

Exercise training increases mitochondrial biogenesis in the brain

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Steiner JL, Murphy EA, McClellan JL, Carmichael MD, Davis JM. Exercise training increases mitochondrial biogenesis in the brain. *J Appl Physiol* 111: 1066–1071, 2011. First published August 4, 2011; doi:10.1152/jappphysiol.00343.2011.—Increased muscle mitochondria are largely responsible for the increased resistance to fatigue and health benefits ascribed to exercise training. However, very little attention has been given to the likely benefits of increased brain mitochondria in this regard. We examined the effects of exercise training on markers of both brain and muscle mitochondrial biogenesis in relation to endurance capacity assessed by a treadmill run to fatigue (RTF) in mice. Male ICR mice were assigned to exercise (EX) or sedentary (SED) conditions ($n = 16$ – 19 /group). EX mice performed 8 wk of treadmill running for 1 h/day, 6 days/wk at 25 m/min and a 5% incline. Twenty-four hours after the last training bout a subgroup of mice ($n = 9$ – 11 /group) were euthanized, and brain (brain stem, cerebellum, cortex, frontal lobe, hippocampus, hypothalamus, and midbrain) and muscle (soleus) tissues were isolated for analysis of mRNA expression of peroxisome proliferator-activated receptor- γ coactivator-1- α (PGC-1 α), Silent Information Regulator T1 (SIRT1), citrate synthase (CS), and mitochondrial DNA (mtDNA) using RT-PCR. A different subgroup of EX and SED mice ($n = 7$ – 8 /group) performed a treadmill RTF test. Exercise training increased PGC-1 α , SIRT1, and CS mRNA and mtDNA in most brain regions in addition to the soleus ($P < 0.05$). Mean treadmill RTF increased from 74.0 ± 9.6 min to 126.5 ± 16.1 min following training ($P < 0.05$). These findings suggest that exercise training increases brain mitochondrial biogenesis, which may have important implications, not only with regard to fatigue, but also with respect to various central nervous system diseases and age-related dementia that are often characterized by mitochondrial dysfunction.

PGC-1 α ; SIRT1; citrate synthase; endurance exercise; central fatigue

IT IS WELL ESTABLISHED that exercise training induces an increase in muscle mitochondria, resulting in fatigue resistance and enhanced endurance performance (13). Improved mitochondrial function is also a major factor in exercise-associated health benefits, including prevention of chronic diseases like cardiovascular disease and diabetes (10, 29). However, little research has focused on the mitochondrial response to exercise training in the brain. The available research is limited to two reports of exercise-induced changes in mitochondrial activity in the hippocampus of rats (9, 15), and several reports involving attenuated age-associated declines in mitochondrial activity (2, 21, 23, 24), including maintenance of respiratory chain complexes I and VI activity in isolated mitochondria (24), and Silent Information Regulator T1 (SIRT1) expression in the cerebellum (21). Given the important role of mitochondrial dysfunction in various psychological disorders and neurode-

generative diseases, the implications for an exercise-induced increase in brain mitochondrial biogenesis are vast. Further, an exercise-induced increase in brain mitochondrial biogenesis may contribute to decreased central fatigue and improved exercise performance, although this hypothesis has not yet been tested.

Mitochondrial biogenesis can be promoted via an increase in the transcriptional coactivators SIRT1 and peroxisome proliferator-activated receptor- γ coactivator 1- α (PGC-1 α) (12, 28). SIRT1 physically interacts with, deacetylates, and activates PGC-1 α , which is considered the “master regulator” of mitochondrial biogenesis as it coactivates transcription factors and nuclear receptors regulating mitochondrial protein expression (12, 28, 31, 32). Both acute and chronic exercise have been reported to activate PGC-1 α in skeletal muscle, which has been linked to an increase in mitochondrial proteins and improvements in exercise performance (3, 38, 39, 43). Calvo et al. (3) has shown that muscle-specific overexpression of PGC-1 α in mice increases voluntary exercise (wheel cage activity), maximal oxygen consumption, and endurance performance, as well as mitochondrial DNA (mtDNA) and mitochondrial enzyme activity. We recently reported that the dietary flavonoid quercetin increased brain, as well as muscle, PGC-1 α and SIRT1 mRNA, and mtDNA, and this was associated with an increase in voluntary wheel running and treadmill endurance capacity (8). However, to date no studies have examined the effects of exercise training on PGC-1 α , SIRT1, citrate synthase (CS), and mtDNA in the brain, and subsequent exercise performance.

