

Endurance Exercise Training Attenuates the up Regulation of iNOS in the Skeletal Muscles of Chronic/Progressive Mouse Model of Parkinson's Disease

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Abstract

Background: Parkinson's disease (PD) is one of the most common neurodegenerative diseases in the elderly. PD complications include muscle weakness and fatigue, these complications lead in part to decrease the endurance of PD patients. Strength of skeletal muscles has a major role in ADL performance, which is one of the challenges that PD patients have. The main goals of this study are to study the expression of inducible nitric oxide synthase (iNOS) in the skeletal muscles of PD and to examine the effect of treadmill exercise training on iNOS expression in these skeletal muscles.

Methods: Twenty normal albino mice and 20 albino mice with 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) -induced PD were divided into four groups: sedentary control (SC), exercised control (EC), sedentary PD (SPD), and EPD (EPD). Parkinsonism was induced by injections of 10 doses of MPTP (25 mg/kg) and probenecid (250 mg/kg) over 5 weeks. After the completion of treadmill exercise training, samples from the gastroc and soleus muscles were evaluated by immunohistochemistry to examine the expression of iNOS in the four groups of animals.

Results: Expression of iNOS in gastrocnemius muscle showed significant increase in expression of iNOS is SPD group compared to SC, # P value < 0.05. In soleus muscle, there was an increase in expression of iNOS is SPD group compared to SC, but the change was not significant, P value < 0.08. Also, exercise did not significantly decrease the expression of iNOS in Parkinsonian group P value < 0.13.

Conclusion: Our present data suggest that endurance exercise

training reduces PD-induced alterations in iNOS expression in skeletal muscles. These results might be important in considering rehabilitation protocols for PD and its related pathophysiology.

Keywords: Parkinson's disease; iNOS; Skeletal muscles

Introduction

Parkinson disease is a common neurodegenerative disorder caused by significant depletion in dopamine, which leads to abnormal voluntary movements produced by skeletal muscles [1]. Nitric Oxide Synthase (NOS) serves as a key signaling molecule in physiological process that includes immune system defense, neuronal communications, and regulation of vascular injuries [2]. iNOS protein was found post-mortem in the brains of patients with Alzheimer's and PD [2]. In PD, ambulation is one of the major concerns, but difficulties and abnormalities in gait are mostly attributed to neuronal issues not to the contractile capacity of the muscles [3]. Recently, literature also has focused in non motor issues in PD including, muscles weakness, decreased strength, and decreased endurance. These studies suggested that exercise might be the mediator that improves the neuronal communication in PD [4].

The 1-methyl-4-1, 2, 3, 6-tetrahydropyridine (MPTP) induces Parkinsonism due to its production of reactive oxygen species, peroxynitrite, which eventually leads to the nitration of tyrosine residues and the subsequent depletion of dopamine [1-3]. Previous studies [5] strongly suggested a harmful upregulation of inducible nitric oxide synthase (iNOS) in the brain of animals and humans following MPTP administration. Moreover, iNOS has been reported to be involved in the muscle loss occurring in muscle wasting syndromes like sarcopenia and cachexia [6].

We hypothesize that iNOS plays a pathological role in the skeletal muscle abnormalities observed in PD. Therefore, using immunohistochemistry and light microscopy, our study has investigated the expression of iNOS in fast-twitch (gastrocnemius) and slow-twitch skeletal muscles (soleus) of mice with MPTP/probenecid-induced chronic PD. In ad-

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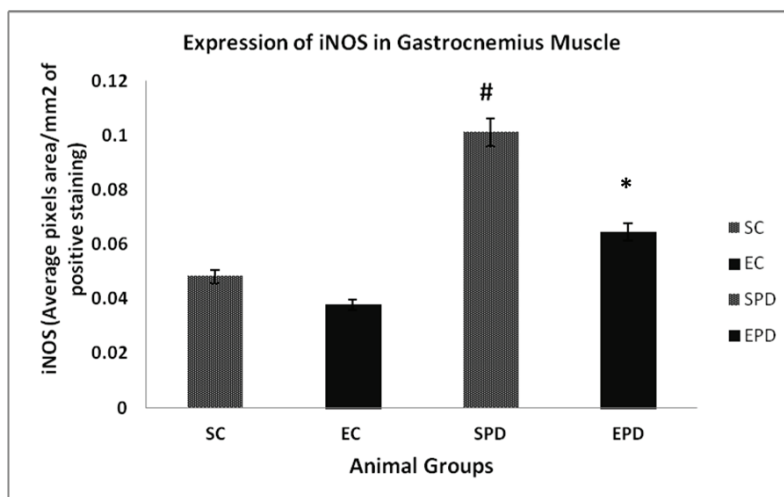


