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The effect of 6 weeks of high intensity interval training on myelin biomarkers and demyelination in experimental autoimmune encephalomyelitis model

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ABSTRACT

Exercise has been shown to increase myelin biomarkers such as klotho and PLP and improve clinical and pathological symptoms using the experimental autoimmune encephalomyelitis (EAE) model of multiple sclerosis (MS). In the present study, we evaluated whether 6 weeks of high-intensity interval training (HIIT) prior to induction of EAE increase klotho and/or PLP and attenuate the severity of symptoms and/or disease progression in EAE model. Our data demonstrate that HIIT increased klotho and PLP and decreased disability. These proteins are associated with maintaining myelination and further research is required to examine potential clinical relevance.

1. Introduction

Multiple sclerosis (MS) is a neurological disorder in the central nervous system (CNS), characterized by demyelination of white matter axons and autoreactive immune cell infiltration (Karussis, 2014). It has been established that the prevalence rate of the disease has been increasing in females and was higher in women than men with ratios as 3:1 (Sellner et al., 2011). The pathology of MS is often examined using an inflammatory demyelinating mouse model, referred to as experimental autoimmune encephalomyelitis (EAE) (Constantinescu et al., 2011).

Demyelination is a key marker of MS pathology and is accompanied by degradation of the main myelin proteins such as proteolipid protein (PLP) and myelin basic protein (MBP) (Chen et al., 2013). Histological analysis demonstrated demyelination was identified in both grey and white matter regions including the cerebellum. Studies demonstrated in MS; the broad range of clinical symptoms are related to cerebellar dysfunction. So, there is considerable evidence of cerebellum involvement in MS. Prior works have shown the role of the protein, klotho, as a regulator of remyelination, a therapeutic target for myelin repair in MS and oligodendrocytes maturation as myelinating cells in the CNS (Chen et al., 2013; Zeldich et al., 2015). German and et.al (2012) showed the highest levels of klotho were present in two brain regions of the mouse: the choroid plexus, and cerebellar Purkinje cells. Also, a higher expression of klotho in the mice brain compared to that detected in the spinal cord. In addition, klotho receptors are expressed on oligodendrocytes and increase the expression of myelination proteins, such as MBP and PLP (Chen et al., 2013). PLP is a key myelin protein that plays an essential role in the stability of the structure and function of myelin. Studies have shown that PLP is destroyed in MS and is thought to be a major mechanism for the development of MS (Greer and Pender, 2008). Klotho also inhibits the proinflammatory cytokine, TNF- α (Degaspari et al., 2015), which when secreted by Th17 cells increases damage to the myelin sheath in MS patients (Glass et al., 2010). Therefore. it seems one of the main physiological roles for klotho is to control myelination through oligodendrocyte maturation, increased MBP and PLP concentrations while simultaneously suppressing neuroinflammatory cytokines. However, the mechanisms through which klotho increases myelination remain unclear.

Studies have characterized small molecular complexes that increase the klotho expressions in vivo (King et al., 2011). Also, exercise has been shown to increase klotho in human and animal models (Ji et al.,

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2018; Tan et al., 2018). It seems that intensity, duration, and kind of exercise influence on the increase of klotho levels. However, certain physiological mechanisms have not been proposed as an explanation of how exercise training can increase the klotho level. Emerging evidence suggested that exercise could increase the klotho expressions through an upregulation in the AMPK- PGC1-α- and oxidative stress (Constantinescu et al., 2011; Tan et al., 2018). Also, High-intensity interval training (HIIT) is one of the best methods that can induce activation of AMPK, PGC-1α. On the other hand, prior work has shown decreased klotho concentrations in the cerebrospinal fluid of MS patients (Aleagha et al., 2015) and the brain of the EAE mouse model (Aleagha et al., 2018). Besides, exercise has for many years been a controversial issue in rehabilitation for persons with MS, though in more recent years the effect of physical activity and/or exercise on persons with MS has received considerably more attention (MacDonald et al., 2017). For example, studies demonstrated exercise attenuates markers oxidative damage that have the main role in MS pathology, in the cerebellum and also, improves in motor function (Cui et al., 2009; Houdebine et al., 2017).

A common means to assess the influence of exercise in rat and mouse EAE model is to use a pre-conditioning/post-conditioning model (Bernardes et al., 2013). 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. Several studies have indicated that pre-conditioning is an effective method in attenuating clinical outcomes from onset to peak and reduced demyelination (Bernardes et al., 2016a; Bernardes et al., 2016b).

Based on prior work, it seems there is a relationship between klotho, control of myelination (Chen et al., 2013), and increased klotho via exercise (Ji et al., 2018). In this study, we investigated whether 6 weeks of HIIT increased klotho and PLP concentrations and attenuated assessment severity of disease symptoms in the EAE mouse model. We hypothesize that HIIT increases klotho and PLP concentrations and reduces disease progression as measured via clinical outcome scores.

