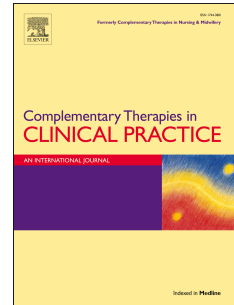


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**Acute Responses of Cytokines and Adipokines to Aerobic Exercise in Relapsing vs. Remitting Women with Multiple Sclerosis**

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**Running title:** Exercise in relapse MS: Cytokines and Adipokines Response

## **Acute Responses of Cytokines and Adipokines to Aerobic Exercise in Women with Relapsing vs. Remitting Multiple Sclerosis**

### **ABSTRACT**

*Objective:* To examine the acute effect of exercise on cytokines and adipokines during relapse and the remitting phase of multiple sclerosis (MS).

*Methods:* Thirty women with MS in the relapsing or remitting phase were matched with fifteen healthy controls. Participants performed a single-bout of aerobic exercise at 60-70% maximal heart rate. Furthermore, five women in the relapsing phase were enrolled (control relapse) and did not receive any intervention. Blood samples were taken before, immediately after, 1-hour and 6-hours after the exercise.

*Results:* Levels of IL-10 and TNF- $\alpha$  in response to exercise were similar in healthy and MS remitting subjects. Compared to baseline, TNF- $\alpha$  levels in relapsing subjects were significantly decreased immediately after exercise. Immediately following exercise, leptin levels significantly decreased in relapsing subjects. Adiponectin and IL-6 showed no significant difference between groups.

*Conclusion:* After relapse, exercise does not induce inflammatory cytokine response and temporarily improves both cytokine and adipokine balance.

**Keywords:** Multiple sclerosis; Cytokines; Inflammation; Adipokines; Aerobic interval exercise

## 1. Introduction

Multiple sclerosis (MS) is an autoimmune disease characterized by inflammation, demyelination and axonal loss of the central nervous system (CNS) [1-5]. MS can be subdivided into four clinical courses: relapsing-remitting (RRMS), primary progressive (PPMS), secondary progressive (SPMS), and progressive relapsing (PRMS) [6]. RRMS is the most common form of MS [7]. RRMS is characterized by relapsing phases in which new or exacerbating symptoms appear [4]. The underlying pathways that activating this relapsing phase are unclear [2, 3, 8]. Cytokines and their related pathways are considered to be major regulators of the immune system [9, 10] which may be important in the evolution of MS lesions and disease activity [9]. During relapse, some important abnormalities and imbalances occur [2, 3, 8]; therefore, an accurate examination of the effects of environmental stressors such as acute bouts of exercise is essential.

Although, the causes of MS are still unclear, T helper (Th) and T regulatory (Treg) lymphocytes have been suggested to play a major role in the development of the disease [11]. In fact, Th lymphocyte cells can produce and secrete pro-inflammatory cytokines such as tumor necrosis factor (TNF)- $\alpha$ , interleukin (IL)-6 [12] and furthermore, some anti-inflammatory cytokines such as IL-10. The latter cause down-regulation of inflammatory Th cell function, which in turn leads to anti-inflammatory triggers [12]. The evidence suggests a key role of acute dysregulation of the cytokine balance on acute inflammatory lesions in the relapsing phases of MS accompanied by an increase in the production of pro-inflammatory cytokines that results in acute inflammation [12, 13].

In the last decade, extensive attention has been paid to the close association between adipose tissue and inflammation [14, 15]. For instance, it has been reported that leptin is involved in the neurodegenerative and inflammatory environment that sustains MS [16]. In this regard, experimental evidence confirms the well-known impact of leptin and adiponectin on immunological function [17]. Previous studies in this field have verified the roles of leptin and adiponectin in the maintenance of energy balance, food intake and body mass [14, 17]. Moreover, the important roles of leptin on immunological function through regulation of Th balance are well known [3]. In addition, there is strong evidence that indicates that leptin levels increase during relapse and can also affect the response pattern of cytokines [16, 17]. Therefore, the acute response of adipokines, such as leptin, to exercise is an important issue, which has been neglected in previous studies in MS patients.

Exercise training has a more robust stress paradigm compared to psychological stress [18, 19]. For instance, exercise is known to induce anti-inflammatory mediators such as IL-10 [20]. The cytokine response to exercise is well documented in a normal healthy population and is dependent on the mode, intensity and duration of exercise [21]. However, considerably less is known regarding these responses in MS, whilst this is critical to understanding the impact of exercise in MS patients. The importance of acute exercise is that it serves as a model for understanding exercise as a stressor that might result in long-term adaptations in a response to a single bout of exercise. That is, the repeated exposure to the acute stimulus might change the acute response over time as an adaptation and chronic alteration. Heesen et al. [22] reported a blunted cytokine response of TNF- $\alpha$  and IL-10 in sedentary MS subjects compared to trained MS subjects following a single 30 minute bout of exercise, when compared to trained MS subjects [22]. In another longitudinal study, Schulz et al. [23] indicated that chronic

exercise training may improve quality of life and cognitive function in persons with MS, without significant modulations in IL-6 or soluble interleukin-6 receptor (sIL-6) activity [23]. However, further investigation is warranted to determine how these responses may vary with an increased exercise stress and a wider variety of stress biomarkers. Most treatments and medications prescribed at this time to reduce inflammation in the MS relapse course, include optimization of the pro- and anti-inflammatory cytokines balance [8, 24]. Though, exercise was also shown to affect this course [22, 23]. Therefore, it should be examined how exercise can affect cytokines balance during relapse, and whether exercise can be beneficial or should be avoided during relapse. However, acute responses of adipokines to exercise have not been described previously in the relapsing and remitting phase in MS subjects, neglecting of the role of acute exercise on cytokines during relapses in MS. Nevertheless, we believe that the disease course may interact with cytokines and adipokines responses to an acute bout of exercise. Therefore in order to analyze the impact of exercise on immune status in MS, the present study aimed to investigate the response of various cytokines (TNF- $\alpha$ , IL-10, IL-6) and adipokines (adiponectin and leptin) to an acute bout of exercise in the relapsing and remitting phase of MS, compared to healthy controls.