The primary purpose of the present study was to determine the effects of eight weeks of endurance exercise training on markers of mitochondrial biogenesis (mRNA expression of PGC-1 α , SIRT1, CS, and mtDNA) in specific brain regions (brain stem, cerebellum, cortex, frontal lobe, hippocampus, hypothalamus, and midbrain) and endurance exercise capacity in mice. For comparison purposes, we also examined these same markers in the soleus muscle as it is well known to reflect mitochondrial changes following exercise training (1, 38). We hypothesized that exercise training would result in an increase in brain and muscle mitochondrial biogenesis and this would be associated with an increase in endurance capacity. A substantial metabolic stress occurs in the brain (motor and non-motor regions) during exercise which is similar to that known to stimulate mitochondrial biogenesis in muscle (5, 6, 25), yet no reports have focused on these changes in the brain. Here we provide evidence of the efficacy of exercise training to increase brain mitochondrial biogenesis (mtDNA, and PGC-1 α , SIRT1, and CS mRNA) in addition to the already well-characterized muscular changes.

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METHODS

Animals. The University of South Carolina's Institutional Animal Care and Use Committee approved all experiment procedures. All mice were cared for in the animal facility at the university. Eight-week-old male ICR mice were purchased from Harlan Sprague-Dawley Laboratories and were allowed to acclimate to our facility for at least 3 days before any experimental procedures. Mice were housed 4 per cage and were maintained on a 12:12-h light-dark cycle in a low-stress environment (22°C, 50% humidity, low noise). Food (Purina Chow) and water were provided ad libitum.

Exercise training. Mice were randomized into one of two groups as follows: sedentary (SED) or exercise (EX) ($n = 16\text{--}19/\text{group}$). Mice in each group were further assigned into one of two subgroups: the tissue collection experiment ($n = 9\text{--}11/\text{group}$) or the run to fatigue (RTF) endurance test ($n = 7\text{--}8/\text{group}$). All mice randomized to the exercise training treatment were acclimated to the treadmill for 20 min/day for the 3 days before the 8-wk training period. The exercise training consisted of 8 wk of treadmill running for 1 h/day, 6 days/wk at 25 m/min and a 5% incline. A brief 15-min warm-up period was incorporated into the 1 h of training each day. Throughout the training period mice were encouraged to continue running and maintain pace with the treadmill by gentle hand prodding of the tail or hindquarters. The mice readily responded to this stimulus and as such, no electric shock or other potentially harmful method of motivation was necessary. Mice in the SED groups remained in their cages in the treadmill room throughout the exercise bouts. These mice were exposed to similar noise in an attempt to control for extraneous stresses that may be associated with treadmill running and were handled daily similar to the EX mice.

Tissue collection. All mice randomized to the tissue collection group (EX and SED) were euthanized via isoflurane overdose 24 h after the last training bout to minimize any potentially confounding effects of the last bout of exercise on the outcome measures. Although we cannot completely rule out the potential of an acute exercise effect, others have shown the mitochondrial markers we are measuring to be either unchanged (mtDNA), or to have returned to baseline (mRNA of PGC-1 α , SIRT1, CS), 24 h after an acute exercise (27, 36). The soleus leg muscle and brain regions including the brain stem (BS), cerebellum (CB), cortex (CX), frontal lobe (FL), hippocampus (HC), hypothalamus (HY), and midbrain (MB), were immediately collected, frozen in liquid nitrogen, and stored at -80°C until further analysis. Brains were not perfused before dissection but we do not believe this significantly influenced our findings as minimal blood remained following drainage of the vena cava before dissection.

