High intensity exercise preconditioning influences on steroid hormones following the experimental autoimmune encephalomyelitis model

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Research Article

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Abstract

Steroid hormones improve clinical and pathological symptoms using the experimental autoimmune encephalomyelitis (EAE) model of multiple sclerosis (MS). In addition, exercise seems to play an important role in increasing hormones such as 17beta-estradiol and estrogen receptor beta (ERβ). In the present study, we evaluated whether 6 weeks of high-intensity interval training (HIIT) prior to induction of EAE increased 17beta-estradiol and ERβ and attenuate the severity of symptoms and/or disease progression in the EAE model. Female C57BL/6 mice were randomly divided into exercise (EX) and control (Con) groups. After 4 weeks of training, EAE was induced in half of the Con and the EX groups. The EAE-EX group after EAE induction trained for two more weeks. The EX group trained for 6 weeks. Six weeks of HIIT increased 17beta-estradiol and ERβ in the EX group compared to the control group (P ≤ 0.05). The EAE-EX group had a significant increase in 17beta-estradiol and ERβ and a significant decrease in clinical symptoms compared to the EAE group (P ≤ 0.05). In addition, the EAE group had a significant decrease in ERβ (P ≤ 0.05) compared to the control group. Our data demonstrate that 6 week of HIIT increased 17beta-estradiol and ERβ in the cerebellum tissue. These hormones are associated with decrease clinical outcomes and further research is required to examine potential clinical relevance.

1. Introduction

Multiple sclerosis (MS) is an autoimmune, demyelinating, and a neurological disorder in the central nervous system (CNS) [1]. It has been established that the prevalence rate of the disease has been increasing in women [2]. However, women with MS often experience clinical improvement during pregnancy and the postpartum period [3]. Epidemiological studies demonstrated steroid hormones have known as an important protective factor in neurodegenerative damage. A decreased steroid hormone levels accompanied by cellular changes, such as apoptosis and a reduction in the number of dendrites in certain parts of the CNS [4]. Therefore, it seems steroid hormones are involved in regulating the nervous system response in women with MS. However, the relevant mechanisms are not completely understood.

The steroid hormone 17β-estradiol (Estradiol) is the most strong physiological estrogen. Along with estradiol role in regulating endocrine functions and sexual behaviors, growing evidence indicates estradiol plays a key role in CNS development, myelin sheaths protection, and, also as a regulator of the function of the blood-brain barrier [5, 6]. Estradiol impacts many brain regions that are involved with related motor and cognitive functions, such as the cerebellum [7]. Remarkable evidence has shown estrogen activates multiple neuroprotective signaling in the nervous system that is initiated through estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ). ERβ, also known as NR3A21, is an important modulator of some non-reproductive neurobiological systems. Most of the neuroanatomical targets of estrogen are mediated by ERβ [8]. Estrogen binding at ERβ modifies the activation of different signals such as ERK2, and PKB3, as well as the expression of several neurotrophic and neuroprotective proteins, such as IGF-1 and, BDNF4. All of these signals play a key role in the neuroprotective actions in the CNS [9]. Moreover, estradiol also inhibits the proinflammatory cytokine, TNF-α [10], which is secreted by Th17 cells and increases damage to the myelin sheath in MS patients [11]. Therefore, it seems these
physiological roles for estradiol and ERβ making them key candidates for the treatment and a therapeutic target for in MS. However, the mechanisms through which Estradiol and ERβ support the therapeutic potential in MS remain unclear.

Previous experiments on animals have indicated steroid hormones, especially estradiol therapy has demonstrated promising results in attenuating clinical outcomes in experimental autoimmune encephalomyelitis (EAE), and possibly multiple sclerosis (MS) [12–14]. In addition, exercise is important for people with MS at any stage of the disease. In more recent years, the effect of physical activity and/or exercise on persons with MS have received considerably more attention [15–17]. For instance, it suggests that exercise might have the potential to have an impact on persons with MS and slow down the disease process in the EAE model exercise, and increase steroid hormones in humans[18, 19].

