RESEARCH PAPER

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Regular aerobic exercise increased VEGF levels in both soleus and gastrocnemius muscles correlated with hippocampal learning and VEGF levels

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Physical exercise improves learning and memory abilities by increasing the levels of several growth factors in the hippocampus. One growth factor, vascular endothelial growth factor (VEGF), is primarily produced in the muscles and not only increases in the periphery during exercise but can also cross the blood-brain barrier. The aim of this study is to investigate the effects of regular aerobic chronic exercise on different types of muscle fibers and the relationships between learning/memory and muscle induced-VEGF. Following a one-week adaptation period, male rats underwent treadmill training at a speed of 8 m/min for 30 min daily, 3 days a week for 6 weeks. Memory functions were evaluated using the Morris water maze. VEGF, superoxide dismutase (SOD), glutathione peroxidase (GPx), and malondialdehyde (MDA) levels were measured in type 1 and type 2 muscle fibers and VEGF levels were also measured in the hippocampus. Exercise positively affected both learning and memory and also increased VEGF levels in both muscle fiber types. Muscle VEGF levels positively correlate with hippocampal learning and hippocampal VEGF levels. Exercise reduced both SOD and MDA levels in type 1 and type 2 muscle fibers, whereas GPx levels decreased only in type 2 muscle fibers. Our findings suggest that regular aerobic exercise elevates VEGF levels and diminishes oxidative stress in both fiber types. Exercise-induced VEGF levels in both type 1 and 2 muscle fibers appear to be associated with the positive effect of exercise on learning and memory function and is accompanied by an increase in VEGF levels in the hippocampus. Further research is needed to elucidate the exact mechanism by which fiber type-specific VEGF mediates hippocampal neurogenesis and angiogenesis.

Key words: exercise, muscle fiber type, VEGF, antioxidant, spatial learning and memory

INTRODUCTION

Many aspects of the health benefits of exercise are known, such as those in endocrine and cardiovascular systems, as well as metabolic and developmental functions (Hughes et al., 1993; Ostergard et al., 2006; Labonte-Lemoyne et al., 2017; Lin and Lee, 2018). In recent years, scientific research has focused on the effects of exercise on the neurocognitive process. Exercise has been shown to improve learning and memory function by increasing neurogenesis and angiogenesis in the hippocampus, which is the primary center of learning and memory (Radák et al., 2001; Fabel et al., 2003; Winter et al., 2007; Cassilhas et al., 2012; Khabour et al., 2013; Ballard, 2017; Jeong et al., 2018). In previous studies, we have demonstrated the memory en-

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hancing effects of exercise accompanied by increased neuronal density in the hippocampus (Uysal et al., 2005, 2017; Cetinkaya et al., 2013).

Skeletal muscle is an exercise-responsive tissue that produces hormones and signaling factors in order to maintain physiological adaptations. Skeletal muscle fibers, which are classified by myosin heavy-chain isoform expression, are basically divided into two types: type 1 (oxidative) and type 2 (glycolytic) fibers. Type 1 fibers have a slow contraction speed and predominantly use oxidative metabolism for energy production, while type 2 fibers are fast-twitch and use glycolytic metabolism (Qaisar et al., 2016). In addition to metabolic differences, there are also functional capability differences among the fiber types. For instance, oxidative fibers have greater capillary densities than glycolytic fibers (Cherwek et al., 2000). Regular aerobic exercise leads to many adaptive changes in skeletal muscles to increase utilization of oxygen and energy substrates, such as increasing vascularization to achieve better perfusion of the muscle, fiber type transformation and mitochondrial biogenesis (Yan et al., 2011).