mRNA expression. Soleus muscle and brain tissue ($n = 9\text{--}11/\text{group}$) were homogenized under liquid nitrogen with a Polytron (PT3100; Kinematica, Lucerne, Switzerland), and the guanidine thiocyanate method with TRIzol reagent (Life Technologies, GIBCO-BRL, Carlsbad, CA) was used to extract total RNA. The extracted RNA (2.5 μl of sample) was dissolved in diethylpyrocarbonate-treated water and quantified spectrophotometrically at 260-nm wavelength. The 260:280 ratio was used to determine the quality of RNA; RNA with a ratio >1.6 was included in the analysis. RNA was reverse transcribed into cDNA in a 50- μl reaction volume containing 19.25 μl RNA (1.5 μg) in RNase-free water, 5 μl $10\times$ RT buffer, 11 μl 25 mM MgCl_2 , 10 μl deoxy-NTP mixture, 2.5 μl random hexamers, 1 μl RNase inhibitor, and 1.25 μl multiscribe reverse transcriptase (50 U/ μl). Reverse transcription was completed at 25°C for 10 min, 37°C for 120 min, and 85°C for 5 min, followed by a quick chilling on ice. Samples were stored at -20°C until subsequent amplification.

Quantitative RT-PCR analysis was carried out as per the manufacturer's instructions (Applied Biosystems, Foster City, CA) using TaqMan Gene Expression Assays. Amplification of DNA was performed in 12.5 μl Taqman Universal PCR Master Mix (AmpliTaq Gold DNA Polymerase, Passive Reference 1, buffer, dNTPs; AmpEase UNG), 1 μl cDNA, 9 μl RNase-free water, 1.25 μl 18S primer

(VIC), and 1.25 μl primer (FAM) (for endogenous reference and target gene) in a final volume of 25 $\mu\text{l}/\text{well}$. A MicroAmp 96-well reaction plate was loaded with samples and run using an ABI Sequence Detection System. Following 2 min at 50°C and 10 min at 95°C , plates were coamplified by 40 repeated cycles, with one cycle consisting of a 15-s denaturing step at 95°C and a 1-min annealing/extending step at 60°C . The data were analyzed on by ABI software using the cycle threshold (C_T), or the value calculated and based on the time (measured by PCR cycle number) at which the reported fluorescent emission increases beyond a threshold level (based on the background fluorescence of the system), reflects the cycle number at which the cDNA amplification was first detected.

Quantification of mRNA expression of PGC-1 α , SIRT1, and CS was calculated using the $\Delta\Delta C_T$ method, which employs a single calibrator sample to compare every unknown sample's gene expression against. Briefly, $\Delta C_T [C_T (\text{FAM}) - C_T (\text{VIC})]$ was calculated for each sample and the average ΔC_T of the control mice was then used as the calibrator sample. $\Delta\Delta C_T [\Delta C_T (\text{calibrator}) - \Delta C_T (\text{sample})]$ was then determined for each sample and the relative quantification was calculated as $2^{\Delta\Delta C_T}$. Initial exclusion criteria consisted of FAM $C_T \geq 40$ and VIC $C_T \geq 23$ and results showed all sample C_T values were VIC $C_T \leq 15$, and FAM $C_T \leq 33$. This analysis and quantification method has been shown to yield similar results as the standard curve method.