## **2. Materials and methods**

### **2.1. Subjects**

As the effect size for responses of cytokines and adipokines to acute bouts of exercise in MS has not been calculated before, and according to previous studies, Cohens' classification ( $f=0.3$ ) was used for power analysis which indicates a small to moderate effect. Moreover, a significance level of 0.05 and statistical test-power of 0.8 was used. The correlation of time points was estimated at  $r=0.5$ . The subsequent calculation indicated a total sample size of 28 (G\*power 3.0.10 software, Germany). Factoring in a drop-out of 15%, the minimum total sample size required was 33 participants. The current study recruited thirty five participants with MS, including fifteen women during remitting and twenty women during relapse with RRMS (mean EDSS score =  $2.11\pm 0.76$ ), according to the McDonald 2010 criteria [25] from the MS society center of Khuzestan, Iran under the observation of a neurologist. A further fifteen healthy women (healthy control) ranging from 20-35 years old were recruited. Healthy control subjects had neither laboratory nor clinical symptoms of infectious, autoimmune or inflammatory diseases. The objectives, risks and benefits of the study were explained to all enrolled members, followed by collection of written informed consent. This study was approved by the Ethics committee of Jundishapur University of Medical Sciences, Ahvaz, Iran (registration number: IRCT2016031427047N1).

### **2.2. Study design and procedure**

At baseline, persons with RRMS ( $n = 35$ ) were divided into three groups: a relapsing exercise group ( $n = 15$ ), a remitting exercise group ( $n = 15$ ) and a relapsing control group ( $n = 5$ ). Aerobic capacity (maximal oxygen uptake,  $VO_{2max}$ ) was determined [26] four days before initiation of the exercise sessions. After a 10-hour overnight

fasting period, subjects arrived at the ergometry laboratory, and consumed the same standardized breakfast under the researchers' supervision 60 minutes prior to the beginning of the exercise session. Anthropometric measures and physical activity (PA) levels were assessed. After 60 minutes of seated inactive rest, subjects performed an exercise bout consisting of upper and lower limb cycling performed at an intensity of 60-70% of maximum heart rate ( $HR_{max}$ ) determined from the maximal exercise test. Blood samples were collected at four different time points: before initiation of the exercise, immediately after, 1-hour after and 6-hours after completion of the exercise (Figure 1). Relapse definition was based on the 2010 revisions of McDonald criteria as "patient-reported symptoms or objectively observed signs typical of an acute inflammatory demyelinating event in the CNS, current or historical, with duration of at least 24 hours, in the absence of fever or infection" [25].

### **2.3. Physical activity**

PA was monitored four days prior to the exercise sessions using the Baecke physical activity questionnaire [27]. The Baecke questionnaire includes a total of three domains scored on a Likert scale. The average score for the three domains was the overall activity score, with a score of five indicating the highest possible physical activity [27].

### **2.4. Anthropometric measurements**

Based on each patient's body mass (kg) and squared height ( $cm^2$ ), body mass index (BMI) was calculated. Also, skinfold thickness was measured at thigh, suprailium and triceps sites via Harpenden calipers (®) according to the instructions previously highlighted [28]. Body density subsequently was estimated by a three-site skinfold thickness equation [28], after which the Siri equation was used to estimate body fat percentage (BFP) [29].

### **2.5. Aerobic capacity**

In order to determine aerobic capacity ( $VO_{2max}$ ), subjects cycled for four min without resistance on a cycle ergometer. Work rate was subsequently increased by 15 watts (w) per minute until exhaustion, while pedaling rate was maintained at a constant 60 revolutions per minute (rpm). Heart rate was monitored throughout the test in order to provide a maximal heart rate value, which was used to determine the intensity of exercise in the subsequent exercise sessions. Finally, maximal workload (watt), age (years) and weight (kg) of subjects were used to determine their  $VO_{2max}$  ( $ml.kg.min^{-1}$ ) [26].

### **2.6. Exercise bout**

Subjects were asked to refrain from strenuous exercise and physical activity at least 4 days prior to the exercise session. Exercise consisted of four sets of 5 minute intervals of upper body cycling and four sets of lower

body cycling interspersed with 2 minutes of passive rest between each interval. The intensity of each bout of interval exercise was adjusted to achieve an intensity of 60-70% HRmax. It should be noted that, after completing the exercise, subjects remained seated passively for six hours. Furthermore, in the relapsing group exercise was performed 2 weeks after the onset of an acute relapse. Temperature and humidity were controlled during each trial at an average of  $21.2\pm 1.07$  C and  $37.24\pm 3.41\%$ , respectively.

### **2.7. Blood sampling and assays**

Venous blood samples were collected at 4 different time points and centrifuged immediately after collection; the resulting serum was aliquoted and stored at  $-70^{\circ}\text{C}$ . Serum high density lipoprotein (LDL), high density lipoprotein (HDL) and triacylglycerol (TG) were measured using photometric method by Pars Azmoon kits. Cytokine concentrations were assessed via enzyme linked immunoassay ELISA kits and were conducted according to the manufacturer's instructions. Leptin and adiponectin levels were obtained using R&D Systems kit (Minneapolis, USA) and BioVendor (Brno, Czech Republic), respectively. TNF- $\alpha$  and IL-10 levels were measured using IBL International GMBH kit (Hamburg, Germany). Also, IL-6 levels were measured using an R&D system kit. The intra and inter assay coefficient of variation (CV) for the performed samples was  $<7\%$ .