Vascular endothelial growth factor (VEGF) is a potent angiogenic factor which is predominantly produced by myocytes and diffuses to the peripheral circulatory system (Hoier et al., 2013). Studies have demonstrated that exercise-induced VEGF is effective on neurogenesis and angiogenesis in the hippocampus (Rich et al., 2017). The suppression of exercise-induced hippocampal neurogenesis by the administration of anti-VEGF antibodies to the peripheral circulatory system is evidence that muscle-derived VEGF acts as a somatic regulator of hippocampal neurogenesis (Fabel et al., 2003). Furthermore, VEGF can also cross the blood-brain barrier (Fournier and Duman, 2012; Ballard, 2017). Additionally, muscle fiber type is a determining factor for VEGF production in exercising muscles (Birot et al., 2003). It is unknown whether there is a relationship between muscle fiber type-specific VEGF levels and learning/memory. The aim of this study is to investigate the relationship between hippocampus-dependent memory function and fiber type-specific VEGF levels and to determine if antioxidant status depends on muscle fiber type in exercised rats.

METHODS

Animals

Adult male Wistar albino rats (Dokuz Eylul University, Experimental Animal Laboratory, Izmir, Turkey) were housed in individual cages with free access to water and food. They were kept on a 12h-light/12h-dark cycle at constant room temperature (22±1°C), humidity (60%).

All experimental procedures were performed following the principles of animal care in the Guidelines for the ethical use of animals in applied etiology studies and were approved by the Dokuz Eylul University School of Medicine Animal Care Committee.

Experimental design

The rats were divided into two groups: an exercise group (n=7) and a control group (n=7). As an adaptation period, the exercise group underwent treadmill training at 5m/min on a 0° slope for 10 min/day, 5 days a week. Following treadmill training, the rats exercised on the treadmill at a speed of 8 m/min for 30 min daily, 3 days (Monday, Wednesday, Friday) a week for 6 weeks. This exercise protocol was previously identified as "regular mild treadmill exercise" (Kim et al., 2003; Uysal et al., 2005, 2011, 2015; Aksu et al., 2012). The control group was taken to the experiment facility and subjected to identical handling.

Two days after completion of the exercise procedure, each rat completed learning and memory tests. At the end of the memory tests, the rats were sacrificed under CO2 anesthesia for measurements. Brain tissues were removed and hippocampal tissues were extracted. The soleus and gastrocnemius muscles were taken for the identification of different muscle fiber types (type 1 and type 2, respectively). Tissue samples were stored at -80°C until homogenization.

Learning and memory task

Two days after the end of the exercise phase, all rats were tested using the Morris water maze (MWM) (Morris, 1984). The MWM is a plexiglas pool with a diameter of 140 cm and a height of 75 cm and is filled with water up to 50 cm where a hidden platform was placed one cm below the surface. Each rat was tested in the MWM for four consecutive days and on day five, the hidden platform was removed. The experimental procedure was similar to our previous studies (Uysal et al., 2005). The latency period to find the platform and the time spent in the correct quadrant were analyzed using the Noldus Ethovision video tracking system.

Biochemical measurements

Tissue ELISA measurements for VEGF, superoxide dismutase (SOD), glutathione peroxidase (GPx) and malond-

ialdehyde (MDA) were performed according to the kit protocol. Rat VEGF levels were measured using commercially available ELISA kits (VEGF, Boster Immunoleader, Wuhan, China with assay sensitivity <1 pg/ml and range 15.6–1000 pg/ml). Additionally, rat specific SOD kit (Bioassay Technology Laboratory, China; assay sensitivity <0.022 ng/mL and detection range 0.05–20 ng/mL), rat specific GPx kit (Bioassay Technology Laboratory; assay sensitivity <0.24 ng/ml and detection range 0.5–200 ng/ml), rat specific MDA kit (Bioassay Technology Laboratory, China; assay sensitivity <0.01 nmol/mL and detection range 0.05–10 nmol/mL) were used. The results were expressed as per mg tissue protein. Tissue analyses were completed based on the protein content in milligrams, and protein analyses were performed according to the manufacturers guide for the BCA Protein Assay kit (PierceTMBCA Protein Assay Kit, USA).

Statistical analyses

All statistical analyses were performed using SPSS 15.0. The differences in learning days between experimental groups in the MWM test were analyzed using GLM-repeated measure *post hoc* Bonferroni. The group comparisons utilized independent samples t-test after performing normality and homogeneity tests. The paired samples t-test was performed to compare VEGF levels of type 1 and type 2 fibers in the experimental groups. Correlations between groups were calculated using Pearson correlation analysis. Data are presented as mean ± S.E.M.; *p* values below 0.05 were considered statistically significant.