A common method to specify the influence of exercise in EAE animal models is to use a pre-conditioning/post-conditioning model [20]. In these models, exercise is used prior to and throughout (pre-conditioning) and following (post-conditioning) the induction of a disease using a non-human animal model. Studies have demonstrated that pre-conditioning is an effective method in attenuating clinical outcomes from onset to peak and decreased myelination [21, 22]. Moreover, a growing body of evidence demonstrates HIIT is a promising strategy to improve and physical fitness in MS patients [23, 24]. However, in persons with MS further research is needed to determine the impact of exercise on biomarkers in MS. Based on prior studies, it seems there is a relationship between increased estradiol and ERβ via exercise, improvement in clinical symptoms, and myelination [19, 25]. In this study, we investigated whether 6 weeks of HIIT increased estradiol and ERβ concentrations and attenuated the severity of disease symptoms in the EAE mouse model.

2. Experimental Procedures

2.1. Animals and experimental group

Forty 4–5 weeks Female C57BL/6 mice were purchased from Pasteur Institute (Tehran, Iran). The animals were housed cages at 22 ± 1°C under a 12-h light/dark cycle (lights on at 07:00 hours), and controlled temperature and humidity. All animals received food and water provided ad libitum. Ethical approval for animal experimentation was received from the Institute of Iran Ministry of Science Research and Technology (IR.SSRI.REC.1397348). Forty animals were randomly divided into four groups (n = 10 per group): Control, EAE, EX, and EAE-EX. The EX group trained five times a week for 6 weeks. The EAE-EX mice exercised five times a week for 4 weeks, then experimental autoimmune encephalomyelitis (EAE)
was induced. After induction of EAE, the EAE-EX group trained for the entire duration of the training program (Fig. 1).

2.2. Training protocol designed

Running on the treadmill was performed by the EX groups (Table 1). Briefly, mice were familiarized with running on a treadmill twice a day with a speed of 8 m/min for 10 minutes and 0% grade for 1 week. At the end of the familiarization period, the maximal running speed (Vmax) in an incremental exercise test was calculated to determine the intensity of training protocols as previously described by Ferreira et al. training [26]. In the EX groups, animals were trained 5 days per week for the final 6 weeks. Before and after exercising, a 5 minutes warm-up and cool down was performed with intensity of 30–40% of the maximum running speed [27]. Ex groups performed an incremental exercise test every week and the intensity of training was determined by the new speed of an incremental exercise test each week. Progressive overload was performed by increasing the running time and number intervals in both HIIT groups.

2.3. EAE induction

To induce EAE, Hooke Kit™ (Hooke laboratories, Cat No. EK-2110, Lawrence, MA, USA) was used. In brief, animals were immunized with myelin oligodendrocyte glycoprotein (MOG35–55) antigen in an emulsion with complete Freund’s adjuvant (CFA). The MOG35–55/ CFA emulsion was administered into the flanks of each mouse (0.1 ml/flank or 0.2 ml/animal) subcutaneously. Each mouse was also injected intraperitoneally (i.p.) with pertussis toxin (PTX; 500 ng PTX diluted in 0.1 ml Phosphate Buffered Saline (PBS) on days 0 and 1 after (24 h) immunization. Mice in the control group were injected with PBS.

2.4. Clinical EAE Score

To approve the onset and stage of development of the disease, the clinical score was recorded daily until 21 days following immunization by blinded scorers (Fig. 2). A standard scoring system was used to detect the clinical score. The scores were defined as follows: 0 = no clinical signs, 1 = tail paralysis (or loss of tail tone), 2 = tail paralysis and hind-limb weakness, 3 = hind limb paralysis, 4 = complete hind-limb paralysis and front limb weakness [20].