### **2.8. Statistical analysis**

Statistical analyses were performed by SPSS Statistics (IBM SPSS Statistics, version 21, Armonk, NY). Data were presented as means and standard deviation (mean $\pm$ SD). Group differences at baseline were evaluated using one-way analysis of variance (ANOVA) with groups (relapse vs. control relapse vs. remitting vs. healthy controls) as the between-subjects factor. Post-hoc (Bonferroni-adjusted) tests were performed where appropriate. Repeated measures ANOVA were applied with regard to 'group' as the between-subjects factor (relapse vs. control relapse vs. remitting vs. healthy controls) and 'time' as the within subjects factor (baseline vs. immediately after vs. 1-hour after vs. 6-hour after). Post-hoc (Bonferroni-adjusted) tests were performed in case of a significant time\*group interaction. An alpha level of 0.05 was considered significant.

## **3. Results**

### **3.1. Demographic data and baseline cytokines**

No differences were found between groups for age, weight and BMI ( $p > 0.05$ ). Mean disease duration in the MS groups was  $3.04\pm 0.94$  years. Mean EDSS score of all RRMS was  $2.11\pm 0.76$ , with scores of  $2.41\pm 0.83$  and  $1.82\pm 0.65$  for the relapsing and remitting groups, respectively. No significant differences in disease duration were found between the MS groups (all values  $p > 0.05$ ).

At baseline,  $VO_{2max}$  and PA were significantly higher in the HC than the MS group ( $p < 0.05$ , Table 1). Between MS groups,  $VO_{2max}$  in the MS remitting group was significantly higher than the relapse group ( $p = 0.003$ ). No differences in PA were observed between MS groups ( $p = 0.174$ ). Significant baseline differences in cytokine levels between groups (all values  $p < 0.05$ ) were observed. Leptin levels were higher in the relapsing group when compared to the remitting ( $p = 0.001$ ) and control ( $p = 0.001$ ) groups. The remitting group showed higher levels of leptin than the control group ( $p = 0.033$ ). IL-10, IL-6 and adiponectin levels were similar between groups ( $P > 0.05$ ). Significant differences in basal TNF- $\alpha$  levels were present between groups ( $P = 0.007$ ). Subsequently, Bonferroni tests revealed significant differences between the relapsing group and healthy subjects ( $p = 0.026$ ) as well as between the remitting group and healthy subjects ( $p = 0.024$ ) while the MS groups were not significantly different ( $p = 0.213$ ) (Figure 2). Finally, significant baseline differences in TG levels between groups ( $p < 0.05$ ) were observed, with higher TG levels in the relapsing groups compared to remitting and control groups (Table 2).

### 3.2. Cytokine and lipid profile responses to exercise

Significant time effects were observed for leptin ( $p = 0.001$ ), IL-6 (0.004) and TNF- $\alpha$  (0.021). However, for leptin and TNF- $\alpha$ , a post hoc test indicated a significant decrease ( $p = 0.002$ ) and increase ( $p = 0.013$ ) 1-hour after completion of the exercise bout, respectively. With the exception of adiponectin ( $p = 0.09$ ), a significant time\*group interaction was observed for all cytokines ( $p < 0.05$ ) (Figure 3). Moreover, TG indicated a significant time\*group interaction ( $P = 0.004$ ). TG ( $p = 0.031$ ) and heart rate ( $p = 0.001$ ) showed a time effect.

Immediately following exercise, TNF- $\alpha$  concentrations significantly decreased in relapsing ( $p = 0.001$ ) subjects but significantly increased in remitting ( $p = 0.013$ ) subjects. A marked increase was observed 1-hour after exercise in TNF- $\alpha$  in relapsing subjects compared to immediately after exercise ( $p = 0.006$ ), whilst no change was observed in the control relapsing group ( $p > 0.05$ ). A significant decrease in IL-10 was observed immediately after exercise in relapsing subjects ( $p = 0.023$ ). In remitting subjects, a significant increase was observed 1-hour after exercise compared to baseline ( $p = 0.010$ ). All three exercise groups showed similar IL-6 responses increasing immediately after exercise ( $p < 0.01$ ) with no significant change in other time points ( $p > 0.05$ ). Leptin concentration was decreased significantly in the relapse group ( $p = 0.001$ ) immediately after exercise, however no change was observed in other groups ( $p > 0.05$ ). All groups, with the exception of the control relapsing group, showed a significant decrease 1-hour after exercise in leptin concentrations ( $p < 0.02$ ), while the degree of change in the relapsing group was significantly higher compared to other groups. Finally, leptin levels of MS subjects who performed exercise remained significantly lower compared to baseline and 6-hours following exercise ( $p = 0.019$ ).

## 4. Discussion

In the present study, we investigated cytokine responses to a single exercise-bout in both the relapsing and remitting phases of MS. Responses of IL-10 and TNF- $\alpha$  levels to an acute bout of exercise were comparable in HC and MS remitting subjects. Subjects in the relapsing phase of MS showed a significant decrease and increase in



TNF- $\alpha$  levels respectively compared to baseline, immediately and 1-hour after exercise. Moreover, immediately after exercise, leptin significantly decreased in the MS relapsing subjects. Finally, there were no significant differences in IL-6 or adiponectin responses to exercise between groups.

The underlying pathogenesis of relapses depends on the presence of new inflammatory plaques that are caused by activation of the immune system [2, 12]. It is not clear which inflammatory pathways are associated with relapses and this has a pivotal role in determining a more stimulated disease activity [2]. Several cytokines such as TNF- $\alpha$ , IL-10, IL-6, INF- $\gamma$ , and leptin, were associated with a more active phase of the disease [12, 17]. In this regard, the results of the present study indicate that leptin was higher in relapsing vs. remitting subjects accompanied by significantly higher TG in the relapsing groups. Also, MS groups indicated higher levels of leptin compared to the healthy control group.