2. Materials and methods

2.1. Animals and experimental group

Female C57BL/6 mice (n = 50, 4-5 weeks old, 13-15 g) were purchased from Pasteur Institute (Tehran, Iran) and were housed cages at 22 ± 1 °C under a 12-h light/dark cycle (lights on at 07:00 h), 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). Fifty animals were randomly divided into five groups (n = 10 per group): Control, EAE, EX, EAE-EX1 and EAE-EX2. During the first week, animals were familiarized with the treadmill running. The EX group trained five times a week for 6 weeks. The EAE-EX1 and EAE-EX2 mice exercised five times a week for 4 weeks, then experimental autoimmune encephalomyelitis (EAE) was induced. After induction of EAE, the EAE-EX1 group trained for the entire duration of the training program (6 weeks) whereas the EAE-EX2 group stopped training following completion of the 4th week (Fig. 1).

2.2. Exercise training protocol

Exercise training (Table 1) was performed on a motor-driven treadmill by the EX group. Treadmill familiarization involved running twice a day with a speed of 8 m/min for 10 min and 0% grade for 1 week. At the end of the familiarization period, mice underwent an incremental exercise test to obtain the maximal running speed. Mice initially ran at 6 m/min for 3 min at 0% grade, with the speed progressively increasing by 3 m/min every 3 min until exhaustion - when the mice were unable to maintain the required running speed (Ferreira et al., 2007). The maximal speed obtained was then used to calculate the individualized running speed for mice in the EX group. HIIT involved a treadmill running 5 days per week for the final 6 weeks. The first week of exercise training included 2, 2 min intervals at 80% maximal running speed interspersed with a 2 min active recovery at 40% maximal running speed. Before and after exercising, a 5 min warm-up and cool down was performed with intensity of 30–40% of the maximum running speed (Afzalpour et al., 2015). Progressive overload was performed by increasing the running time and number intervals in both HIIT groups.

2.3. EAE induction and clinical score

To induce EAE, the animals were immunized with Hooke Kit™ (Hooke laboratories, Cat No. EK-2110, Lawrence, MA, USA) following the instruction given by the company. 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. Clinical score was recorded daily until 21 days as a high score following immunization by blinded scorers. 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 (Bernardes et al., 2013).

2.4. Euthanasia and tissue collection

Animals were deeply anesthetized with ketamine (100 mg/kg) and xylazine (20 mg/kg) and perfused using 10% formalin in Phosphate Buffered Saline (PBS). The animal cerebellum was removed and maintained in the fixative solution overnight.

2.5. Histopathological analysis

Fixed tissue was paraffin-embedded and 5 μm 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 (Soleimani et al., 2014). The total surface of demyelinated regions was calculated by Infinity software (v. 4.6, Lumenera Corporation, Canada).

2.6. Western blotting

To detect the level of proteins extracted from the cerebellum tissue, we used the western blot technique (Khani et al., 2017). Briefly, cerebellum tissue was homogenized on ice in a RIPA lysis buffer (Cat NO. R0278, Sigma-Aldrich, USA) (The composition of the RIPA buffer was as follows: 50 mM Tris-HCl, pH 8.0, with 150 mM sodium chloride, 1.0% Igepal CA-630 (NP-40), 0.5% sodium deoxycholate, and 0.1% sodium dodecyl sulfate) containing the Protease Inhibitor Cocktail (Cat NO. 11836153001, Roche, USA). The sample's protein content was standardized trough Bradford assay and was subjected to acrylamide SDS-page electrophoresis.). For each lane thirty-micrograms of proteins were loaded in 10 μl sample buffer and separated by electrophoresis followed by transferring to Polyvinylidene difluoride PVDF membranes (Cat NO. 427152, Sigma-Aldrich, USA). The membranes were then blocked in phosphate-buffered saline, 0.01% Tween 20, (PBS-T) containing 5% non-fat milk and probed with primary antibodies against PLP (1/500, Cat NO. sc-58,571, Santa Cruz, USA), Klotho (1/500, Cat