Figure 1. Expression of iNOS in gastrocnemius muscle showed significant increase in expression of iNOS in SPD group compared to SC, # P value < 0.05. Exercise did not significantly decrease the expression of iNOS in control group P value < 0.18. However, exercise significantly decreased iNOS expression in PD-induced group, * P value < 0.01. iNOS: Inducible nitric oxide synthase. SC: Sedentary control, EC: Exercised control, SPD: Sedentary Parkinson's disease, EPD: Exercised Parkinson's disease.

dition to that, our study has revealed the impact of exercise on PD-induced alteration in iNOS expression in soleus and gastrocnemius muscles.

Materials and Methods

Animals

Twenty normal Albino mice and 20 Albino mice with MPTP-induced Parkinson disease were used. The animals were housed in individual cages under identical conditions (22 ± 1 °C, free access to standard chow and water, 12 hours dark/light cycle). Animal-related protocols were conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee at Jordan University of Science and Technology.

The 40 mice were divided in 4 equal groups, which were: sedentary control (SC, n = 10), exercised control (EC, n = 10), sedentary PD (SPD, n = 10), and exercised PD (EPD, n = 10). Parkinsonism was induced by mice with 10 doses of MPTP (25 mg/kg) and probenecid (250 mg/kg) purchased from Sigma Chemical Co. (St. Louis, MO, USA) over 5 weeks, three days and half apart. Control mice received (25 mg/kg) saline injections.

Exercise protocol

Animals were introduced to modified human treadmill in which each animal run in separate chamber divided by glass so running animals can see each other. Ten chambers were

placed on the belt of human treadmill, so 10 animals can exercise in each session of training. Animals were exercised for 40 minutes per day, 5 days per week for 4 weeks at a speed of 18 m/min. Although sedentary mice did not exercise, they were exposed to the same environment as the exercised mice by transferring them to the training room daily.

iNOS immunostaining of skeletal muscles

The mice were sacrificed, and their skeletal muscles (gastrocnemius/soleus) were dissected and fixed in 4% paraformaldehyde, embedded in paraffin, and sliced in 5 micrometer thick sections. Then, the 5 μ m thick sections were processed via immunohistochemistry using an antibody to iNOS (Biocare medical, San Antonio, TX). So, the five micron thick paraffin-embedded sections mounted on glass slides were deparaffinized in xylene for 2 minutes twice, and subsequently rehydrated through serially descending dilutions of alcohol (starting with 100%, and ended with 70%) followed by water (2 minutes for each step). After that, sections were processed for antigen retrieval in the reveal solution (RV 1000M, Biocare Medical, Concord, CA) under pressure in the Decloaking chamber (Biocare medical) for 2 minutes. Tissue sections were then cooled down to room temperature, and incubated with 3% hydrogen peroxidase in methanol for 5 minutes. After washing the sections in phosphate buffered saline (PBS), they were incubated with iNOS antibody (Biocare medical, San Antonio, TX), with the dilution recommended by the vendor, at room temperature for one hour. Next, the sections were washed in PBS and incubated with biotinylated secondary antibody (LSAB kit, Dako Carpinte-