The presence of leptin is essential for the induction and progression of several experimental models of autoimmune diseases [30]. While most pro-inflammatory cytokines such as TNF- $\alpha$  only increase during relapse [31], it has been revealed that circulating leptin increases prior to the relapsing phase in MS followed by a marked reduction during the relapse. These findings suggest that variations in leptin anticipate cytokine pattern changes [16]. For these reasons, it is commonly agreed that leptin is a crucial factor in the pathogenesis of some immune-mediated disorders and inflammatory responses [16, 17].

It has also been suggested that the release of cytokines during exercise may contribute to the maintenance of immune homeostasis [31]. On the other hand, exercise is concomitant with a mild physical stressor and exhibits an array of modular effects on the immune system [23]. Consequently, cytokine responses to exercise could contribute to neuroprotection [22, 31]. In addition, investigating the immunological response to exercise in people with MS may provide important information regarding the impact of acute exercise on how people with MS respond to physical stress in general.

Le Page et al. (1994) reported that, during the inflammatory phase of EAE, exercise did not exacerbate the disease course [32]. Limited research has addressed the acute effect of exercise on cytokine responses in MS subjects. Hessen et al. [22] examined cytokine responses to standardized physical stress (60% VO<sub>2</sub> max for 30 min) in MS, showing that such stress did not induce a pro-inflammatory immune deviation [22]. In another study, Kjolhede et al. [30] reported that cytokine responses to resistance exercise were without significant change in IL-10 and TNF- $\alpha$ . However, other cytokines have been found to be similar in trained and untrained MS subjects [31]. In all of these studies MS patients were recruited in the remitting phase of the disease. In the present study, however, in order to fully comprehend cytokine responses, we also included subjects in the relapsing phase of the disease. Finally, we observed almost identical response of cytokines to acute bouts of exercise in MS remitting and control subjects. Relapsing subjects showed different cytokine responses to exercise, specifically for leptin and TNF- $\alpha$ . For leptin, we clearly showed a marked tendency for reduction during relapses. However, leptin concentrations were still higher in the relapsing group compared to other subjects who performed the exercise.

The decreased response level of serum leptin to physical stress, such as exercise, can result in immunosuppression as lower levels of leptin are related to Th1 cell responses that can decrease and improve Th2 cell function [3, 17]. In fact, experimental evidence indicates that leptin concentrations are associated with

inflammatory processes and directly stimulate the production of inflammatory cytokines such as TNF- $\alpha$  and IL-6 [14, 17]. Therefore, the reduced leptin levels in response to exercise in the relapsing group can be considered as an anti-inflammatory effect of exercise. However, more extensive investigations are required to ascertain the likelihood of this possibility. The obtained results indicate that TNF- $\alpha$  levels were reduced immediately after exercise in the relapsing group. However, 1-hour after exercise TNF- $\alpha$  tended to increase significantly in all groups. For IL-10, a significant increase was observed immediately after exercise in control subjects only. However, 1-hour after exercise the relapsing group experienced an increase compared to baseline. Finally, a significant increase was demonstrated for IL-6 in all exercise groups.

Given the complex function of both TNF- $\alpha$  and IL-6 on inflammatory processes, the present findings are difficult to interpret comprehensively. TNF- $\alpha$  possesses mutual functions, that are related to different receptors called p55 and p75 [33]. The majority of inflammatory responses of TNF- $\alpha$  are attributed to p55 while p75 mediates cell growth and proliferation [20, 33]. Therefore, recent studies have speculated that elevation of TNF- $\alpha$  levels may have either detrimental or beneficial effects on MS subjects [34]. On the other hand, the immunoregulatory properties of IL-10 inhibit the synthesis of many Th1-related cytokines (TNF- $\alpha$ , IL-1, IL-6). Therefore, it seems that the presence of IL-10 appears to be beneficial in MS subjects. Also, the lower levels of IL-10 in the relapsing group in response to exercise might be a result of an increase in pro-inflammatory cytokines at baseline which could be harmful in MS patients. The results of the present study indicate no changes in IL-10 in the relapsing group, which can be explained by lower levels of  $VO_{2max}$  at baseline, as the IL-10 response to exercise depends to some extent on fitness level [35]. Furthermore, at baseline, a modest positive correlation was observed between IL-10 levels and  $VO_{2max}$  in the relapsing group and healthy subjects. Interpretation of the cytokine responses suggests a regulative effect of exercise on cytokine balance without inducing inflammatory patterns. Finally,  $VO_{2max}$  and PA differences between groups may, in part, explain the observed differences between cytokine responses which are not related to the applied protocols or disease course.

In the present study cytokine measurements were only performed in blood samples. Further biochemical analysis in other areas, such as the cerebrospinal fluid, might produce different results. Furthermore, we chose to examine the impact of aerobic interval exercise. This choice was motivated by the fact that interval exercise may induce lower increases in body temperature due to the inclusion of rest and active periods. However, various characteristics of other types of exercise might influence the differences in cytokine responses.

These findings reveal that responses of MS subjects to exercise can be similar to that of healthy controls. It should be noted that the balance of the Th1/ Th2 cytokines is less impaired in the benign phase of MS [18]. Furthermore, it seems possible that subjects with higher disability (EDSS>5) might respond differently to the exercise task.

Several studies have suggested that cytokine responses can occur hours after an exercise bout [22]. Therefore, sampling time represents another limitation of our study and gives rise to the need for further research in order to support these results regarding cytokine responses to exercise. Finally, although pharmacological treatment, such as corticosteroids, can influence cytokine patterns, Kraszula et al. [17] reported higher levels of leptin after 2

weeks of corticosteroid treatment in relapsing subjects [17]. In order to counter this, the present study included a control relapsing group to minimize and eliminate the effect of any pharmacological treatment.