RESULTS

Behavioral measurements

Exercise was observed to have a significant positive effect on learning and memory function. The escape latency period after four consecutive days was shorter in the exercise group compared to the control group ($p<0.05$) (Fig. 1A).

In the probe trial, the exercise group spent significantly more time in the target quadrant compared to the control group, but significantly less time in the opposite quadrant compared to the control group ($p<0.05$ for both) (Fig. 1B).

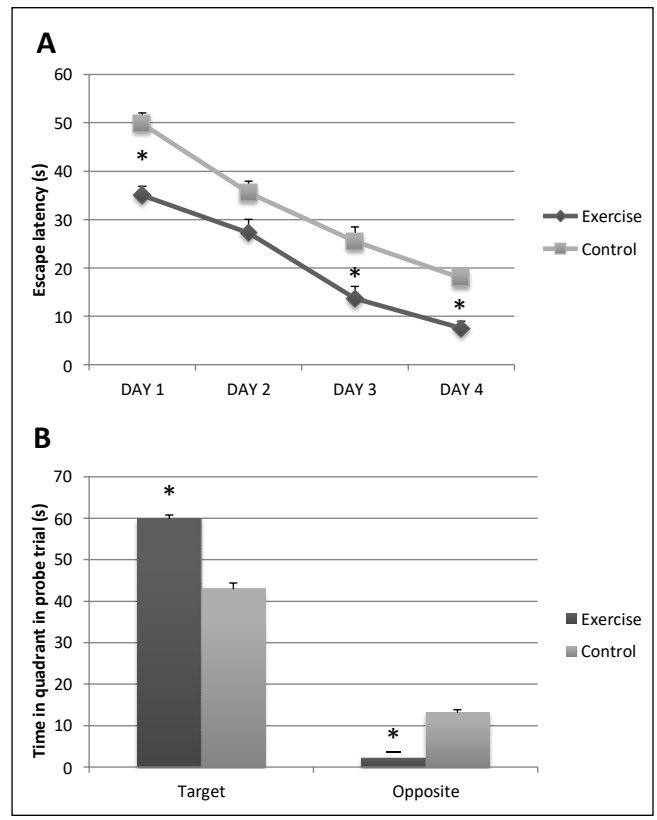

Fig. 1. Effect of regular treadmill exercise on reference memory task. (A) Latency period to find the platform during four consecutive training days. (B) Time spent in target and opposite quadrants in the probe trial. * $p<0.05$.

Biochemical measurements

VEGF levels of type 1 and type 2 muscle fibers were significantly increased in the exercise group compared to the control group (for both, $p<0.001$). In the exercise group, VEGF levels of type 2 muscle fibers were significantly higher than type 1 muscle fibers ($p<0.001$), whereas no significance was detected between VEGF levels of type 1 and type 2 muscle fibers in the control group (Fig. 2A). Exercised rats had significantly higher VEGF levels in the hippocampus compared to control rats ($p<0.001$) (Fig. 2A).

SOD levels of type 1 and type 2 muscle fibers decreased in the exercise group compared to the control group (for type 1 fibers $p<0.001$, for type 2 fibers $p<0.01$) (Fig. 2B).

GPx levels of type 2 muscle fibers significantly decreased in the exercise group compared to the control group ($p<0.001$). Exercise did not affect GPx levels of type-1 muscle fibers (Fig. 2C).

The exercise group had decreased MDA levels of type 1 and type 2 muscle fibers compared to the control group ($p<0.05$ and $p<0.01$, respectively) (Fig. 2D).