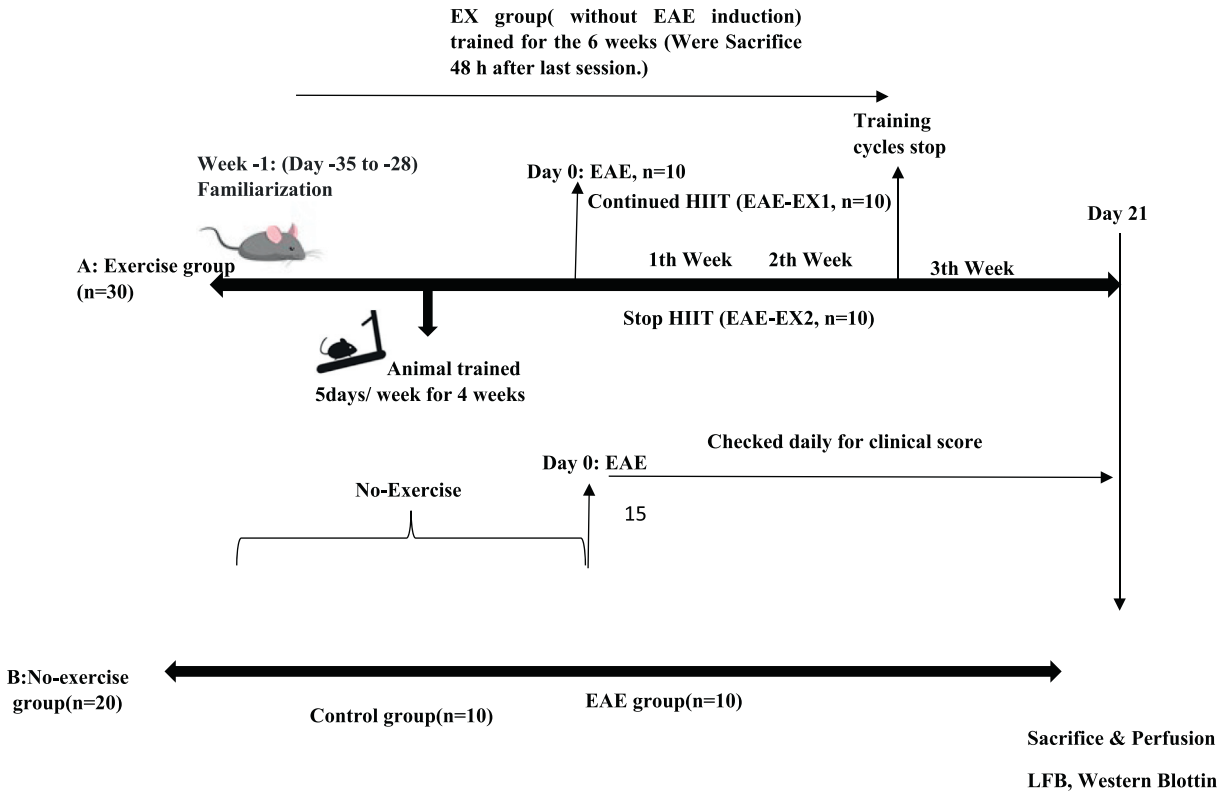

Fig. 1. Schematic representation of the experimental design.

NO. sc-22,220, Santa Cruz, USA), TNF-α (1/500, Cat NO. sc-130,349, Santa Cruz, USA), and GAPDH (1/500, Cat NO. sc-32,233, Santa Cruz, USA) at 4 °C in a shaker incubator (Behdad, Tehran, Iran) overnight and incubated with peroxidase-conjugated secondary antibodies (Cat NO. sc-516,102 and sc-2020, Santa Cruz, USA)for 1 h at 25 °C. Equal volumes of ECL substrate solution (Cat NO. 102030396, Bio-Rad, USA) and Luminol/enhancer solution (Cat NO. 102030394, Bio-Rad, USA) were used to visualize the protein bands. Quantification of the results was performed by densitometry scan of films. Band density was measured by using Image J software and normalized to that of GAPDH as the housekeeping gene and expressed as fold change.

2.7. Statistical analysis

All data were analyzed using Graph Pad Prism software (version 5.0) and were expressed as mean ± SD. To compare the differences in klotho, PLP, TNF-α concentrations and demyelination in 21 dpi between groups (control, EAE, EAE-EX1 and EAE-EX2) we used the one-way ANOVA with the Tukey post-test. Power of the study (1- β) is fixed at > 55%. Differences in klotho, PLP, TNF-α concentrations in the EX and the control groups were made by independent samples t-test. Clinical score data from the EAE, EAE-EX1 and EAE-EX2 groups were analyzed using the non-parametric Kruskal Wallis test. The significance level was accepted at $P \leq .05$.

3. Results

3.1. 6 weeks of HIIT increased klotho and PLP and decreased TNF-α concentrations in the cerebellum

To examine the effect of HIIT protocol, western blotting analysis was performed. 6 weeks of HIIT increased klotho ($P \leq .001$) and PLP ($P \leq .01$) in the EX group compared to the control group (Fig. 2-B and C, respectively). The EAE group compared with the control group demonstrated a significant decrease in the klotho and PLP concentrations in the cerebellum at 21 dpi ($P \leq .0001$). Compared with EAE, the EAE-EX1 group demonstrated a significant increase in the klotho and PLP concentrations ($P \leq .0001$). Also, klotho and PLP concentrations were higher in the EAE-EX1 group compared with the EAE-EX2 group ($P \leq .0001$) (Fig. 2-B and C, respectively). There was a significant increase in TNF-α concentration in the EAE group compared to the control group ($P \leq .0001$) (Fig. 2-D). HIIT significantly decreased TNF-α concentration in both EAE exercise groups compared to the EAE group and in the EAE group compared to the control group ($p \leq .05$) (Fig. 2-D).