## 6. Conclusions

We showed that relapsing phase of MS is associated with a cytokine imbalance. Importantly, acute exercise does not seem to influence the inflammatory response of the cytokines measured. Nevertheless, the reduction of leptin and TNF- $\alpha$  levels in response to exercise in relapsing groups can be considered as an anti-inflammatory effect of exercise training. Therefore, exercise may temporarily improve the cytokine balance in MS subjects, particularly those in the relapse phase. Given that one of the targets of treatment and medication in relapse is the reduction of inflammation, acute exercise can also be considered as an effective strategy. Although our preliminary study showed that exercise, from a cytokine and adipokine perspective, could represent valid therapy in the relapsing phase of MS, further studies need to fully determine the potential of exercise as a complementary therapy during relapse. Finally, it can be argued that exercise is safe in relation to cytokine and adipokine concentrations following relapse in RRMS.

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## References

- [1] L. Xie, X.-K. Li, S. Takahara, Curcumin has bright prospects for the treatment of multiple sclerosis, *International immunopharmacology*, 11 (2011) 323-330.
- [2] I. Agirrezabal, R. Palacios, B. Moreno, J. Sepulcre, A. Abernathy, A. Saiz, S. Llufrui, M. Comabella, X. Montalban, A. Martinez, Increased expression of dedicator-cytokines-10, caspase-2 and Synaptotagmin-like 2 is associated with clinical disease activity in multiple sclerosis, *Multiple Sclerosis and Demyelinating Disorders*, 1 (2016) 7.
- [3] M. Farrokhi, M. Dabirzadeh, E. Fadaee, A.A. Beni, Z. Saadatpour, A. Rezaei, Z. Heidari, Polymorphism in leptin and leptin receptor genes may modify leptin levels and represent risk factors for multiple sclerosis, *Immunological investigations*, 45 (2016) 328-335.
- [4] F. Peiravian, H. Rajaian, A. Samiei, N. Gholijani, B. Ghahesi-Fard, P. Mokaram, A. Rahimi-Jaberi, E.K. Sarvestani, Altered serum cytokine profiles in relapse phase of relapsing-remitting multiple sclerosis, *Iranian Journal of Immunology*, 13 (2016) 186.
- [5] P. Zimmer, W. Bloch, A. Schenk, M. Oberste, S. Riedel, J. Kool, D. Langdon, U. Dalgas, J. Kesselring, J. Bansi, High-intensity interval exercise improves cognitive performance and reduces matrix metalloproteinases-2 serum levels in persons with multiple sclerosis: A randomized controlled trial, *Multiple Sclerosis Journal*, (2017) 1352458517728342.

- [6] I.K. Sand, Classification, diagnosis, and differential diagnosis of multiple sclerosis, *Current opinion in neurology*, 28 (2015) 193-205.
- [7] I. Tramacere, C. Del Giovane, G. Salanti, R. D'Amico, G. Filippini, Immunomodulators and immunosuppressants for relapsing-remitting multiple sclerosis: a network meta-analysis, status and date: New, published in, (2015).
- [8] E. Kotelnikova, M. Bernardo-Faura, G. Silberberg, N.A. Kiani, D. Messinis, I.N. Melas, L. Artigas, E. Schwartz, I. Mazo, M. Masso, Signaling networks in MS: A systems-based approach to developing new pharmacological therapies, *Multiple Sclerosis Journal*, 21 (2015) 138-146.
- [9] A. Waschbisch, I. Wenny, A. Tallner, S. Schwab, K. Pfeifer, M. Mäurer, Physical activity in multiple sclerosis: a comparative study of vitamin D, brain-derived neurotrophic factor and regulatory T cell populations, *European neurology*, 68 (2012) 122-128.
- [10] Z. Golzari, F. Shabkhiz, S. Soudi, M.R. Kordi, S.M. Hashemi, Combined exercise training reduces IFN- $\gamma$  and IL-17 levels in the plasma and the supernatant of peripheral blood mononuclear cells in women with multiple sclerosis, *International immunopharmacology*, 10 (2010) 1415-1419.
- [11] S.R. Mirandola, D.E. Hallal, A.S. Farias, E.C. Oliveira, C.O. Brandão, H.H. Ruocco, B.P. Damasceno, L.M. Santos, Interferon-beta modifies the peripheral blood cell cytokine secretion in patients with multiple sclerosis, *International immunopharmacology*, 9 (2009) 824-830.
- [12] A.P. Kallaur, S.R. Oliveira, E.R. Delicato de Almeida, H. Kaminami Morimoto, J. Lopes, W.L. de Carvalho Jennings Pereira, R. Marques Andrade, L. Muliterno Pelegrino, S. Donizete Borelli, D.R. Kaimen Maciel, Cytokine profile in relapsing-remitting multiple sclerosis patients and the association between progression and activity of the disease, *Molecular medicine reports*, 7 (2013) 1010-1020.
- [13] M. Duddy, M. Armstrong, A. Crockard, S. Hawkins, Changes in plasma cytokines induced by interferon- $\beta$ 1a treatment in patients with multiple sclerosis, *Journal of neuroimmunology*, 101 (1999) 98-109.
- [14] C. Procaccini, C. La Rocca, F. Carbone, V. De Rosa, M. Galgani, G. Matarese, Leptin as immune mediator: Interaction between neuroendocrine and immune system, *Developmental & Comparative Immunology*, 66 (2017) 120-129.
- [15] C. Procaccini, V. Pucino, C.S. Mantzoros, G. Matarese, Leptin in autoimmune diseases, *Metabolism*, 64 (2015) 92-104.
- [16] M. Rotondi, A.P. Batocchi, F. Coperchini, M. Caggiula, F. Zerbini, R. Sideri, P. Loporati, V. Nociti, G. Frisullo, M. Mirabella, Severe disability in patients with relapsing-remitting multiple sclerosis is associated with profound changes in the regulation of leptin secretion, *Neuroimmunomodulation*, 20 (2013) 341-347.
- [17] Ł. Kraszula, A. Jasińska, M.-O. Eusebio, P. Kuna, A. Głabiński, M. Pietruczuk, Evaluation of the relationship between leptin, resistin, adiponectin and natural regulatory T cells in relapsing-remitting multiple sclerosis, *Neurologia i neurochirurgia polska*, 46 (2012) 22-28.
- [18] R.W. Motl, B.M. Sandroff, G. Kwakkel, U. Dalgas, A. Feinstein, C. Heesen, P. Feys, A.J. Thompson, Exercise in patients with multiple sclerosis, *The Lancet Neurology*, 16 (2017) 848-856.
- [19] M.P. Herring, K.M. Fleming, S.P. Hayes, R.W. Motl, S.B. Coote, Moderators of Exercise Effects on Depressive Symptoms in Multiple Sclerosis: A Meta-regression, *American Journal of Preventive Medicine*, (2017).
- [20] M. Mokhtarzade, R. Ranjbar, N. Majdinasab, D. Patel, M.M. Shamsi, Effect of aerobic interval training on serum IL-10, TNF $\alpha$ , and adipokines levels in women with multiple sclerosis: possible relations with fatigue and quality of life, *Endocrine*, (2017) 1-10.
- [21] J. Peake, P. Della Gatta, K. Suzuki, D. Nieman, Cytokine expression and secretion by skeletal muscle cells: regulatory mechanisms and exercise effects, *Exercise immunology review*, 21 (2015) 8-25.
- [22] C. Heesen, S.M. Gold, S. Hartmann, M. Mladek, R. Reer, K.-M. Braumann, K. Wiedemann, K.-H. Schulz, Endocrine and cytokine responses to standardized physical stress in multiple sclerosis, *Brain, behavior, and immunity*, 17 (2003) 473-481.
- [23] K.-H. Schulz, S.M. Gold, J. Witte, K. Bartsch, U.E. Lang, R. Hellweg, R. Reer, K.-M. Braumann, C. Heesen, Impact of aerobic training on immune-endocrine parameters, neurotrophic factors, quality of life and coordinative function in multiple sclerosis, *Journal of the neurological sciences*, 225 (2004) 11-18.
- [24] E. Portaccio, A. Ghezzi, B. Hakiki, A. Sturchio, V. Martinelli, L. Moiola, F. Patti, G.L. Mancardi, C. Solaro, M.R. Tola, Postpartum relapses increase the risk of disability progression in multiple sclerosis: the role of disease modifying drugs, *J Neurol Neurosurg Psychiatry*, (2014) jnnp-2013-306054.
- [25] C.H. Polman, S.C. Reingold, B. Banwell, M. Clanet, J.A. Cohen, M. Filippi, K. Fujihara, E. Havrdova, M. Hutchinson, L. Kappos, Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria, *Annals of neurology*, 69 (2011) 292-302.