2.5. Histopathological analysis

After anesthetizing with ketamine (100 mg/kg) and xylazine (20 mg/kg), perfusion and fixlation by aldehyde solutions was done via the left ventricle and the cerebellum was removed. Fixed tissue was paraffin-embedded and 5µm sections obtained in the midpoint of the cerebellum and photomicrographs were taken from right paravermis near the granule cell layer by using a rotary microtome (Leica- rm2235, UK) from the cerebellum. 5 µm cross-sections obtained from paraffin blocks were stained with Luxol Fast Blue (LFB) to assess the degree of myelination [28]. The total surface of demyelinated regions was calculated by Infinity software (v. 4.6, Lumenera Corporation, Canada).
**2.6. ELIZA**

The ELISA procedure was performed according to the commercial kit. The calculated 17beta-estradiol (RE52041) and estrogen receptor beta (MBS928082) concentrations (ng/mg) in the cerebellum homogenate was divided by the total protein concentration (mg/ml) of the corresponding homogenate. The calculated data were presented as nanograms per milligram protein.

**2.7. Statistical analysis**

Data were reported using GraphPad Prism (v5.0). To compare the differences in 17beta-estradiol, estrogen receptor beta, and demyelination in 21 dpi between groups (control, EAE, EX, and EAE-EX) we used the one-way ANOVA with the Tukey post-test. Clinical score data from the control, EAE, EAE-EX groups were analyzed using the non-parametric Mann-Whitney U test. The data were presented as mean ± SD, and the significance level was established at P ≤ 0.05.

**3. Results**

**3.1. High intensity interval training for 6 weeks reduced disease severity and delayed the onset of EAE symptoms**

Animals were scored for clinical symptoms daily until 21 days post-immunization (21dpi). We observed that animals in the EAE group exhibited early symptoms on the 10 dpi (score of 1) and had a gradual increase in disease severity until 16 dpi. Notably, following 6 weeks of HIIT, animals in the EAE-EX group demonstrated early symptoms on 12 dpi and the peak score was attained on day 18 after induction (score of 1). (Fig. 2-A). Mean clinical signs in the EAE-EX group was lower compared to the EAE group (Fig. 2-A; P ≤ 0.05). Weight was significantly lower in the EAE group compared to both Control and EAE-EX1 groups (Fig. 2-B, P ≤ 0.0001 for both comparisons).

**3.2. High intensity interval training for 6 weeks increased 17beta-estradiol and estrogen receptor beta in the cerebellum**

17beta-estradiol and estrogen receptor beta were estimated in the cerebellum using ELISA kit. 6 weeks of HIIT increased 17beta-estradiol and estrogen receptor beta in the EX group compared to the control group (P ≤ 0.05) (Fig. 3-A and B, respectively). The EAE group compared with the control group demonstrated a significant decrease in the estrogen receptor beta concentartion in the cerebellum at 21 dpi (P ≤ 0.05). Compared with EAE, the EAE-EX group demonstrated a significant increase in the 17beta-estradiol and estrogen receptor beta (P ≤ 0.05).

**3.3. High intensity interval training for 6 weeks reduced cerebellum demyelination**

EAE effected significant demyelination in a certain area of the brain such as the cerebellum. (Fig. 4) Demyelination was significantly higher in the EAE group compared to the control group (P ≤ 0.0001) and was significantly lower in the EAE-EX group compared to the EAE group (P ≤ 0.01) (Fig. 4-B).
4. Discussion