mtDNA content. A QIAamp DNA mini kit (Qiagen, Valencia, CA) was used for DNA isolation of the soleus muscle ($n = 8\text{--}11/\text{group}$) and various brain regions (BS, CB, CX, FL, HC, HY, and MB) ($n = 9\text{--}11/\text{group}$). Half sections of each brain tissue and the whole soleus muscle were lysed with proteinase K in the presence of a lysis buffer (Qiagen, Valencia, CA) at 56°C for 3 h. A second lysis (Qiagen, Valencia, CA) buffer was added to the sample before being incubated at 70°C for 10 min. Ethanol was added to the sample before passing it through a QIAamp spin column. The bound DNA was washed twice before being eluted using an elution buffer (10 mM Tris and 0.5mM EDTA, pH 9.0) (Qiagen, Valencia, CA). DNA in the brain and muscle was quantified spectrophotometrically at 260-nm wavelength. The DNA was subjected to quantitative real-time PCR (100 ng/reaction) carried out with Universal PCR Master Mix (Applied Biosystems). Comparison of kinetics of amplification of β -actin [Endogenous Control (VIC)/MGB Probe, catalog no. 4352341E] (Applied Biosystems) and cytochrome b (forward: TATTCCTTCATGTGCGGACGA; reverse: AAATGCTGTGGCTATGACTG; probe: ACCTGAAACAT-TGGAGTACTTCTACTG) were used to determine the relative amounts of nuclear and mtDNA.

Treadmill RTF test. Twenty-four hours following the last exercise bout of the 8-wk training period mice randomized to the endurance test group (RTF) completed a treadmill RTF test. The test consisted of a brief 10-min warm-up period (5 min at 15 m/min and 5 min at 20 m/min) followed by continuous treadmill running at a speed of 25 m/min until fatigue was reached. The point of fatigue was defined as the time (in min) at which the mouse could no longer keep pace with the treadmill despite 1 min of continuous hand prodding to the tail and hindquarters. Mice completing the RTF test were not included in the tissue analysis so as to reduce any acute bout effects and to maintain a sedentary control group that was not exposed to the exercise stimulus.

Statistical analysis. Data were analyzed using one-way ANOVA with Student-Neuman-Keul post hoc comparisons where appropriate. All data were analyzed using commercial software (SigmaStat, SPSS, Chicago, IL). Statistical significance was set with an alpha value of $P < 0.05$. Data are presented as means \pm SE.

RESULTS

PGC-1 α , SIRT1 and CS mRNA gene expression. Eight weeks of exercise training resulted in significant increases in PGC-1 α , SIRT1, and CS mRNA expression in the soleus