3.2. 6 weeks of HIIT reduced cerebellum demyelination

Demyelination in the EAE group was observed using a Luxol Fast

Table 1
Training protocol.

Warm-up	HIIT	Speed running	Recovery internal	Recovery speed	Total time	Weeks	Cool-down
5 min 30–40% Vmax	2 interval, 2 min 80%Vmax	22 m/min	2 interval, 2 min 40%Vmax	9 m/min	18/min	1	5 min 30–40% Vmax
	4 interval, 2 min 90%Vmax	24 m/min	4 interval, 2 min 40%Vmax	10 m/min	26/min	2	
	6 interval, 2 min 100%Vmax	26 m/min	6 interval,2 min 40%Vmax	11 m/min	34/min	3	
	8 interval, 2 min 110%Vmax	29 m/min	8 interval, 2 min 30%Vmax	9 m/min	42/min	4–6	

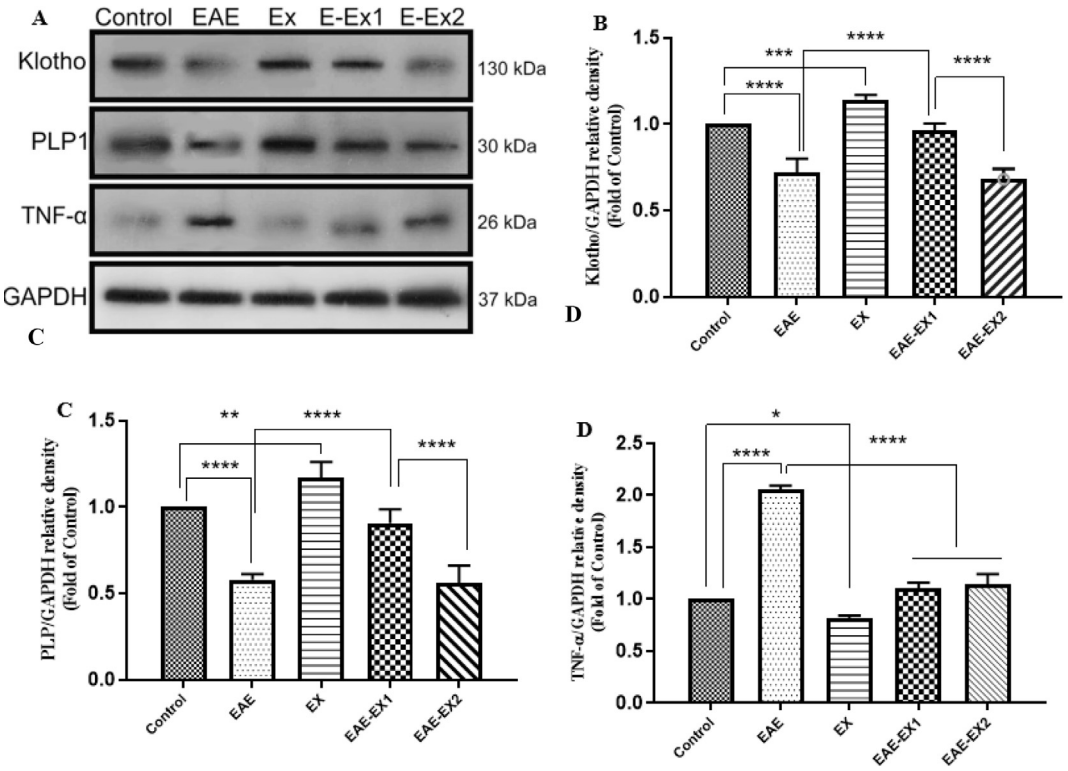

Fig. 2. 6 weeks of HIIT increased klotho and PLP and decreased TNF-α concentrations in the cerebellum. Representative western blots (A). Klotho and PLP concentrations in the cerebellum at 21 dpi were significantly decreased in the EAE group compared to the control group. Also, klotho and PLP concentrations were higher in the EX group compared with the control group and EAE-EX1 group compared to the EAE and the EAE-EX2 groups (B and C). TNF-α concentration was significantly increased in the EAE group compared to the control group. Also, it was significantly decreased in the EAE-EX1, EAE-EX2 groups compared to the EAE group and in the EX group compared to the control group (D). Values are given as mean ± SD. (*P ≤ .5, ** P ≤ .01, ***P ≤ .001 and ****P ≤ .0001).