- [26] T.W. Storer, J.A. Davis, V.J. Caiozzo, Accurate prediction of VO<sub>2</sub>max in cycle ergometry, *Medicine and Science in Sports and Exercise*, 22 (1990) 704-712.
- [27] J.A. Baecke, J. Burema, J. Frijters, A short questionnaire for the measurement of habitual physical activity in epidemiological studies, *The American journal of clinical nutrition*, 36 (1982) 936-942.
- [28] A.S. Jackson, M.L. Pollock, Generalized equations for predicting body density of men, *British journal of nutrition*, 40 (1978) 497-504.
- [29] W.E. Siri, Body composition from fluid spaces and density: analysis of methods, *Techniques for measuring body composition*, 61 (1961) 223-244.
- [30] V. Sanna, A. Di Giacomo, A. La Cava, R.I. Lechler, S. Fontana, S. Zappacosta, G. Matarese, Leptin surge precedes onset of autoimmune encephalomyelitis and correlates with development of pathogenic T cell responses, *Journal of Clinical Investigation*, 111 (2003) 241.
- [31] T. Kjølhede, U. Dalgas, A.B. Gade, M. Bjerre, E. Stenager, T. Petersen, K. Vissing, Acute and chronic cytokine responses to resistance exercise and training in people with multiple sclerosis, *Scandinavian journal of medicine & science in sports*, 26 (2016) 824-834.
- [32] C. Le Page, A. Ferry, M. Rieu, Effect of muscular exercise on chronic relapsing experimental autoimmune encephalomyelitis, *Journal of Applied Physiology*, 77 (1994) 2341-2347.
- [33] J.J. Peschon, D.S. Torrance, K.L. Stocking, M.B. Glaccum, C. Otten, C.R. Willis, K. Charrier, P.J. Morrissey, C.B. Ware, K.M. Mohler, TNF receptor-deficient mice reveal divergent roles for p55 and p75 in several models of inflammation, *The Journal of Immunology*, 160 (1998) 943-952.
- [34] V. Castellano, D.I. Patel, L.J. White, Cytokine responses to acute and chronic exercise in multiple sclerosis, *Journal of Applied Physiology*, 104 (2008) 1697-1702.
- [35] D. Markovitch, R.M. Tyrrell, D. Thompson, Acute moderate-intensity exercise in middle-aged men has neither an anti-nor proinflammatory effect, *Journal of applied physiology*, 105 (2008) 260-265.

**Table 1**

Baseline characteristics of the relapsing, remitting MS and healthy control group

	<b>Age</b> (years)	<b>Weight</b> (kg)	<b>BMI</b> (kg/cm <sup>2</sup> )	<b>BFP</b> (%)	<b>VO<sub>2max</sub></b> (ml/kg.min)	<b>PA</b> (score)
<b>Relapsing MS</b>	28.23±3.65	68.51±4.28	23.16±1.20	33.24±5.47	19.19±6.37	2.21±0.87
<b>Remitting MS</b>	29.16±4.81	64.20±5.11	22.75±1.07	31.65±3.87	23.21±2.87 <sup>#</sup>	2.19±0.36
<b>Healthy control</b>	28.10±6.11	62.38±3.91	22.37±1.67	33.79±6.37	29.37±3.17*	3.07±0.41*

Data are presented as Mean±SD and represent baseline differences of BMI, BFP, VO<sub>2max</sub> and physical activity in relapsing (n=20), remitting (n=15) MS and healthy controls (n=15). \* p<0.05 between control group and MS groups.