Correlation analyses

VEGF levels of both type 1 and type 2 fibers had a strong positive correlation with time spent in the tar-

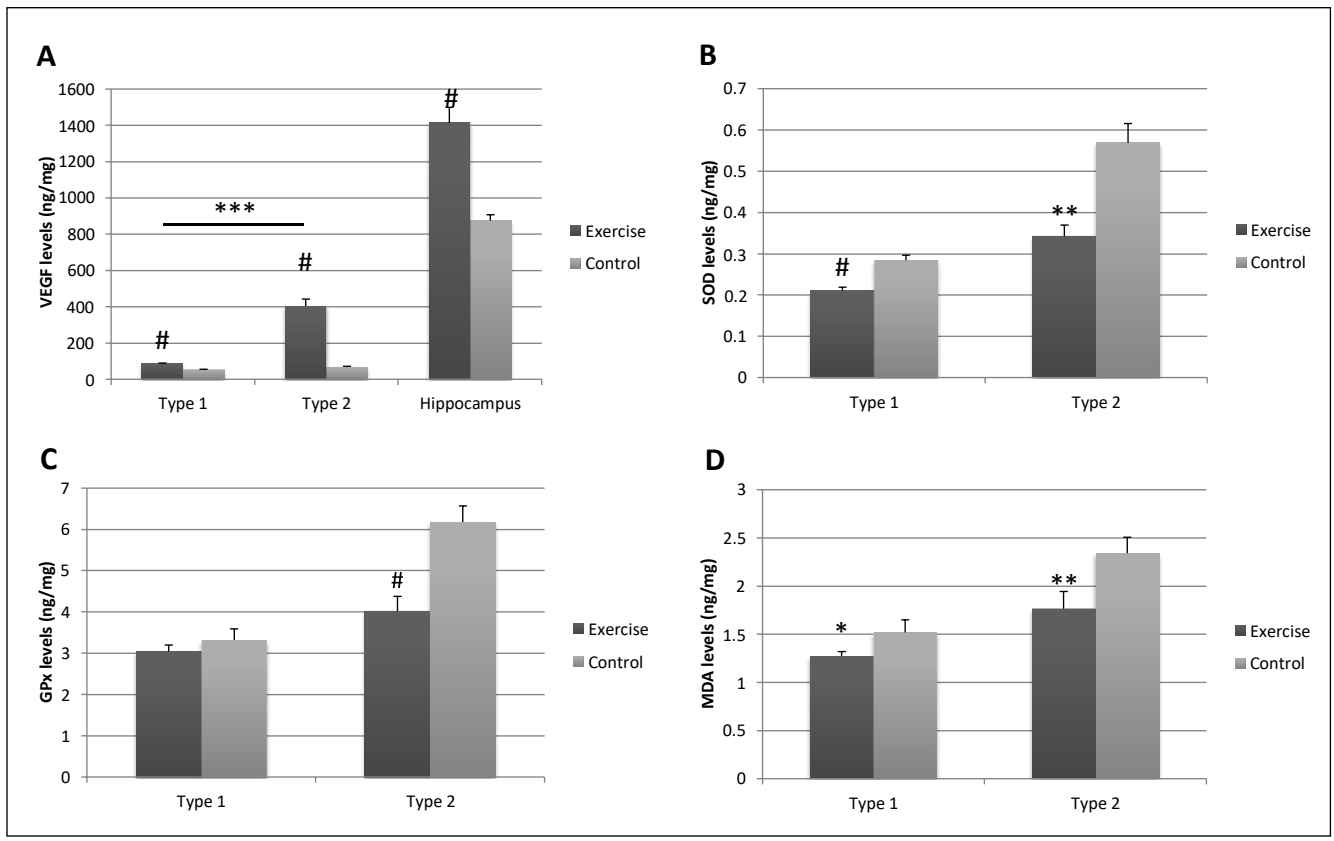

Fig. 2. Biochemical investigation results. (A) Effect of regular treadmill exercise on hippocampal and muscle fiber type-specific VEGF levels, (B) fiber type-specific SOD levels, (C) fiber type-specific GPx levels, (D) fiber type-specific MDA levels. VEGF: vascular endothelial growth factor. SOD: superoxide dismutase. GPx: glutathione peroxidase. MDA: malondialdehyde. * p<0.05, ** p<0.01, # p<0.001 (compared to control group). *** p<0.001 (comparison between the VEGF levels of type-1 and type-2 fibers in exercise group).

get quadrant of the MWM test (type 1; r=0.691, p<0.001, type 2; r=0.716, p<0.001, respectively) and strong negative correlation with time spent in the opposite quadrant (type 1; r=-0.584, p<0.01, type 2; r=-0.768, p<0.001, respectively).

