OVERTRAINING PHYSICAL AEROBIC EXERCISE AGAINST THE DECLINE OF MEMORY ON MICE (*RATTUS NORVEGICUS*)

Ni Made Ridla Nilasanti Parwata[1*]

[1]Politeknik Kesehatan Kemenkes Palu

*Correspondence email : karenmargareth08@gmail.com

ABSTRACT

Overtraining syndrome is a decrease in physical capacity, emotions and immunity due to training that is too often without adequate periods of rest. Overtraining is often experienced by athletes who daily undergo heavy training with short break periods. This research aims to look at the effect of overtraining aerobic physical exercise on memory in mice. The research method was experimental in vivo with the subject of adult male rat (*Rattus Norvegicus*) Wistar strain aged 8-10 weeks, body weight 200-250 gr. Divided into three groups, namely the control group, aerobic group and overtraining group. The results of memory tests with water E-Maze showed an increase in the duration of travel time and the number of animal mistakes, made by the overtraining group (p = 0.003). This study concludes that overtraining aerobic physical exercise can reduce memory in rat hippocampus.

Keywords: Overtraining; Memory; Hippocampus

INTRODUCTION

Several studies shown that moderate intensity of aerobic physical exercise has many benefits to our health, not only for improving the fitness, but also endurance against disease, and improving brain function.[1] A study from Neeper SA et al (2000) showing that physical exercise increases the ability to learn and were correlated with BDNF levels on hippocampus.[2] However, in some circles, especially on the group of athletes, the obsession for an achievement requires that athletes to train hardly beyond their capacity, which causes athletes to experience overtraining syndrome.[3] The percentage of overtraining events in athletes is quite high. In the UK, 29% of 110 young athletes experience overtraining. Based on the competition level, 37% of national athletes were overtrained, at the international level 45% of athletes were overtrained.[4] About 65% of national and international runners experienced overtraining at least once throughout their careers.[5] Overtraining will reduce athlete’s performance due to decreasement of brain function in the process of learning, and memory as it is used to enhance the skills of motion, and the execution of movement by a stimuli. As a result, athletes will have a difficultiy on learning some movements. Hence, technical mistakes will continously appear even though it has been repeteadly repaired. The disruption of the learning process and memory were the causes of declining in athlete performance during a match. Based on the background above, researchers wanted to see how the effect of overtraining aerobic physical exercise on memory by using the *Water E-maze* device as a measurement tool.

MATERIAL AND METHODS

Research Design

This research is an in vivo experimental study.

Subjects

This study uses the adult male mice from the Wistar strain (*Rattus norvegicus*), aged 8-10 weeks, with initial body weight ranging from 200-250 grams, they were randomly divided into three groups. Each group consisted of 10 experimental animals, namely: a control group that was not given any treatment (Control), a group that was treated with mild aerobic physical exercise (Aerobic), and a group that were treated with aerobic physical exercise that leads to overtraining (Overtraining).

Research Procedure

Overtraining Aerobic Exercise Procedure

Overtraining aerobic physical exercise given to the treatment group until overtraining (Overtraining) determined as aerobic physical exercise using a treadmill with exercise duration and gradually increasing speed, according to the protocol of overtraining aerobic exercise for 11 weeks.[7] This overtraining aerobic physical exercise is given 5 days each Sunday with two days of rest, Saturday and Sunday. While the aerobic group (Aerobic) was given mild aerobic physical exercise during the study.

Memory Test Procedure

The memory tests were performed on all of the animal groups. The memory test was carried out using a *Water E-Maze* device that was designed in the form of T letter and filled with water for about 30 cm.[8] At the right side of the device, the target was placed in the form of ladder. Initially, the animals were given 3 times exercise to swim from the starting point and find the ladder target, then they will be rested. The first memory test was carried out at the beginning of the study i.e., before physical exercise to the control group (Control), the aerobic physical exercise (Aerobic) and overtraining (Overtraining) treatment groups. Furthermore, a memory test is carried out every week (the end of the 1st weeks until the 11th weeks) throughout the study. In each test, three repetitions were performed for each animal in a row without any resting period. The average required time to swim from the starting point to find the target ladder and the average number of mistakes, namely backing, selection and zoning made by animals trying to reach the target were recorded as data for memory measurement.[8]

Tools and Materials

The tools and materials used in this study are scales, animal treadmills, *Water-E maze* to measure memory, and stopwatch.