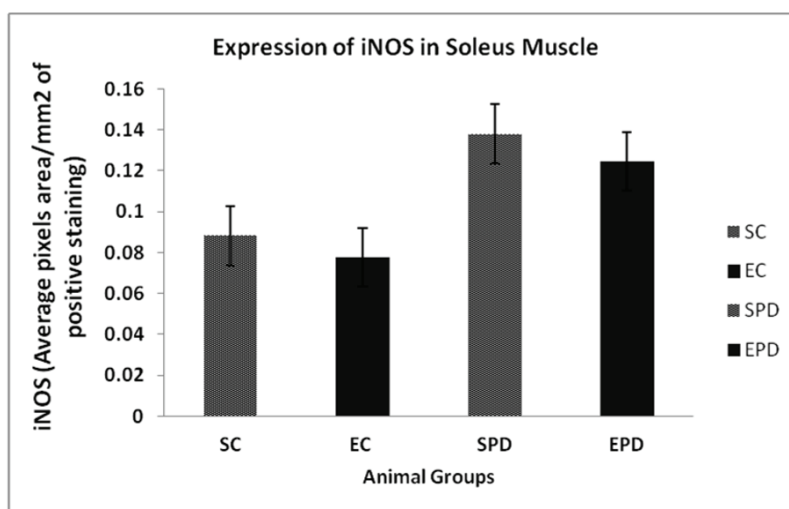


Figure 2. Expression of iNOS in Soleus muscle showed an increase in expression of iNOS in SPD group compared to SC, but the change was not significant # P value < 0.08. Also, exercise did not significantly decrease the expression of iNOS in Parkinsonian group P value < 0.13. iNOS: Inducible nitric oxide synthase. SC: Sedentary control, EC: Exercised control, SPD: Sedentary Parkinson's disease, EPD: Exercised Parkinson's disease.

ria, CA) for 15 minutes at room temperature, then washed with PBS. Then, sections were incubated with streptavidin horse radish peroxidase (LSAB kit, Dako) for 15 minutes at room temperature and washed with PBS, 3'-Diaminobenzidine (DAB) applied for 2 minutes or longer, until the desired intensity was developed, and then the slides were washed with tap water to stop the reaction. Negative control sections were processed without the primary antibody. All sections were then counterstained with hematoxylin and viewed under the light microscope. Ten slides of gastrocnemius muscle and ten other slides of soleus muscle from each animal group were evaluated for iNOS expression by immunohistochemistry.

Data analysis

The sections were photographed with digital camera. Photoshop software was used. The slides from each group were analyzed by counting the total pixels area occupied by positive staining. iNOS expression was analyzed, in the different skeletal muscles, and statistically compared among the 4 different groups using paired and unpaired student t-test. Differences in iNOS expression were considered statistically significant at P value < 0.05.

Results

To investigate the effect of PD as pathology on skeletal muscles, we compared the expression of iNOS expression in both SC and SPD groups. Expression of iNOS in gastro-

nemius muscle showed significant increase in expression of iNOS in SPD group compared to SC, P value < 0.05. Exercise did not significantly decrease the expression of iNOS in control group P value < 0.18. However, exercise significantly decreased iNOS expression in PD-induced group, P value < 0.01 (Fig. 1). Concerning soleus muscle, results showed an increase in expression of iNOS in SPD group compared to SC, but the change was not significant, P value < 0.08. Also, exercise did not significantly decrease the expression of iNOS in Parkinsonian group, P value < 0.13 (Fig. 2).

Discussion

Our study reveals two main findings. Firstly, PD increased iNOS expression in the soleus and gastrocnemius muscles. Secondly, exercise training significantly decreased iNOS expression in the gastrocnemius muscle in the Parkinsonian mice. Thus, we postulate that there is a muscle fiber type-specific iNOS response to PD with endurance exercise training.

iNOS has been reported to be expressed at low levels in normal rodents skeletal muscles [7-12]. NOS mediates the production of (NO), which is a vasodilator important in controlling blood pressure in skeletal muscles [13, 14]. A previous report [15] has suggested no significant increase in iNOS expression in the soleus muscle following endurance exercise training. No iNOS induction in gastrocnemius muscle has been reported [16] in response to endurance exercise training. Consistent with those previous reports [15, 16], our results demonstrate that endurance exercise training does not

induce any significant increase in the iNOS expression in both soleus and gastrocnemius muscles in the control mice.