Blue (LFB) (Fig. 3-A) stained cross-section of the cerebellum and was significantly higher in the EAE group compared to the control group (P ≤ .0001). Demyelination was significantly lower in the EAE-EX1 group compared to the EAE group (P ≤ .01) with no difference between EAE and EAE-EX2 (P = .4) (Fig. 3-B).

3.3. 6 weeks of HIIT delayed and attenuated the severity of EAE symptoms from onset to disease peak

To evaluate the effect of prior HIIT on the development of clinical signs of EAE, we scored all EAE animals 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-EX1 group demonstrated early symptoms on 12 dpi and the peak score was attained on day17 then gradually recovered from days18 after induction (score of 1). The EAE-EX2 group displayed symptoms on 15 dpi (score of 1) and remained unchanged 21dpi (Fig. 4-A, Table. 2). Mean clinical signs in the EAE-EX1 and EAE–EX2 groups was lower compared to the EAE group (Fig. 4-A; P ≤ .05). Weight was significantly lower in the EAE group compared to both Control and EAE-EX2 groups (Fig. 4-B, P ≤ .0001 for both comparisons).

4. Discussion

In the present study, we demonstrated that HIIT increased klotho and PLP and decreased TNF-α concentrations in the cerebellum tissue in the EX and EAE-EX1 groups (See Fig. 2-B, C and D) and there was also a reduction in demyelination and severity of EAE symptoms (see Fig. 3-B, 4-A and B). Thus, in general, the findings confirmed our hypotheses, specifically regarding the beneficial effects of HIIT on myelination through increasing klotho. Furthermore, data presented in this paper in both EAE-exercise groups can be considered as klotho importance during the early and peak phase of EAE. Also, it appears the intensity of training likely is an important factor in the exercise regimen during the early phase of EAE.

4.1. Exercise induced increase in proteins associated with myelination

Prior works have shown that exercise modulates different proteins, such as brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) which are thought to play a role in neuronal repair and plasticity in MS patients (Castellano and White, 2008; Gold et al., 2003). Remyelination during the early stages of the disease process has been documented. Also, several intrinsic molecular pathways that execute endogenous remyelination have been identified (Stangel et al., 2017). However, there is no evidence that exercise reduces demyelination and enhances remyelination through increasing klotho and PLP concentrations. In our study increased klotho and PLP concentrations were observed in the EX group compared with the control group and in the EAE-EX1 group compared with the EAE and EAE-EX2 groups (Fig. 2-B).

We observed that klotho expression in the EAE-EX2 group at the peak phase of EAE was lower than the EAE-EX1 group. One of the possible reasons was that the animals in the EAE-EX2 group were sacrificed three weeks after the last sessions of HIIT. So, it appears after three weeks of detraining in the EAE-EX2 group, klotho concentration returned to baseline levels in the cerebellum. On the other hand, Although, klotho concentrations were not measured after 4 weeks of HIIT and at the early phase of EAE (EAE onset was noticed on days 9–10) in the EAE-EX2 group, they had better clinical scores and less weight loss compared to the EAE and the EAE-EX1 group. The previous study demonstrated a substantial decrease in klotho expression in the