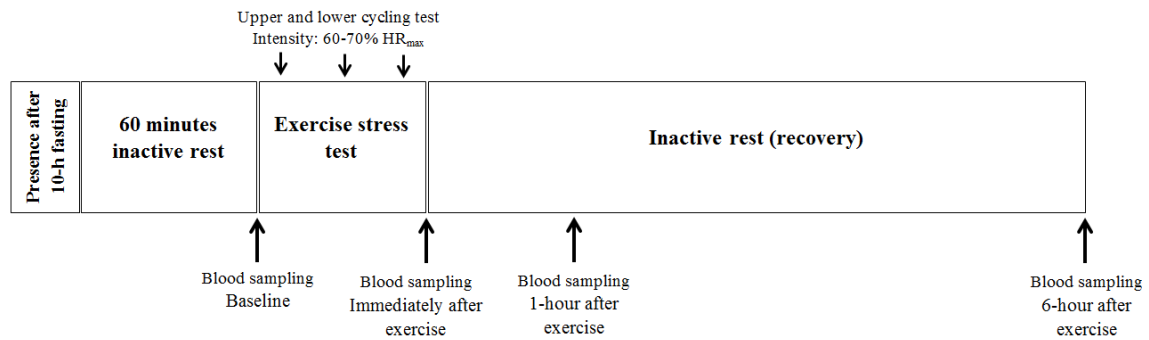
<sup>#</sup> p<0.05 between MS remitting and relapsing group; BMI: body mass index; BFP; body fat percent; VO<sub>2max</sub>; maximum rate of oxygen consumption; PA: physical activity; kg/cm<sup>2</sup>: kilogram/centimeter; ml/kg.min: milliliter/kilogram.minute.

**Table 2**

Heart rate and lipid profile response to exercise in relapse (n=15), control relapse (n=5), remitting (n=15) and control group (n=15).

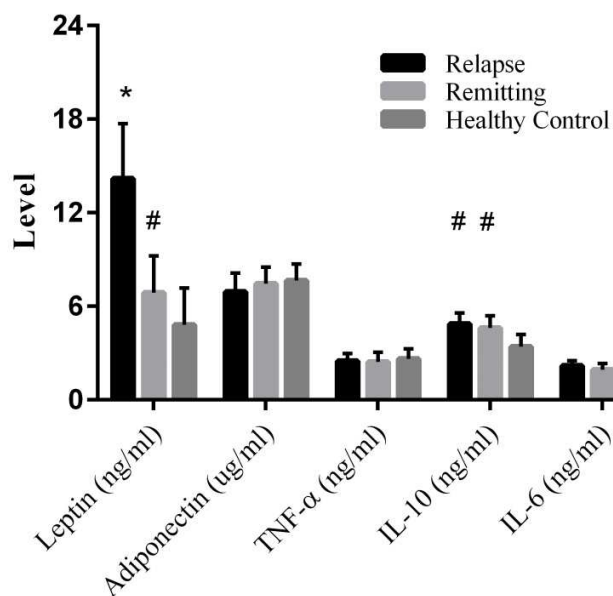
		Baseline	Immediately after exercise	1-hour after exercise	6-hour after exercise
Heart rate (bpm)	Relapsing MS	75.16±4.61	129.16±7.14	86.12±4.61	74.15±6.05
	Control relapsing MS	77.11±6.12	75.18±9.07	75.94±6.05	78.12±6.10
	Remitting MS	73.16±4.81	127.20±4.18	81.11±7.34	76.61±4.39
	Healthy control	68.67±6.57	130.19±6.11	70.28±2.77	69.57±2.33
TG (mg/dL)	Relapsing MS	132.27 ± 11.11# <sup>a</sup>	114.16 ± 12.16*#	118.24 ± 16.21	128.11 ± 18.24#
	Control relapsing MS	129.45 ± 18.67# <sup>a</sup>	126.72 ± 20.16# <sup>a</sup>	120.14 ± 19.60	121.12 ± 7.29
	Remitting MS	116.19 ± 15.74	110.12 ± 13.94	112.87 ± 20.12	117.25 ± 14.11
	Healthy control	105.16 ± 9.94	99.14 ± 14.16	104.64 ± 10.64	103.27 ± 15.61
HDL (mg/dL)	Relapsing MS	49.19±4.88	51.12±8.07	51.19±9.11	48.16±4.12
	Control relapsing MS	47.28±6.16	48.11±6.74	44.22±7.65	47.19±6.54
	Remitting MS	54.20±3.16	53.78±6.91	52.17±4.09	53.26±6.61
	Healthy control	51.44±4.74	57.26±5.17	54.19±3.45	52.16±6.90
LDL (mg/dL)	Relapsing MS	114.64 ± 7.87	113.94 ± 10.12	114.42 ± 14.51	110.09 ± 6.67
	Control relapsing MS	114.54 ± 12.93	112.16 ± 9.61	115.94 ± 9.62	112.66 ± 11.25
	Remitting MS	108.57 ± 12.06	109.71 ± 15.12	106.16 ± 10.09	105.28 ± 16.21
	Healthy control	108.84 ± 6.64	101.12 ± 9.78	107.81 ± 11.65	110.29 ± 14.36

Data are given as Mean±SD. TG indicated significant time\*group interacts (P <0.05). TG and Heart rate showed a time effect (P <0.05). \* refers to the significant differences in comparison to baseline (p<0.05). # refers to the significant differences in comparison to the control group (p<0.05). <sup>a</sup> refers to the significant differences in comparison to the Remitting group (p<0.05). TG: Triglyceride; HDL: High-density lipoproteins; LDL: Low-density lipoproteins.