To our knowledge, this is one of the first studies that examine PD-induced alterations in iNOS expression in the different types of skeletal muscles. Previous studies [17-20] have demonstrated iNOS overexpression in skeletal muscles of chronic heart failure patients and animals. Such iNOS overexpression has been shown to be induced by inflammatory cytokines and the priming effect of gamma interferon (γ -IFN) in chronic heart failure [21], iNOS overexpression has also been demonstrated in skeletal muscles of autoimmune inflammatory myopathies [22]. NOS activity inhibits mitochondrial respiration in skeletal muscles [23, 24]. iNOS overexpression results in the production of excessive NO [25]. Excessive NO causes oxidative stress [26, 27], which may be contributing to the skeletal muscle abnormalities seen in PD animals and humans.

Aging-induced iNOS upregulation has been shown [28] in the white, fast-twitch gastrocnemius skeletal muscle. These findings were consistent with our study, which revealed that PD-induced iNOS upregulation was detected more in gastrocnemius muscle (Fig. 1). Skeletal muscle abnormalities in PD have been reported [29] to include myopathies with mitochondrial abnormalities. Mitochondrial reactive oxygen species have been demonstrated [30] to promote the production of proinflammatory cytokines, which in turn dramatically induce iNOS expression. Thus, we can conclude that iNOS upregulation detected in the parkinsonian gastrocnemius muscle maybe attributed to the difference in mitochondrial abnormalities, or the inflammatory respond in these different muscle fibers.

It has been demonstrated [21, 31-37] that iNOS overexpression is induced by various signaling molecules such as proinflammatory cytokines. Previous studies [38-41] suggested that iNOS participated in many pathological conditions. For example, iNOS has been shown to be deleterious to ischemia/reperfusion injury in skeletal muscle [42]. Selective inhibition of iNOS has been shown [42] to protect tissues against iNOS ischemia/reperfusion injury in skeletal muscle. The observed overexpression of iNOS PD skeletal muscles is consistent with the previous report [29] that skeletal muscle abnormalities in PD include inflammatory myopathies and myopathies associated with mitochondrial abnormalities. Upregulation of iNOS in PD skeletal muscles is also in agreement with the previous studies [43-45] reporting iNOS overexpression in other parkinsonian tissues including the brain, where iNOS upregulation has been suggested [46] as the cause of dopaminergic neuronal death leading to PD. Indeed, inhibition of nitric oxide synthase has been shown [45, 47, 48] to protect against MPTP-induced neurotoxicity in animals. Therefore, reducing iNOS expression either pharmacologically [49, 50], genetically [48, 51], or by exercise as our finding indicated can protect various tissues in the different pathological conditions including PD.

Clinical inflammatory myopathies and myopathies associated with mitochondrial abnormalities increase in PD [29]. Thus, we assume that mitochondrial abnormality-associated inflammatory cytokines are the reason behind statistically significant and statistically insignificant iNOS upregulation in parkinsonian soleus and gastrocnemius muscles.

In Rodents, fast-twitch red muscle has a higher content of mitochondria than slow-twitch red muscle. Previous reports [52-54] have suggested that exercise training promotes mitochondria biogenesis by increasing mitochondrial number, content, volume and function. Hence, we can postulate that exercise training may ameliorate the oxidative environment caused by the mitochondrial abnormalities in the PD skeletal muscles. Therefore, it can be concluded that the detected reduction in iNOS overexpression in the parkinsonian slow-twitch (soleus) skeletal muscle following endurance exercise training is due to the expected downregulation in the signaling inflammatory cytokines resulting from mitochondrial biogenesis. The other factor that might be responsible for this difference could relate to the differences in the type of motor neuron innervating fast-twitch and slow-twitch muscle fibers that accompanied with differences in firing pattern, contractile response, and specific mechanical stress.

In summary, our study is one of the first studies to report fiber type-specific alterations in iNOS expression in PD skeletal muscles. In addition, this is the first study to examine the impact of endurance exercise training on iNOS expression in PD slow- and fast-twitch skeletal muscles.

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Declaration

We confirm that there is no known conflict of interest associated with this manuscript and there has been no financial support for this work that influenced the outcome.

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