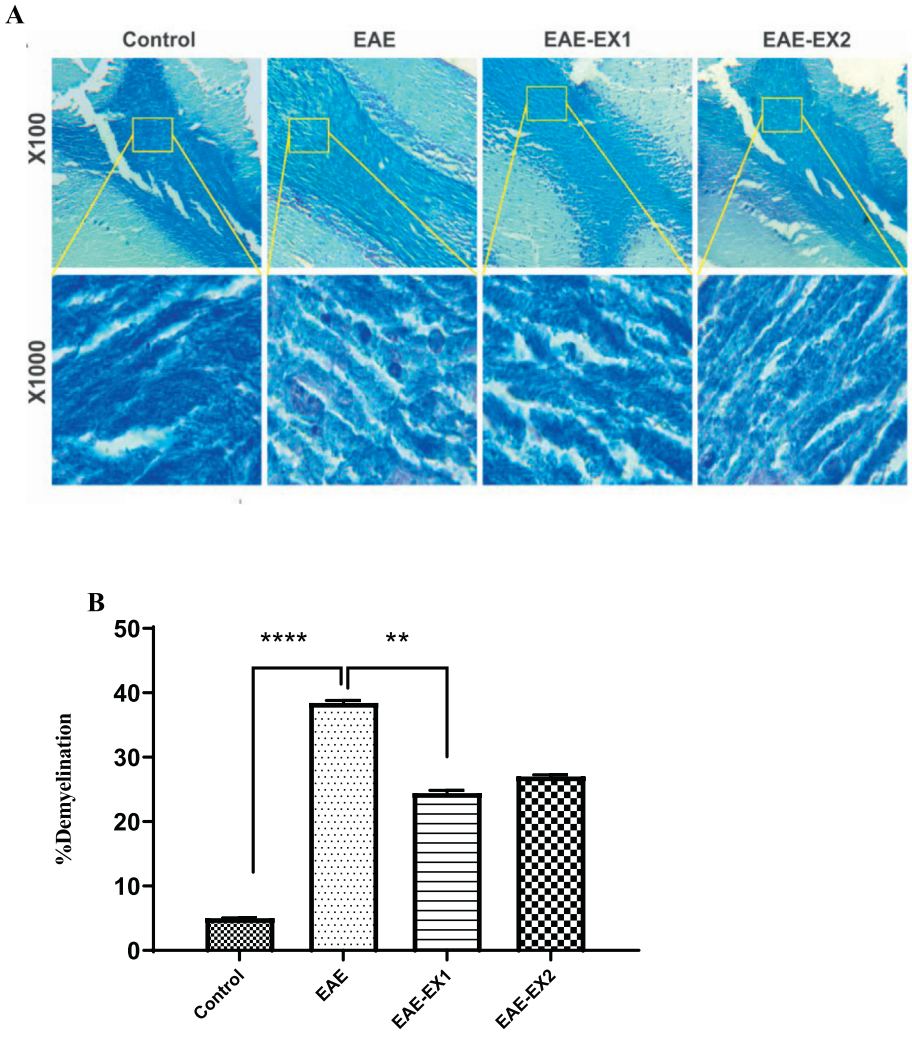

Fig. 3. 6 Weeks of HIIT reduced cerebellum demyelination. Demyelination pathological analysis following EAE induction in the cerebellum by LFB at magnification 100× and 1000× (A), in all groups at 21pdi (B). Lower demyelination was observed in the EAE-EX1 group compared to the EAE group. Demyelination was significantly higher in the EAE group compared to the control group. Values are given as mean ± SD. (**P ≤ .01 and **** P ≤ .001).

brain of EAE animals during the peak stages and early stage (Aleagha et al., 2018). So, it seems 4 weeks of HIIT increased klotho concentration in the early phase of EAE and developed a clinical outcome. Furthermore, overexpression of klotho improved clinical outcomes following cuprizone-induced demyelination in the onset stage of EAE in the brain but not in the spinal cord (Zeldich et al., 2015). Thus, these findings may support the idea that klotho affects the development of the disease during the early phase of EAE, and increasing in klotho

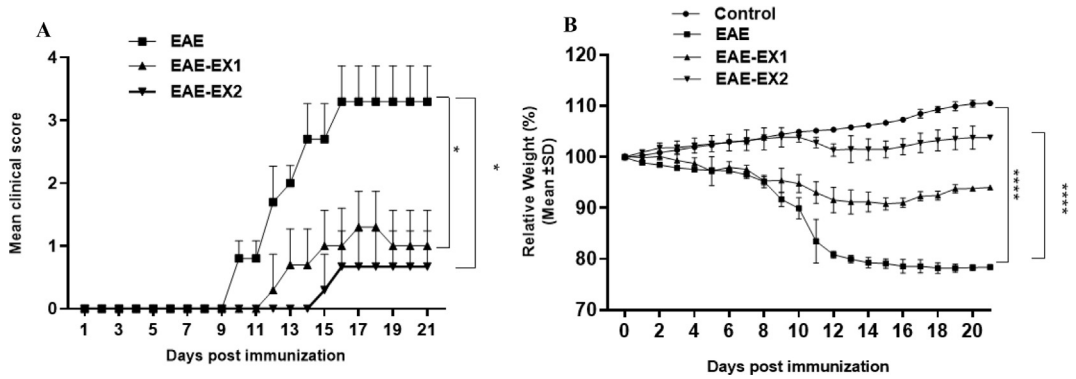

Fig. 4. 6 weeks of HIIT delayed and attenuated the severity of EAE symptoms from onset to disease peak. Clinical assessment of exercise and EAE groups. Clinical signs were assessed daily for 21 after EAE induction. Results are expressed as the day by day mean clinical score. Mean clinical score in the EAE-EX1 and EAE–EX2 groups was lower compared to the EAE group (*P ≤ .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-EX2 groups (****p ≤ .001) (B). Values are given as mean ± SD.