Hippocampal VEGF levels positively correlated with time spent in the target quadrant (r=0.699, p<0.001) and negatively correlated with time spent in the opposite quadrant of the MWM test (r=-0.758, p<0.001).

Positive correlations were found between the hippocampal and muscle fiber specific-VEGF levels (type 1; r=0.760, p<0.001, type 2; r=0.477, p<0.05, respectively).

SOD levels 1 and type 2 fibers negatively correlated with time spent in the target quadrant of the MWM test (type 1; r=-0.698, p<0.001, type 2; r=-0.478, p<0.01, respectively), and positively correlated with time spent in the opposite quadrant (type 1; r= 0.645, p<0.001, type 2; r=0.612, p<0.001, respectively). GPx levels in type 2 fibers negatively correlated with time spent in the target quadrant of the MWM test (r=-0.603, p<0.001) and positively correlated with time spent in the opposite quadrant (r=0.619, p<0.001).

There was a strong positive correlation between VEGF levels of type 1 and type 2 muscle fibers (r=0.644 p<0.001).

The VEGF levels also negatively correlated with oxidative stress parameters in type 2 fibers (VEGF and SOD levels, r=-0.366, p<0.05; VEGF and GPx levels, r=-0.364, p<0.05).

In addition, there were strong positive correlations among SOD, GPx and MDA levels in type 1 and type 2 fibers (for type 1 muscle fiber: GPx and MDA, r=0.616, p<0.001; for type 2 muscle fiber: SOD and GPx, r=0.908 p<0.001; SOD and MDA, r=0.819, p<0.001; GPx and MDA, r=0.899, p<0.001, respectively)

DISCUSSION

In this study, we demonstrated that regular aerobic exercise enhanced the memory performance of rats, elevated the VEGF levels in both fiber types and in hippocampal tissue, and reduced antioxidant and lipid peroxidation levels in type 1 and type 2 muscle fibers.

Only the GPx levels of type 1 fibers did not decrease in response to exercise. Additionally, there were strong correlations between the performance of reference memory tasks and the elevation of VEGF levels, as well as the reduction of SOD levels in oxidative fibers. To our knowledge, no prior study has investigated the effects of exercise-induced fiber type-specific VEGF levels on learning and memory function.

Aerobic exercise-related cognitive improvement has been consistently reported by both human and animal studies using various exercise protocols (van Praag et al., 1999, 2005; Weuve et al., 2004; Uysal et al., 2015; Vanzella et al., 2017). Confirming previous reports, our study demonstrated that exercised rats performed significantly better at memory tasks, as their escape latency period was shorter and the time spent in the target quadrant was higher compared to control rats, whereas the time spent in the opposite quadrant in the probe trial decreased. Learning/memory function correlates with hippocampal neurogenesis and plasticity (Schmidt-Hieber et al., 2004; Winocur et al., 2006). Exercise facilitates synaptic plasticity in the hippocampus, especially in the dentate gyrus, by increasing the levels of synaptic proteins, glutamate receptors and several growth factors including BDNF, IGF-1 and VEGF (Cotman et al., 2007). These growth factors which mediate the memory enhancing effects of exercise are also called "neurotrophins" (Vanzella et al., 2017). VEGF is a neurotrophic factor which is primarily derived from the muscles, but has the capability of crossing the blood brain barrier and mediating both angiogenesis and neurogenesis in the hippocampus (Fabel et al., 2003; Ding et al., 2006).

Previously, we reported that regular exercise associated with increased hippocampal VEGF levels and the number of neurons in the hippocampus, both of which correlate with enhanced memory function (Uysal et al., 2015). Additionally, peripheral VEGF has been demonstrated to be necessary for neurogenesis in the hippocampus (Fabel et al., 2003). Increased VEGF levels could potentially be responsible for the beneficial effects of exercise on learning/memory function by inducting hippocampal neurogenesis and angiogenesis (Ballard, 2017). Previous studies suggest that peripheral VEGF is capable of crossing the blood-brain barrier (Fournier and Duman, 2012), and in our study, we observed that peripheral VEGF levels derived from type-1 and type-2 fibers strongly correlated with hippocampal VEGF levels and the performance of memory tasks. Our study further characterizes the well-known effects of exercise-induced VEGF levels on memory function.

