***p < 0.005; *200 min* – 200 minutes cold exposure; *str* – striatum; *ctx* – cortex; *crb* - cerebellum.

Supplementary Ffigure 2. – Striatal, cortical and cerebellar relative mRNA expression level of PGC1-1α isoforms of mice after 900 minutes cold exposure (4°C). Expression level of the examined isoforms was not changed. Values are plotted as medians and interquartile range; *p < 0.05, **p < 0.01, ***p < 0.005; *900 min* – 900 minutes cold exposure; *str* – striatum; *ctx* – cortex; *crb* - cerebellum.

Supplementary Ffigure 3. – Striatal, cortical and cerebellar relative mRNA expression level of PGC1-1α isoforms of mice after 5 days rotarod training (5 RPM ~~rpm~~). Expression level of the examined isoforms was not changed. Values are plotted as medians and interquartile range; *p < 0.05, **p < 0.01, ***p < 0.005; *5D* – 5 days rotarod training; *str* – striatum; *ctx* – cortex; *crb* - cerebellum.

~~**Supplementary figure 4.** – Striatal, cortical and cerebellar relative mRNA expression level of Sirtuin isoforms of mice after 200 minutes cold exposure (4°C). The SIRT3-M1 isoform was significantly upregulated in mice cortex and the cerebellar SIRT3-M3 was also~~

~~significantly decreased. Values are plotted as medians and interquartile range; *p < 0.05, **p < 0.01, ***p < 0.005; *200* – 200 minutes cold exposure; *str* – striatum; *ctx* – cortex; *crb* - cerebellum.~~

~~**Supplementary figure 5.** – Striatal, cortical and cerebellar relative mRNA expression level of Sirtuin isoforms of mice after 900 minutes cold exposure (4°C). The SIRT1-FL isoform was significantly downregulated in mice cortex and the striatal SIRT3-M1 was also significantly decreased. Values are plotted as medians and interquartile range; *p < 0.05, **p < 0.01, ***p < 0.005; *900* – 900 minutes cold exposure; *str* – striatum; *ctx* – cortex; *crb* - cerebellum.~~

Supplementary Ffigure 46. – Striatal, cortical and cerebellar relative mRNA expression level of Sirtuin isoforms of mice after 5 days rotarod training (5 RPM~~rpm~~). Expression level of the examined isoforms was not changed. Values are plotted as medians and interquartile range; *p < 0.05, **p < 0.01, ***p < 0.005; *5D* – 5 days rotarod training; *str* – striatum; *ctx* – cortex; *crb* - cerebellum.

Supplementary Ffile 1. – Thermal cycling conditions.