Table 2
Clinical parameters of EAE. Incidence of EAE was significantly higher in the EAE group compared to the EAE-EX1 (* P ≤ .05,) and EAE-EX2 (**P ≤ .01) groups. Onset is considered when animals reached a clinical score 1 by 2 consecutive days, was significantly earlier in the EAE group compared to the EAE-EX1 (* P ≤ .05,) and EAE-EX2(****P ≤ .0001) groups. Day of peak disease was significantly earlier in the EAE group compared to the EAE-EX1 group (* P ≤ .05,) and was significantly earlier in the EAE-EX2 group compared to the EAE-EX1 group (# P ≤ .05). Cumulative Disease Index is calculated as the sum of the clinical scores of each animal between Day 0 and 21 and is a measure of disease severity, which was significantly higher in the EAE group compared to both EAE-EX groups (****P ≤ .0001). Values are given as mean ± SD.

Variable	Incidence		Onset			Day of peak disease			Cumulative disease index		
	percent	P value	Day	mean ± SD	P value	Day	mean ± SD	P value	Sum	mean ± SD	P value
EAE	81.6%	P ≤ .04	10.66	10.6 ± 0.5	P ≤ .05	17	17 ± 1	P ≤ .05	92	30.6 ± 3.1	P ≤ .0001
EAE-EX1	56.6%	P ≤ .01	14.33	14.3 ± 2.1	P ≤ .0001	18	18 ± 1		28	9.3 ± 4.5	
EAE-EX2	33.3%		16	16 ± 1		16.33	16.3 ± 0.5		17	5.5 ± 1.2	

concentration plays an essential role in the early stage of chronic inflammatory disease to decrease the severity of symptoms. Also, it seems klotho exhibits different patterns at the early and peak stage of EAE that in the early stage was higher compared to the Peake stage (Aleagha et al., 2018). On the other hand, likely despite klotho increases in the EAE-EX1, two more weeks of HIIT has more stress and increased EAE symptoms than the EAE-EX2 group. Further studies, however, are required for testing this hypothesis.

Some studies demonstrated the effect of exercise training on klotho plasma levels in sedentary healthy adults and postmenopausal women and animal model. However, some studies have proposed certain physiological mechanisms as an explanation of how exercise can induce an increase the klotho concentration in human and animal model. It has been shown that the peroxisome proliferator-activated receptor-γ (PPAR- γ) increased klotho gene expression in the kidney (Zhang et al., 2008) and angiotensin II down-regulates the klotho gene expression (Torres et al., 2007). Interestingly, several studies have demonstrated that exercise increased the activity of PPAR-γ (Kawamura et al., 2004) and decreased the angiotensin II type I receptors (Muñoz et al., 2018). Thus, exercise-induced increases in klotho expression may result from a combination of increased PPAR- γ concentrations and a decrease in angiotensin II type I receptors.

4.2. Mechanisms for klotho-dependent myelination

Since in multiple sclerosis most of the lesions are not remyelination, the first clinical proof-of-concept trials to enhance remyelination in MS have been conducted in the past few years (Patrikios et al., 2006). In the current study to understand role klotho play in demyelination and remyelination processes, we used EAE mouse experimental design as a model for study MS mechanisms potentially involved in MS pathophysiology (Constantinescu et al., 2011). Our data shows a decrease in klotho and PLP concentrations (Fig. 2-B and C) and an increase in demyelination in the EAE group compared to the control group (Fig. 3-A and B). Recently decreased levels of klotho were reported in the cerebral spinal fluid of MS patients compared to healthy controls and klotho values showed a significant negative correlation with the expanded disability status scale (EDSS) (Tan et al., 2018). Furthermore, serum klotho concentration tends to be higher in MS patients when compared to the control group that these findings might be attributed to the treatment of MS patients (Torres et al., 2007). This decrease in klotho concentration in the EAE model may be a result of the reduction in total antioxidant capacity and an increase in oxidative stress (Aleagha et al., 2015).

Another possible explanation for the decrease of klotho in the EAE model may result from the increased concentration of inflammatory cytokines such as TNF-α. Increased TNF-α concentration was indeed observed in the EAE group compared with the control group (Fig. 2-D). A previous study suggesting that the peak expression of cytokines, probably occurs by the onset of the disease (Bernardes et al., 2013) and the expression of pro-inflammatory cytokines such as TNF-α increases neurodegenerative decline have been associated with EAE disability (Glass et al., 2010). In the present study, a comparison of cerebellum TNF-α concentration between EAE-induced exercised groups and the EAE group at the peak phase of EAE showed a significant reduction in TNF levels in the exercised group. Also, the EX group demonstrated lower TNF concentrations in the cerebellum (Fig. 2-D) compared to the control group. This result may suggest a potential anti-inflammatory effect of exercise (Dalgas and Stenager, 2012) on decrease cytokines and the development of the clinical score and weight assessment in both the EAE-EX group. In addition of the anti-inflammatory effect of exercise, likely increase in klotho concentration inhibits TNF-α decrease cytokines and development of the clinical score and weight assessment. Furthermore, previous studies demonstrated that klotho suppresses TNF-induced expression and has a role in the modulation of inflammation (Degaspari et al., 2015; Maekawa et al., 2009) .Although klotho and TNF-α concentration were not measured in the early phase of EAE, however better clinical scores in the EAE-EX2 group may support the idea that 4 weeks of HIIT increased klotho in the early phase of EAE and decreased TNF- α and improved clinical score and demyelination. More studies are required to further characterize the effect of klotho on proinflammatory cytokines in the CNS and its clinical relevance to neurological disorders, such as MS. On the other hands, clinical score and less weigh lose were better in the EAE-REX2 compared with EAE-EX1 group seems that intensity of training likely is an important factor in exercise regimen and should be prescribed with more precaution in the early phase of EAE.