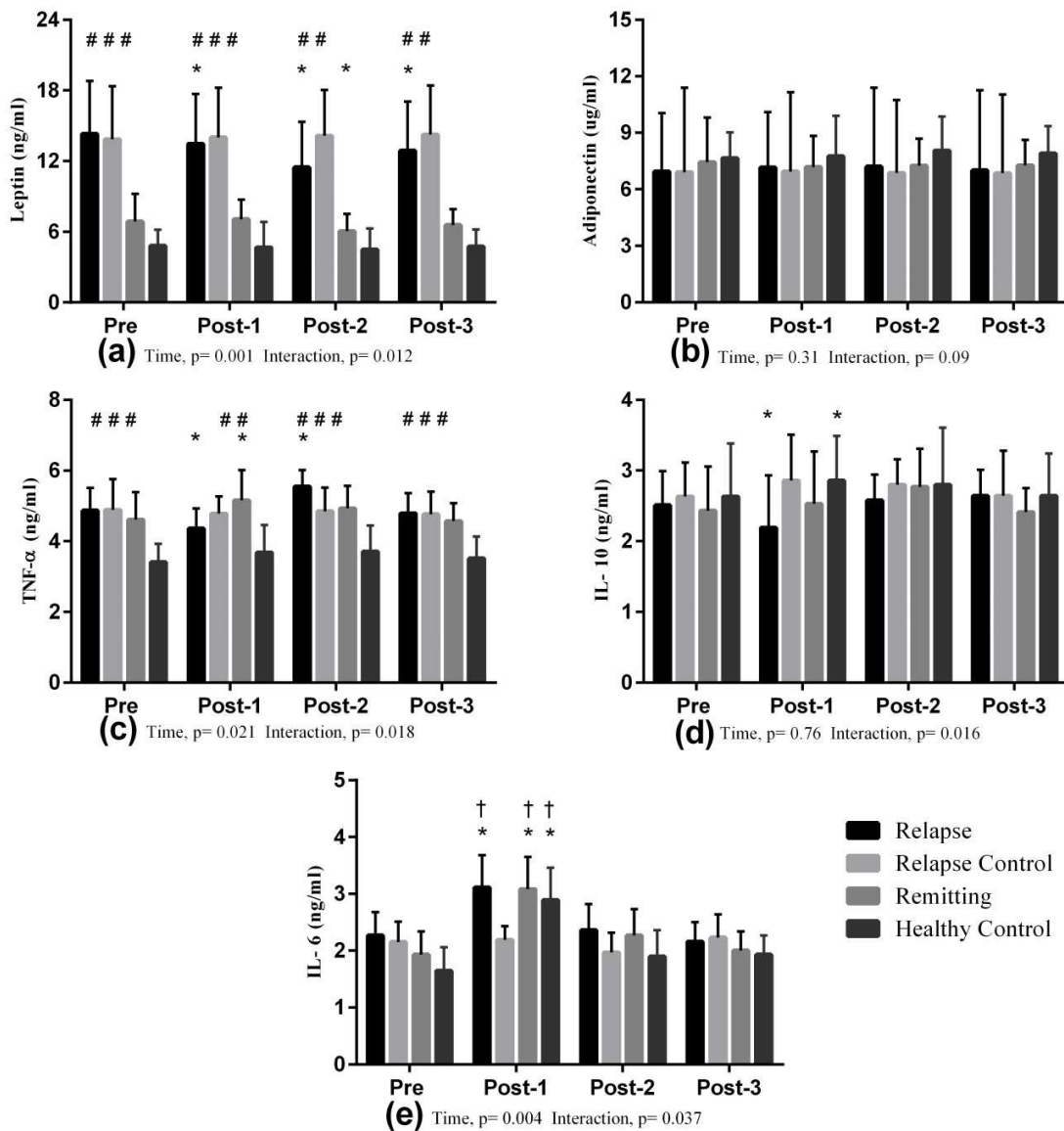


**Fig. 1.** Timeline of exercise stress session; the exercise consisted of four sets of 5 minutes intervals of upper body cycling and four sets of lower body cycling interspersed with 2 minutes of passive rest between each interval. Subjects were asked to start cycling after sitting 5-min on the ergometer. Lower and upper limb cycling was considered as exercise stress which was set using percentage of HR<sub>max</sub>. The intensity of each bout of interval exercise was adjusted to achieve an intensity of 60-70% HR<sub>max</sub>.





**Fig. 2.** Baseline differences in cytokine parameters between in relapse (n=20), remitting (n=15) and control group (n=15). Data are given as Mean±SD. \* refers to the significant differences in comparison to the other groups; # refers to the significant differences in comparison to the control group; IL-10: Interleukin 10; TNF-α: tumor necrosis factor alpha; IL-6: Interleukin 6.



**Fig. 3.** Leptin (a), Adiponectin (b), TNF- $\alpha$  (c), IL-10 (d) and IL-6 (e) response to exercise in relapse (n=15), control relapse (n=5), remitting (n=15) and control group (n=15). Data are given as Mean $\pm$ SD. \* refers to the significant differences in comparison to baseline. # refers to the significant differences in comparison to the control group. † refers to the significant differences in comparison to the control relapse group. IL-10: Interleukin 10; TNF- $\alpha$ : tumor necrosis factor alpha; IL-6: Interleukin 6. Pre: baseline; Post-1: immediately after exercise; Post-2: 1-hour after exercise; Post-3: 6-hour after exercise.

Exercise does not influence the inflammatory response of the cytokine profile.

Exercise temporarily improves cytokine and adipokine balance in the relapsing phase of multiple sclerosis.

Exercise successfully influences leptin in relapses of multiple sclerosis.

Exercise seems safe, tolerable and feasible following relapse in multiple sclerosis patients.

ACCEPTED MANUSCRIPT