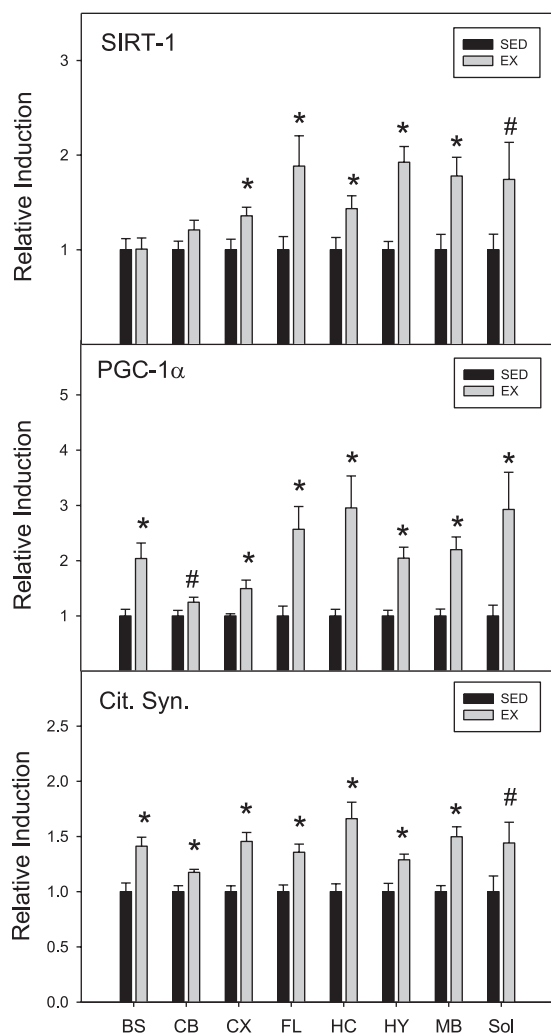


Fig. 1. Exercise training increases expression of genes associated with mitochondrial biogenesis. Sirtuin 1 (SIRT1) (*top*), peroxisome proliferator activated receptor- γ coactivator (PGC-1 α) (*middle*), and citrate synthase (Cit Syn) (*bottom*) expression were measured in major brain regions [brain stem (BS), cerebellum (CB), cortex (CX), frontal lobe (FL), hippocampus (HC), hypothalamus (HY), and midbrain (MB)] and soleus (Sol) muscle using RT-PCR following 8 wk of treadmill running ($n = 9-11$ /group). Black bars represent sedentary (SED) mice and gray bars represent exercised (EX) mice. Values are means \pm SE. Differences from SED mice: * $P < 0.05$, # $P < 0.10$.

muscle and in a majority of the brain regions ($P < 0.05$). Within the brain, SIRT1 mRNA expression was significantly increased (up to ~ 2 -fold), in the CX, FL, HY, HC, and MB, but not in the BS or CB, in the EX group (Fig. 1, *top*). Similarly, mRNA expression of PGC-1 α was significantly increased (up to ~ 3 -fold), in the BS, CX, FL, HC, HY, and MB, and there was a trend for an increase in the CB ($P < 0.10$) (Fig. 1, *middle*). All brain regions displayed significant elevations in CS mRNA expression (up to 1.7-fold) following exercise training ($P < 0.05$) (Fig. 1, *bottom*). Expression of PGC-1 α mRNA was significantly increased in the soleus muscle following exercise training ($P < 0.05$) (Fig. 1, *middle*), and there was a strong trend for increased CS and SIRT1 mRNA expression; CS mRNA expression was 1.4-fold greater ($P = 0.08$) (Fig. 1, *bottom*), and SIRT1 was 1.7-fold higher ($P = 0.087$) (Fig. 1, *top*) than levels observed in SED mice.

Due to the limited quantities of tissue available from each brain region, measurement of the corresponding protein concentrations were not feasible although others have shown that elevations in mRNA and protein commonly occur in concert, at least in skeletal muscle following exercise training in mice (4, 17, 20, 35). Further research will be required to confirm this relationship in the brain.

mtDNA content. An increase in mtDNA copy number relative to nuclear DNA is one of the best molecular markers of mitochondrial biogenesis (22). The relative amount of mtDNA in the soleus muscle and various brain regions was determined by RT-PCR and quantified by using the measurement of cytochrome *b* as the target gene for mtDNA and β -actin as the internal control for nuclear DNA. mtDNA was significantly greater following exercise training in the BS, CB, CX, FL, and HC ($P < 0.05$), and there were trends toward increases in the HY ($P = 0.074$) and MB ($P = 0.06$) (Fig. 2). Increases in mtDNA were greatest in the BS (5.5-fold) and CX (~ 4 -fold). mtDNA content of the soleus muscle was also increased 5.7-fold ($P = 0.05$) (Fig. 2).

Treadmill RTF test. The effects of exercise training on treadmill RTF were also examined in order to relate changes in brain and muscle mitochondrial markers to functional performance. As expected, exercise training resulted in a significant improvement in endurance capacity compared with SED ($P < 0.05$). Following the 8 wk of training EX mice ran 126.5 ± 16.1 min before reaching fatigue whereas untrained SED mice ran 74.1 ± 9.6 min.