Statistical Analysis

Statistical analysis begins with the normality and homogeneity test, if the distribution is normal and homogeneous then further data analysis will be performed with *Anova*. If the parametric test were not met the requirements, then the *Kruskall Wallis* test will be carried out and followed by the *Mann Whitney* test. The significance limit used is 5%.

RESULTS

The Effects of Overtraining Aerobical Exercise on Memory (Required Time of The Tested Animals in Completing the Memory Test on a Water-E Maze Device

Average Required Times of Experimental Animals

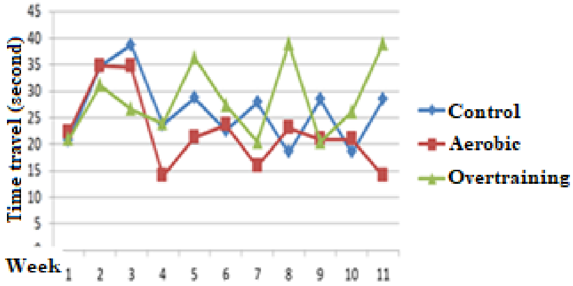

Figure 1. Average required time of animals trying to complete memory tests on *Water-E maze* device

Based on Figure 1, the average required time between the control group and the aerobic exercise group was pretty much similar, except at 4th,5th,7th,9th , and 11th weeks. The average required time of the control group tends to be the same with the overtraining aerobic exercise group during the 1st,2nd,4th, and 6th weeks. The mean required time of the control group was far lower than the overtraining aerobic exercise group during the 3rd,5th,8th,9th,10th, and 11th weeks. The mean travel time of the aerobic exercise group was similar to the overtraining aerobic exercise group during the 1st, 2nd, and 9th weeks. The average required time of the aerobic exercise group was higher than the overtraining aerobic exercise group during the 3rd weeks. Although, the average required time of the overtraining aerobic exercise group was higher than the aerobic exercise group at 4th ,5th ,6th ,7th ,10th and 11th weeks.

Statistical Test on The Effects of Overtraining Aerobic Exercise Against Memory (Required Time)

Table 1. Statistics effects of aerobic exercise overtraining against memory (required time)

Required Time per Week	P Value
1st Week	0.320
2nd Week	0.955
3rd Week	0.201
4th Week	0.322
5th Week	0.040
6th Week	0.655
7th Week	0.271
8th Week	0.658
9th Week	0.467
10th Week	0.482
11th Week	0.050

Based on the analysis of statistical tests with a significance limit of 0.05, it could be assumed that there were significant differences on the average required time of animals in completing memory tests during the 5th and 11th weeks. Then proceed with the *Post Hoc LSD* test to observe the significant differences between groups.

Comparative Test on The Effects of Overtraining Aerobic Exercise Against Memory (Required Time)

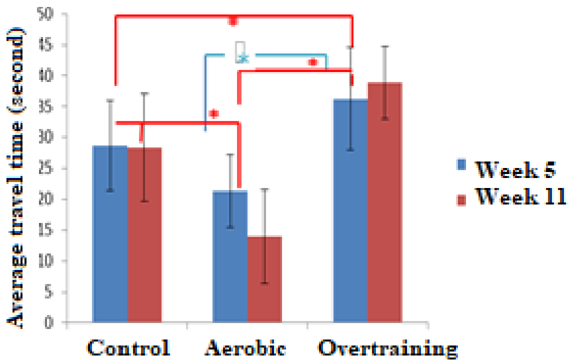

Figure 2. Comparative test of the effects of overtraining aerobic exercise against memory (required time)

The results of the *Post Hoc LSD* comparison test in Figure 2 show that: The mean required time between the aerobic and overtraining aerobic exercise group was significantly different during the 5th week. The mean required time between the control and the aerobic exercise groups, the control and the overtraining aerobic exercise group, as well as the aerobic and the overtraining aerobic exercise groups were significantly different during the 11th week.