To understand the role klotho plays in the myelination process, demyelination and PLP concentration were assessed. Our data indicated demyelination was lower in both EAE-EX groups compared with the EAE group. Zeldich and colleagues strongly supporting the important role Klotho plays process on the remyelination in KL-OE mice following cuprizone demyelination (Zeldich et al., 2015). A recent study demonstrated that klotho promoted the maturation of oligodendrocytes and myelination both in vitro and in animal models (Chen et al., 2013; King et al., 2012) and had a protective effect against oxidative stress (Zeldich et al., 2014). Also, prior work suggested that the activation of klotho receptors present on oligodendrocytes activates a signaling pathway that increases PLP concentration (Chen et al., 2013). Therefore, we propose that an increase in the klotho and PLP concentration in the cerebellum tissue after HIIT in the early and peak stage of EAE may be responsible for the decreased demyelination. Lower demyelination in the EAE-EX1 group would imply, likely that higher level of klotho, enhances remyelination in demyelinating diseases of the CNS.

4.3. Exercise decreased clinical symptoms in the EAE model

The present study demonstrated that HIIT attenuated the clinical score and percent weight loss in the EAE exercise groups and delayed the severity of EAE symptoms from onset to disease peak (Fig. 4-A and B). Previous studies investigated the beneficial effect of exercise programs on clinical symptoms and decrease severity in the EAE model.

Bernard and colleagues (2013 and 2016) showed that a program of 6 weeks of preconditioning exercise promoted a significant reduction of clinical score and severity of EAE from onset to disease peak (Bernardes et al., 2013; Bernardes et al., 2016a).

Souza and colleges (2017) demonstrated both strength and endurance training protocols consistently prevented clinical signs in the EAE model (Souza et al., 2016). These findings were confirmed in our study. We demonstrated improved clinical scores and reduced severity of symptoms in the EAE-EX groups compared to the EAE group. Potential mechanisms for the exercise-induced reduction and improvement in clinical symptoms in our study may be attributed to a decrease in TNF-α. Previous study have shown that TNF-α plays a major role in the induction of EAE and that a reduction in TNF-α correlates with improved functional recovery (Tanuma et al., 1997). Our data demonstrated TNF-α concentration in both EAE-EX groups was lower compared with EAE group (Fig. 2-D), thus leading to lower clinical scores. More importantly, the EAE-EX2 group appears to have a better clinical outcome and less body weight loss than the EAE-EX1 group (Fig. 4-A and B). As we discussed, likely, better clinical score in the EAE-EX2 group may support the idea that 4 weeks of HIIT increased klotho in the early phase of EAE and maybe decreased TNF-α level and clinical score. Also, it appears in the EAE-EX1 two more weeks of HIIT has more stress and increased EAE symptoms than the EAE-EX2 group. However, further studies are required to understand the effect of exercise on klotho and clinical score in the early and peak phase of EAE.

5. Conclusion

The present study demonstrated that 6 weeks of HIIT in mice resulted in increases in klotho and PLP concentrations in the cerebellum tissue and decreased demyelination using the EAE model. Therefore, our study strongly supports the important role HIIT increases klotho and PLP concentrations as myelin biomarkers using a mouse model. Nevertheless, the mean of the clinical score was higher in the EAE-EX1 group compared to the EAE-EX2 group. So, it seems intensity and type of exercise (forced exercise) should be considered in MS patients. On the other hand, it has been suggested that klotho expression decreased in the brain tissue of EAE animals during the onset and peak stages. So, the correlation between exercise and klotho in both phases of the EAE model needs further investigation. On the other, it revealed that klotho exhibited different patterns of gene expression and protein concentration in the brain and spinal cord of EAE mice. So, it seems the effect of exercise on klotho on the other areas of the CNS should be considered. Moreover, despite many attempts that have been made to cure MS, the outcomes are unsatisfactory, and no definite solution has been found yet. On the other hand, due to the relationship between klotho, control of myelination and increased klotho further study should be considered in the human study.

Declaration of Competing Interest

The authors have no competing interests to declare.

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