There are a limited number of studies in the literature addressing the effects of exercise on muscle fiber type-specific VEGF levels and their results are inconsistent due to different exercise protocols. For example, exhausting exercise (Birot et al., 2003) and short periods of volunteer running exercises (Waters et al., 2004) only increased VEGF levels in type 2 fibers. However, resistance training decreased VEGF levels in type 1 myofibers (Holloway et al., 2018). In our study, regular aerobic exercise significantly elevated VEGF levels in both type 1 and type 2 fibers. Additionally, our results indicate that type-2 fibers are much more sensitive for VEGF elevation in response to exercise, as the VEGF level in type 2 fibers are significantly higher than type 1 fibers in the exercised group. In the literature, glycolytic muscles have higher maximal oxygen diffusion distances than oxidative muscles and this may render type-2 fibers more prone to local ischemia during aerobic exercise (Richardson et al., 1995). Hypoxic conditions are known to upregulate VEGF gene expression (Forsythe et al., 1996). Our results are consistent with a study reporting an increase in VEGF levels in glycolytic fibers in response to a single bout of exercise, which is attributed to the involvement of local PO2 changes in VEGF gene expression (Birot et al., 2003). However, we cannot omit the possibility of muscle adaptation during the 6-week training period in our study. Lack of exercise workload progression also contributes to adaptation and exercise adaptation attenuates VEGF upregulation in trained states (Richardson et al., 2000). Nevertheless, the literature lacks information concerning the effect of regular exercise on muscle specific VEGF levels and their implications related to adaptive periods of exercise. Our findings may be attributed to recruitment patterns or some unknown fiber type-specific mechanism. To our knowledge, this is the first study on the impact of regular aerobic exercise on fiber type-specific VEGF levels.

In this study, we did not use two different types of exercise, aerobic and anaerobic, to evaluate type 1 and type 2 muscle fibers in rats. However, slow treadmill exercise has been reported to activate both fiber types (Laughlin and Armstrong, 1983, 1985; Duysens et al., 1991). Since our goal was to determine the contribution of VEGF levels in muscle fiber types to cognitive function, we tried to minimize the effect of different variables by applying a single exercise protocol. When different exercise protocols are implemented, various external motivators (e.g., reward or punishment) which encourage the animals to exercise should be taken into consideration. When different exercise regimens are administered together, it is not possible to stabilize levels. In addition, each of these motivators may independently affect hippocampal neurogenesis as well as learning/memory function (Parihar et al.,

2011; Alvandi et al., 2017). Therefore, we found it more appropriate to evaluate both type 1 and type 2 muscle fibers in a single exercise model.

The literature contains different results regarding the effects of exercise on fiber type-specific antioxidant enzyme activities. Laughlin et al. (1990) reported an increase in GPx activity following exercise in both type 1 and type 2 muscles, although SOD activity remained stable. Criswell et al. (1993) found that exercise-induced elevation of SOD and GPx activities were limited to type 1 fibers. Powers et al. (1994) also reported that rigorous exercise induced muscle antioxidant enzyme activity in a fiber type-specific manner, as the exercise-induced increases in SOD and GPx activities were limited to oxidative fibers. In our study, exercise decreased SOD levels in both fiber types; however, a reduction of GPx levels were observed in only in type 2 fibers. The discrepancy in the results may be due to different exercise protocols (intensity, duration). Likewise, the percentages of muscle fiber types in these studies may be diverse due to the error probability caused by manual separation (Criswell et al., 1993). Consistent with our results, Farhat and Amerand (2017) found that moderate exercise training (running on a treadmill five times a week for 60 min/day for 6 weeks at a speed equivalent to 60–70% of their MAS) reduced SOD and GPx levels in the gastrocnemius muscles in male rats. This effect is thought to be an adaptation aimed to minimize exercise-induced reactive oxygen species production.