The Effects of Overtraining Aerobic Exercise Against Memory (Numbers of Animal Mistakes Try on a Water-Eze Device)

Average Number of The Experimental Animal Mistakes For Each Group

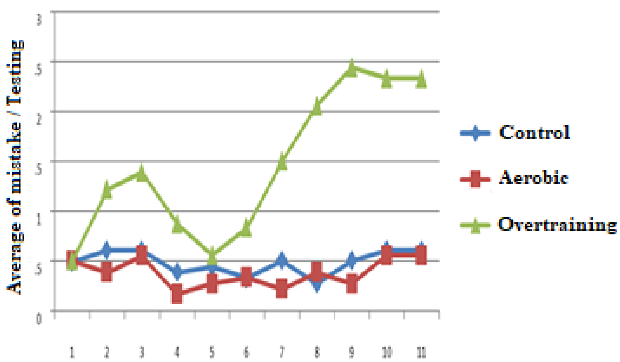

Figure 3. Average number of errors made by the trial animals to complete memory tests on *Water-E maze* device

Based on Figure 3, it can be assumed that the average mistakes of the overtraining aerobic exercise group is higher than the average error of the control group and aerobic exercise, except during the 1st week. The mean error of the aerobic exercise group was lower than the control group except during the 6th and 8th weeks.

Statistical Tests The Effect of Aerobic Exercise Overtraining on Memory (Number of Animal Mistakes Try)

Based on the statistical analysis of the Anova Test (parametric statistics) and the Kruskall Wallis test (non-parametric statistics) with a significance limit of 0.05, it can be concluded that there are significant differences in the average travel time of animals in completing memory tests at week 4,5,7, 8,9,10 and 11.

Table 2. The Effectivity test of overtraining aerobic exercise on memory (average number of animal mistakes try on a Water-E Maze device)

Average Errors per Week	α value
1st Week	0.308
2nd Week	0.104
3rd Week	0.235
4th Week	0.020
5th Week	0.029
6th Week	0.245
7th Week	0.05
8th Week	0.02
9th Week	0.02
10th Week	0.03
11th Week	0.03

Comparative Test Effects of Overtraining Aerobic Exercise on Memory (Number of Mistakes) Between Groups

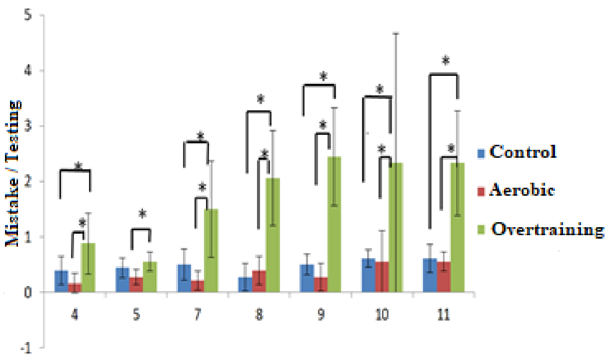

Figure 4. The comparative test effects on overtraining aerobic exercise on memory (number of mistakes) between groups

The result of the *Post Hoc LSD* comparative test in Figure 4 shows that the mean number of mistakes at the 4th weeks demonstrates significant differences between the control group and the overtraining aerobic exercise, as well as for both the aerobic exercise group and the overtraining aerobic exercise group. The mean mistakes time between the aerobic and overtraining aerobic exercise groups was notably different during the 5th week. The mean mistakes time between the aerobic and overtraining aerobic exercise groups, as well as for the control group and overtraining aerobic exercise at week 7 were significantly different. The mean number of mistakes during the 8th weeks were significantly different between the control and overtraining aerobic exercise groups, also for the aerobic and overtraining aerobic exercise groups. The mean number of mistakes during the 9th weeks was significantly different between the aerobic and overtraining aerobic exercise groups, also for the control and overtraining aerobic exercises. The mean number of mistakes at the 10th weeks was significantly different between the aerobic and overtraining aerobic exercise groups, as well as for the control group and overtraining aerobic exercise. The mean number of mistakes during the 11th weeks was significantly different between the control and overtraining aerobic exercise groups, as well as for the aerobic and overtraining aerobic exercise groups.

DISCUSSION

The Effect of Overtraining Aerobic Exercise Against Memory

The memory test results that show the average required time and number of animal mistakes on *Water-E maze* devices were presented in Figures 1 and 3. Based on Figure 1, it can be seen that the average required time of the control group in the first week were shorter, then gradually increased in the following week until the end of the 11th weeks. Whereas in the aerobic exercise group, the average travel time showed a gradual decrease from the first week to the 11th week. The overtraining aerobic exercise group shows opposite result, the average required time showed a tendency for a sharp increase in the 9th,10th,11th weeks According to the results of the *Post Hoc LSD* comparison test in Figure 2, the mean required time at week 5 was significantly different for the aerobic and overtraining training groups. The mean travel time at the

11th week was significantly different between the control and aerobic exercise groups, the control and overtraining aerobic exercise group, and also for the aerobic and overtraining aerobic exercise groups.