DISCUSSION

While it is well established that exercise training increases skeletal muscle mitochondrial biogenesis and overall mitochondrial function, there is limited research on this response in the brain. To our knowledge this is the first study to examine changes in mitochondrial biogenesis within specific brain regions of mice following exercise training. These findings show that 8 wk of treadmill exercise training is a sufficient stimulus to increase markers of mitochondrial biogenesis within the brain, including PGC-1 α , SIRT1, CS mRNA, and mtDNA. Of particular importance to our central hypothesis is the increase in mtDNA, given our inability to measure PGC-1 α , SIRT1,

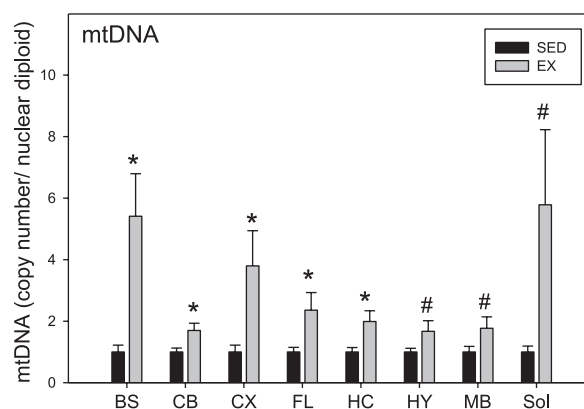


Fig. 2. Eight weeks of treadmill exercise increases mitochondrial DNA (mtDNA) content in brain and soleus (Sol) muscle. The relative amount of mtDNA was determined using RT-PCR ($n = 8-11$ /group). Black bars represent SED mice and gray bars represent EX mice. Values are means \pm SE. Differences from SED mice: * $P < 0.05$, # $P < 0.075$.

and CS protein concentrations due to the small amount of brain tissue available; it is unlikely mtDNA would have increased if these proteins had not been expressed in response to the increased mRNA (22). Consistent with previous reports, mRNA expression of these markers and mtDNA also increased in the soleus muscle following training (1, 4, 36). A functional consequence of these changes was confirmed using a treadmill RTF test, which supports our hypothesis that increases in both brain and muscle mitochondrial biogenesis are associated with improved endurance performance following exercise training.

It is now well established that PGC-1 α plays a necessary role in stimulating mitochondrial biogenesis in skeletal muscle following physiological challenges, such as exercise training (1, 3, 4, 35). PGC-1 α expression is enriched in tissues with high-capacity mitochondrial systems and is essential to endurance exercise capacity as it is a critical regulator of skeletal muscle fuel stores and a driver for the formation of slow-twitch muscle fibers (3, 14, 28, 42). Expression of PGC-1 α is also linked to intracellular calcium [Ca²⁺] levels and the demand for mitochondrial ATP production (14, 32), both of which are known to increase under physiologically demanding conditions like exercise (14). Oxidative phosphorylation is increased following PGC-1 α -mediated increases in mitochondrial biogenesis (28), resulting in improved peak oxygen uptake and delayed fatigue (3). SIRT1 functions together with PGC-1 α to promote mitochondrial biogenesis (31), as it interacts with and deacetylates PGC-1 α , thereby increasing its activity (12). Conversely, evidence for a role of PGC-1 α and SIRT1 on mitochondrial biogenesis in the brain is limited, with one paper showing that PGC-1 α overexpression in neuronal cells of the cortex, mid-brain, and cerebellum was associated with increased mitochondrial density (41). Similarly, SIRT1 overexpression led to a PGC-1 α -mediated increase in the mitochondrial density of cortical neurons (41).

Our results suggest that exercise training increases similar molecular pathways in the brain as in the muscle, making it likely these factors are also involved in mitochondrial biogenesis in the brain. To our knowledge this is the first report of a SIRT1/PGC-1 α -associated increase in mitochondrial biogenesis in specific brain regions of young healthy mice. It is also noteworthy that these changes in the brain are region specific and are generally similar in magnitude as those in skeletal muscle. The majority of the brain regions showed significant increases in SIRT1 and PGC-1 α mRNA expression in conjunction with increased mtDNA and therefore mitochondrial biogenesis following exercise training. While we cannot completely eliminate the possibility that these changes could be due to a lingering acute bout effect from the last training session we are confident that our results reflect molecular changes induced by the training period as others have shown that by 24-h postexercise mRNA levels have returned to baseline for these markers (27, 36). In the BS and CB the increase in mtDNA was not associated with a significant increase in SIRT1 mRNA in contrast to the other brain regions. This apparent discrepancy may be explained by a different time course of transcription and translation of these signaling molecules relative to mitochondrial biogenesis in these brain regions. It would certainly be expected that the temporal pattern of these changes would be different in the various brain regions whose initial mitochondrial content, as well as metabolic activity, and overall plasticity can differ considerably in response to exercise train-