The literature suggests that skeletal muscle fibers are exposed to oxidative stress and elevated antioxidant levels by aerobic exercise. However, these studies mostly utilized heavy exercise protocols (Alessio et al., 1988; Mousavi et al., 2020). Chronic moderate aerobic exercise increases some antioxidant enzyme activities in type-2 fibers, whereas the activities of others did not change or decreased (Abruzzo et al., 2013). Additionally, our study determined that the exercise-induced increase in reactive oxygen species formation did not cause oxidative stress in either fiber type but mild intensity aerobic exercise lowered antioxidant enzyme levels in both fiber types. However, GPx levels only decreased in glycolytic fibers. Our results emphasize the importance of exercise intensity on oxidative stress parameters as well as the predominance of type-2 fibers on susceptibility for oxidative changes. We determined that type 2 fibers were more responsive to the oxidative benefits of regular mild aerobic exercise than type 1. Aerobic training induces a glycolytic-to-oxidative shift in type 2 muscle fiber metabolism and this may render type 2 fibers more sensitive to changes in oxidative stress parameters in response to aerobic exercise (Abruzzo et al., 2013). The influence of aerobic exercise on postural muscles such as the soleus is expected to occur in a limited manner. On the other hand, muscle GPx activity was reported to be affected by intensity and especially by the daily duration of exercise protocol (Powers et al. 1994, 1999). Powers et al. (1994) trained animals on a treadmill with daily durations of 30 min, 60 min and 90 min for 10 weeks and observed that 30 min of chronic daily aerobic exercise was not capable of inducing significant change in the GPx levels in oxidative fibers.

Treadmill exercise may contribute to the memory-enhancing effect through reduction of ROS levels in the hippocampus (Vanzella et al., 2017). The studies on the effect of regular aerobic exercise on memory function have focused on the antioxidant enzyme or ROS levels of the hippocampus (Song and Kim, 2019). To our knowledge, this is the first study demonstrating the correlation between the muscle fiber type-specific antioxidant levels and memory task performance. In our study, treadmill exercise reduced the SOD levels of both fiber types which negatively correlate with memory function. We also detected that the GPx level of type 2 fibers, which are negatively correlated with memory performance, decreased in response to regular aerobic exercise. These results are in accordance with the memory enhancing effect of exercise and provide evidence of the contribution of both fiber types to this beneficial impact.

Increased levels of antioxidant enzymes may indicate a cellular defensive response to the elevation of oxidative stress. A study reported that exogenous VEGF (*via* i.v. infusion) diminished the oxidative stress parameters and the antioxidant enzyme levels, which are elevated by ischemia/reperfusion injury (Kirisci et al., 2013). This result emphasizes the protective effect of VEGF against oxidative stimuli. In our study, we identified a negative correlation between the VEGF levels and antioxidant enzyme levels in type 2 fibers. Glycolytic fibers were reported to be more susceptible to the oxidative alterations in response to exercise (Richardson et al., 1995).

It is well known that MDA, a product of lipid peroxidation, is a valid marker for evaluating lipid peroxidation, which is triggered by oxidative stress (Nielsen et al., 1997). Kanter et al. (2017) demonstrated that aerobic exercise decreased MDA levels in the heart tissues of diabetic rats, suggesting an adverse effect of exercise on oxidative stress status. Strikingly, Alessio et al. (1988) evaluated the effect of different intensities of exercise on type 1 and type 2 fibers' MDA content and concluded that both of the exercise protocols induced MDA levels in both fiber types, though only high intensity exercise resulted in a further increase

in MDA levels. However, they only tested the outcomes of acute exercise. Similarly, Kocturk et al. (2008) found that strenuous exercise (running at a speed of 25 m/min and at a slope of 5° until exhaustion) caused an increase in MDA levels in both muscle fiber types, predominantly in the soleus muscle. On the other hand, supporting the antioxidant effect of exercise, Kim and Yi (2015) reported that both single (30 min/day, 5 days/week for 6 weeks) and intermittent bouts (three times for 10 min/day, 5 days/week for 6 weeks) of exercise training significantly decreased plasma MDA levels in elderly rats.