Another criterion measured in the memory test is the number of errors. Based on Figure 3, it can be seen that the number of errors in the control group show a small increase in the 1st week to the 11th weeks. Correspondingly, the number of mistakes in the aerobic exercise group also experience the same increase. The overtraining aerobic exercise group shows a sharp increase of mistake starting from the 1st up to the 11th weeks. The results of the *Post Hoc LSD* statistical test in Figure 4 show that there were statistically significant differences in the mean mistakes between groups at the 4th,5th ,7th ,8th ,9th,10th, and 11th.

Based on Figures 1 and 3 it can be assumed that the average required time and the number of mistakes in the control group has increased every week in general, although the increase is relatively small compared with the overtraining aerobic exercise group. This indicates that the control group did not experience an increase with their spatial memory capabilities. In the contrary, the required time for the aerobic exercise group from week to week tends to show a declining. It means that every week the experimental animals in the aerobic exercise group needed a shorter time to complete the *Water-E maze* test. This indicates an increase in the spatial memory of the aerobic exercise group. Spatial memory is closely related to the ability to navigate or remember the correct travel path to reach a certain position or target and to remember the order of objects in the surrounding environment.[9]

The increase of spatial memory on the aerobic group was caused by physical exercise, as it works as the main door in the hippocampus to respond stimuli from the environment and also to ensure that the neurons will survive against the stressors. This response, in turn, provides feedback that will strengthen the brain as in the use of encoding the information and maintaining resistancy to pressure.[10] Research from Tali K et al (2008) showed a positive correlation between running and spatial memory abilities.[11] The rats that were given physical exercise showed better performance in completing the spatial memory test on a similar device *Morris Water Maze,* compared to the control mice that were not given any training. Borght et al (2007) suggested that running could increase neurogenesis in the hippocampus of mice, which was thought to be related with the improvement of the animals performance in completing the *Y-Maze* test.[12]

This study was also in line with a research from Makoto M et al (2003) which suggested that increasing levels of BDNF plays an important role in the formation of spatial memory.[13] In this study, the mice that were given aerobic physical exercise for 4 weeks had higher BDNF levels than the control group. Increased BDNF levels consequently will cause an increase in phosphorylation of protein fyn, which in turns will lead to the expansion of NMDA receptors that play a role in the formation of long-term potentiation as the beginning of memory formation including the spatial memory.[13] Research conducted by Yu FL et al (2009) found that mice that were given voluntary running on a treadmill as their aerobic exercise showed a significant effect on their spatial memory through the up regulation of BDNF protein and Syn 1 protein on hippocampus and amygdala.[14] Consequently, this will emphasize the signal between the hippocampus and amygdala which results in the establishment of spatial memory capabilities.

However, the overtraining aerobic exercise group actually showed the opposite results. Based on Figure 1, it can be seen that the mean required time in the overtraining aerobic exercise group shows a gradual increase in the duration during the 9th to 11th weeks. *Post Hoc LSD* statistical test result in Figure 2 shows that the average required time at weeks 5th and 11th were significantly different, between the control group and aerobic exercise, between the control group and overtraining aerobic exercise, also between the aerobic exercise group and overtraining aerobic exercise. Correspondingly, the average error also gets higher. In figure 3 it can be seen that in the overtraining aerobic exercise group, there were moderate increase of mistakes each week. This indicates that the overtraining aerobic exercise group experience deteriorations of spatial memory capabilities. Regarding to the length of treatment (11 weeks), the memory about the stairs position should have formed permanently. Accordingly, it should have taken shorter period of time and smaller number of errors to complete the *Water-E maze* test. Nonetheless, this did not happen in the overtraining aerobic exercise group. In this group there were significant increases on the required time and the number of errors, especially during the final weeks of the study. According to researchers' viewpoint, this could be due to the episode of overtraining aerobic exercise, which in turns will interference or even decrease the memory process in mice. Therefore, the memory about the correct location of the stairs might have formed permanently. Nevertheless, the overtraining aerobic exercise which summit at the 11^{th} weeks caused the rats to experience a memory disturbance.

In addition, low levels of BDNF in the overtraining aerobic exercise group also play an important role in memory loss. This is consistent with the discovery of low BDNF levels in the overtraining aerobic exercise group at this study. This is because BDNF has a role as a master regulator for neuronal survival, synapse plasticity, differentiation and support for neurogenesis of neurons.

In the contrary, the research results of Helga O et al (2005) shows a different result.[15] They found out that overtraining exercises was actually increased BDNF levels and memory proven by the *Passive Avoidance Test*.