ing. This probably could have been clarified if we had been able to measure SIRT1 and PGC-1 α protein concentration, which was not possible due to the small amount of tissue available from each brain region. Further research will also be necessary to determine the specific functional consequences of these brain-specific changes and the relative importance of each to changes in behavior.

It is certainly possible that increased brain mitochondrial biogenesis also contributes to increased endurance performance by attenuating the development of central fatigue. While most research associates only muscular changes in mitochondrial biogenesis to reduced fatigue and increased endurance performance, the brain is a highly metabolic organ containing abundant mitochondria to support the increased energy requirements of exercise and is sensitive to fatigue. We define central fatigue as the progressive reduction in voluntary drive to motor neurons during exercise, which can include both mental and physical factors (7). Decreases in cerebral metabolic ratio, impaired oxygenation, reduced central motor drive, altered neurotransmission, and impaired mood state have all been implicated in the development of centrally mediated fatigue (7, 8, 26, 33, 37, 40). The exact mechanisms for these changes are currently unknown, but it is reasonable to hypothesize that increased brain mitochondria may play an important role in reducing fatigue through their influence on cerebral energy status.

While we cannot distinguish between the relative importance of the mitochondrial changes in the brain and muscle, both likely contribute. In a study similar to ours, Navarro et al. showed that chronic moderate exercise in aging mice improved performance on the neuromuscular and neurologic based tight-rope and T-maze tests, and that this was accompanied by maintenance of the activities of key enzymes of mitochondrial function including Complex IV (cytochrome oxidase), Complex I, and nitric oxide synthase in the brain, which typically decline with aging (2, 23, 24). We have also shown a positive association among brain mitochondrial biogenesis, voluntary activity, and endurance capacity following supplementation of the plant flavonoid quercetin (8). Taken together, these findings warrant further investigation of the specific functional consequences of increases in brain mitochondrial biogenesis on fatigue and endurance performance.

An exercise-induced increase in brain mitochondrial biogenesis may also have important implications in various neurological diseases in which mitochondrial dysfunction is a hallmark including psychiatric disorders, genetic disorders, and neurodegenerative diseases (11, 19, 30). PGC-1 α knockout mice exhibit increased sensitivity to neurodegeneration (34), implicating an important role of PGC-1 α in the etiology of age-associated neurodegenerative disorders like Alzheimer's disease. Studies of PGC-1 α knockout mice have also shown CNS related histological, physiological, and behavioral alterations (16, 18, 34). Moreover, several reports in aging mice suggest that exercise training can attenuate the age-related decline in mitochondrial function (2, 21, 23, 24). These findings provide a likely biological basis for the use of exercise strategies in the prevention and/or treatment of neurological diseases and/or aging which can be characterized by mitochondrial dysfunction.

In summary, this is the first report to show that 8 wk of treadmill exercise training is a sufficient stimulus to increase

mitochondrial biogenesis, as indicated by increases in mRNA expression of SIRT1, PGC-1 α , and citrate synthase, and most importantly mtDNA within the brain of young healthy mice. While the functional and translational significance of these findings remains to be determined, it is possible that these changes could contribute to reductions in centrally mediated fatigue and thus contribute to improved exercise performance. There is also the possibility that exercise can improve neurological health and physical functioning through SIRT1-PGC-1 α coactivator complex-induced changes in mitochondrial properties. These findings could lead to the enhancement of athletic performance through reduced fatigue, as well as to the expanded use of exercise as a therapeutic option to attenuate the negative effects of aging, and the treatment and/or prevention of neurological diseases.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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