In this study, we investigated the possibility that HIIT increases 17beta estradiol (Estradiol) and estrogen receptor beta (ERβ) levels, may attenuate the clinical score and percent weight loss in the EAE exercise group, and delayed the severity of EAE symptoms from onset to disease peak compared to the EAE group. Previous studies investigated the beneficial effect of estrogen and estradiol treatment in the protection of experimental autoimmune encephalomyelitis (EAE) and possibly MS patients. Their results indicated EAE disease severity decreased with estradiol treatment as compared to control [22–24]. These findings also were confirmed in our study. The present study showed the severity of symptoms was reduced in the EAE-EX group compared to the EAE group. Potential mechanisms for the exercise-induced reduction and improvement in clinical symptoms in our study may be attributed to an increase in estradiol and estrogen receptor beta levels. Previous studies have shown that estradiol activates multiple molecular and cellular mechanisms such as brain-derived neurotrophic factor (BDNF) which are thought to play a role in neuronal repair and plasticity in MS patients [29]. In addition, the anti-inflammatory effect of estradiol has been shown to reduce pro-inflammatory cytokines in astrocytes and microglia [10]. High expression levels of pro-inflammatory cytokines such as TNF-α increases neurodegenerative decline and has been associated with EAE disability [7]. It seems likely to an increase in estradiol concentration inhibited pro-inflammatory cytokines decreased the development of the clinical score and weight assessment in the EAE-EX group.

Demyelination is a key marker of MS pathology. A growing body of evidence supports the efficacy of estradiol treatment in MS [26]. The protective actions of estradiol on myelin are in part mediated through ERβ, which induce remyelination by the activation of the PI3K/Akt/mTOR signaling pathway [30] and modify the immune system in EAE [31], and in the cuprizone models [32]. To understand the role estradiol and ERβ play in the myelination process, demyelination was assessed in our study. Our data indicated demyelination was lower in the EAE-EX group compared with the EAE group. Therefore, we propose that an increase in the estradiol and ERβ concentrations in the cerebellum tissue after HIIT may be responsible for the decreased demyelination. Lower demyelination in the EAE-EX group would imply, likely that higher level of estradiol and ERβ, decrease demyelination.

5. Conclusion

The present study demonstrated that 6 weeks of HIIT in mice resulted in increases in 17beta estradiol and estrogen receptor beta levels in the cerebellum tissue and decreased demyelination using the EAE model. Therefore, our study supports the important assertion that HIIT increases 17beta estradiol and estrogen receptor beta levels and improving clinical outcomes using a mouse model. Moreover, it seems the effect of exercise on estradiol and ERβ can be considered in people with multiple sclerosis and the EAE model. However, further studies are required to determine how estradiol and ERβ cascades modulate EAE symptoms.

Declarations
The authors have no competing interests to declare.

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References


### Tables

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### Figures
Figure 1

Schematic representation of the experimental design.

Figure 2

High intensity interval training for 6 weeks reduces disease severity and delayed the onset of EAE symptoms. Clinical assessment of exercise and EAE groups. Clinical signs were assessed daily for 21 after EAE induction. Results are expressed as the day by day mean clinical score. The mean clinical score in the EAE-EX group was lower compared to the EAE group (* P ≤ 0.05) (A). Body mass was measured using the initial weight on the injection day (0dpi) and the final weight on the day indicated (21 dpi).
Weight was significantly lower in the EAE group compared to both Control and EAE-EX groups (**** p ≤ 0.001) (B). Values are given as mean ± SD, (n=10 per group).

**Figure 3**

High intensity interval training for 6 weeks increased estradiol and estrogen receptor-β in the cerebellum. 17beta estradiol and estrogen receptor beta concentrations in the cerebellum at 21 dpi were decreased in the EAE group compared to the control group. Also, 17beta estradiol and estrogen receptor beta concentrations were higher in the EX group compared with the control group and in the EAE-EX group compared to the EAE group (A and B). Representative western blots (c). Values are given as mean ± SD. (*P≤0.5), (n=4 per group).
Figure 4

High intensity interval training for 6 weeks reduced cerebellum demyelination. Luxol Fast Blue staining that was used for rate of demyelination following EAE induction in the cerebellum at magnification 100x (A= Control group, B=EAE group, and C=EAE-EX1 group), in all groups at 21pdi (D). Lower demyelination was observed in the EAE-EX group compared to the EAE group. Demyelination was significantly higher in the EAE group compared to the control group. Values are given as mean ± SD. (**P≤0.01 and **** P ≤ 0.001), (n=10 per group).