This study were in line with Eloi FR et al (2007) research which suggested that overtraining will induce oxidative stress in the brain by means of lipid peroxidation increment which resulted in the damage of hippocampal tissue and decreased cognitive function as proven by cognitive function test.[16] Based on a research from John A K et al (2012), overtraining exercise will cause a decline of performance in athlete, as in brain function.[17] Decreased brain function is related to difficulties of learning and memory disturbance in the overtraining group. Fry et al (1997) suggested that overtraining in humans was related to impaired brain function especially in thinking.[18]

CONCLUSION

According to the conducted research, the conclusion was: Overtraining aerobic physical exercise will decrease the memory in mice.

REFERENCES

1. Tong L, Shen H, Perreau VM, Balazs R, Cotman CV. Effects Exercise On Gene Expression Profil In The Rat Hipocampus. Neurobiol. 2001; Dis.8(6):1046-56.
2. Neeper, S.A, Gomez PF, Choi J, Cotman CW. Physical activity increases mRNA for brain-derived neurotrophic factor and nerve growth factor in rat brain. Brain Res. 1996; 726, 49–56.
3. Diamond,MC. Response Of The Brain To Enrichment. Anais de academia brasileira de ciencas. 2001; 73(2):211-20.
4. Nuno FM, Richard JW, and Craig AW. Prevalence of Nonfunctional Overreaching/ *Overtraining* in Young English Athletes. Medical Scientic Sports Exercise. 2011; 43, 287–94.
5. Morgan WP, O'Connor PJ, Sparling PB, et al. Psychologic Characterization Of The Female Elite Distance Runner.Int J Sports Med. 1987; 8:124-31.
6. Dong HK, Bum SK, Hyukki C, Young IK, SangmeeAJ,Yea HL. Ameliorates Exercise cognition impairment due to restraint stress-induced oxidative insult and reduced BDNF level. Biochemical and biophysical Reseach communication. 2013; 434: 245-51.
7. Boulanger L, Poo MM. Presynaptic Depolarization Facilitates Neurotrophin Induced Synaptic Potentiation. Nat Neuroscience. 1999; 2:346-51.
8. Surjono TW. Pengaruh pendedahan Pralahir Rubratoksin B Terhadap Perkembangan Pralahir dan Perilaku Pasca lahir Serta Penampilan Reproduksi

Turunan F1 Mencit (*Mus musculus*) Swiss Webster [Disertasi]. Bandung: ITB; 1997.
9. Carlson NR. Physiology of Behavior. 11th Edition. USA: Pearson Education, Inc; 2013.
10. Diamond,MC. Response Of The Brain To Enrichment. Anais de academia brasileira de ciencas. 2001; 73(2):211-20.
11. Kobilo T, Henriette VP.Muscle Fatiq And Cognition: What Is The Link?.2011. Learning And Memory. 18, 103–07.
12. Borght KVD, Havekes R, Bos T, Eggen BJL,Van DZ. Exercise Improves Memory Acquisition and Retrieval in The Y Maze Task: Relationship With Hippocampal Neurogenesis. Bahav Neurosci. 2007;121(2):324-34.
13. Mizuno M, Yamada K, Jue He, et al. Involvement of BDNF Receptor TrkB in Spatial Memory Formation. Learning and Memory. 2003; 10: 108-15.
14. Yu FL, Hsiun IC, Chao LW, et al. Differential effects of treadmill running and wheel running on spatial or aversive learning and memory: roles of amygdalar brain-derived neurotrophic factor and synaptotagmin I. Journal Physiology 2009. 587.13; 3221–31.
15. Helga O, Istvan B, Shuzo K, Takao K, Shoichi T, Sataro G, et al. The effects of moderate-, strenuous- and over-training on oxidative stress markers, DNA repair, and memory, in rat brain. Neurochemistry International. 2005; 46: 635–40.
16. Eloi FR, Takahashi S, Aboulafia J, et al. Oxidative Stress Induched By Intensive And Exhaustive Exercise Impair Murine Cognition Fungtion. J Neurophysiology. 2007; 98:1820-26.
17. John A K, Diana S, Woodruff P. A comparison of low- and high-impact forced exercise: Effects of training paradigm on learning and memory. Physiology and Behavior. 2012; 106:423 – 27.
18. Fry RW, Morton, AR, Keast D. *Overtraining* in athletes: an update. Sports Med. 1991; (12):32–65.