Similarly, Balci and Pepe (2012) determined that chronic aerobic exercise training affects lipid peroxidation in a gender-specific manner, as they found lower MDA levels in female rats in response to exercise. In our study, we observed that exercise lowered MDA levels in both fiber types, which correlate with antioxidant enzyme levels. If we compare our data with previous findings, many factors, such as exercise intensity, duration, gender, and muscle tissue, seem to contribute to the varying results seen in the literature. However, based on our findings, we conclude that chronic aerobic exercise not only reduces oxidative stress but also alleviates lipid peroxidation in oxidative and glycolytic fibers.

In our study, we did not implement a progressive workload during the exercise period. However, a lack of workload progression may lead to adaptation to exercise training and perceived exercise intensity may be lighter throughout the exercise period (Hoydal et al., 2007). It is known that chronic exercise causes some adaptive changes in muscle antioxidant capacity and in other muscle-related physiologic and biochemical factors, and these changes correlate with exercise intensity (Farenia et al., 2019). In low exercise intensity, even minor adaptive changes can occur (Kaczor et al., 2007).

Although we could not find evidence in the literature whether or not the lack of workload progression might have affected our results, depending on muscle fiber types, we think that fiber type transformation from glycolytic to oxidative fibers caused by chronic endurance exercise may be weakened due to stable workloads during exercise. In conditions involving a lack of workload progression, the glycolytic fiber content of the gastrocnemius muscle is expected to be higher at the end of the exercise period than under progressive workload conditions. Research has indicated that type 1 fibers have a greater antioxidant enzyme capacity than type 2 fibers (Qaisar et al., 2016). For our study, the higher type 2 fiber content of the gastrocnemius muscle might have exaggerated the statistical significance of the antioxidant levels between the exercise and control groups. In the soleus muscle, rich in type 1 fibers, the lack of workload progression will result in fewer type 1 fibers than expected at the end of a chronic exercise period. This may influence our results with an exaggerated decrease in oxidative markers and MDA levels in response to chronic exercise. Nevertheless, we can speculate that this excessive decrease may be compensated for by the decrease in the oxidative load of exercise which is also a determinant of fiber-type transformation correlated with the reduction in perceived exercise intensity. Thus, the change in oxidative status is expected to be lighter in the lack of workload progression than in the presence of workload progression.

We surmise that these two factors may partially balance one another. On the other hand, the lack of workload progression may diminish muscle hypoxia, which is a triggering factor for muscle VEGF elevation during exercise (Breen et al., 1996). Type 2 fibers have been found to be more susceptible to hypoxic conditions (de Theije et al., 2015) than type 1 fibers. Additionally, VEGF elevation in response to exercise reportedly occurs mostly in glycolytic fibers rather than in oxidative fibers, in which blood flow is greater (Lloyd et al., 2001, 2003); this elevation has also shown to be positively correlated with the elevation of exercise intensity, which triggers an improved hypoxic stimulus (Wahl et al., 2011). Hippocampal VEGF levels also have been shown to be affected by exercise-induced hypoxia (Tang et al., 2010). However, studies also show an inverted-U shape of hippocampal VEGF response due to changes in brain glucose uptake and stress level depending on exercise intensity (Lou et al., 2008). In our study, adaptation to exercise workload might weaken hypoxia, resulting in a smaller VEGF response, especially in type 2 fibers. However, we expect this effect to be negligible, as the mild exercise intensity used in this study did not provoke a noticeable hypoxic condition in the muscles (Richardson et al., 1995; Lou et al. 2008; Hwang et al., 2020).

CONCLUSIONS

Our findings suggest that regular aerobic exercise diminishes oxidative stress and elevates VEGF levels in both fiber types. VEGF levels produced by both types (type 1 and 2) of muscle fibers correspond with a positive effect of exercise on learning/memory function. Muscle-derived VEGF levels also correlate with hippocampal VEGF levels, probably due to the ability of VEGF to pass through the blood brain barrier. These data suggest that, similar to the impact of central VEGF levels, VEGF derived from both type 1 and type 2

muscle fibers may directly or indirectly contribute to the positive effects of exercise on learning and memory function. Further research is needed to elucidate the exact mechanism by which muscle fiber type specific-VEGF mediates neurogenesis and angiogenesis in the hippocampus.

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