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The Effect of Aerobic Exercise and Caloric Restriction on Mice's Brain Tissue PGC-1α Levels and Their Memory Abilities

The Effect of Aerobic Exercise and Caloric Restriction on Mice's Brain Tissue PGC-1α Levels and Their Memory Abilities

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Abstract. Brain degeneration due to oxidative stress will have an impact on cognitive function decline. Aerobic exercise and calorie restriction are equally believed to increase the ability of cells to inhibit the effects of oxidative stress in the brain. Mitochondria are very susceptible to damage due to oxidative stress. Survival ability of mitochondria can be seen by measuring the levels of the PGC-1α protein, which regulates the transcription of mitochondrial biogenesis in brain tissue. A study has been conducted to compare the effects of aerobic exercise and calorie restriction for 8 weeks on PGC-1α levels of brain tissue of mice and their spatial memory. A total of 24 male mice, aged 6 weeks, were divided into 4 groups: control, aerobic exercise (AE), calorie restriction (CR), and a combination of AE + CR. The exercise was done by running in running wheels, every day for 40 minutes at a speed of 10 m / min. Calorie restriction was done by feeding mice as much as 70% of the calories of food consumed by ad libitum. The CR group had a PGC-1α levels and spatial memory higher than the control. The AE group and the combination of AE + CR had PGC-1α levels and spatial memory higher than the CR group, and both groups have no differences. Physical exercise accompanied with calorie restriction or only physical exercise have better effects on brain's PGC-1α and spatial memory compared with only caloric restriction.

1. Introduction

The brain is the center of cognitive function. Cognitive is the ability to recognize and interpret a person's environment in the form of attention, language, memory, visuospatial and executive functions [1]. Cognitive degeneration is a progressive decline in cognitive function due to aging which results in a decrease in memory function [2].

Calorie restriction (CR) and aerobic exercise (AE) have an effect on neurodegenerative inhibition. CR is the reduction of energy intake without malnutrition, which can inhibit the aging process and improve physiological functions. The well-dose calorie restriction will have a good effect on reducing free radicals and stimulating adaptation pathways to protect and repair neuronal damage [3,4]. An anaerobic exercise is a form of hypoxia preconditioning that will increase levels of reactive oxygen species (ROS) within tolerant limits. This will stimulate the effects of adaptation to protect and repair

the damaged brain. Exercise activates proteins that facilitate mitochondrial biogenesis to maintain the structure of neuron cells in the brain. [5].

Mitochondria are most responsible for oxidative stress and adaptation [6]. As a center of energy metabolism, the mitochondrial damage will have a direct impact on cell damage. Mitochondrial biogenesis greatly determines the cell's ability to survive. The ability of mitochondrial biogenesis is highly dependent on various proteins. Protein co-activator that plays an important role in regulating mitochondrial biogenesis is peroxisome proliferator-activated gamma receptor coactivator-1 alpha (PGC-1α). PGC-1α becomes co-activator of nuclear respiratory factor 1 (NRF-1) and nuclear respiratory factor 2 (NRF-2). These two-factor proteins activate mitochondrial transcription factor A (TFAM), which is directly responsible for stimulating transcription of various proteins that maintain mitochondrial structure [7,8].

CR and AE exercise have a positive effect to repair and protect neuronal mitochondria. However, studies comparing the benefits of these two behavioral modifications to mitochondrial biogenesis have never been carried out. This study wanted to compare the benefits of CR and AE to PGC-1 **α** levels in the mouse brain and their spatial memory abilities.

2. Methods

The study has been carried out at the Faculty of Medicine's animal house, Universitas Sriwijaya. The subjects were 24 mice of Mus musculus Swiss-Webster strain, aged 8 weeks, with a body weight of 20-30 grams. Mice were randomly divided into 4 groups, which consisted of group control (without treatment), aerobic exercise (AE) group, calorie restriction (CR) group and a combination of aerobic exercise and calorie restriction (AE + CR) group. The subjects live in a room set at 23 ° C, setting light and dark 12:12h. Meals were administered ad libitum only for control and AE groups. Acclimatization and orientation of the experimental room were carried out for 7 days before the experiment began.

2.1. Calorie restriction

Calorie restriction was carried out by giving a diet of only 70% (30% reduction) of the total diet consumed by mice on an ad libitum basis every day for 8 weeks [2]. The food menu was the standard food given to all mice in the animal house. The measurement of the amount of food on an ad libitum basis was carried out seven consecutive days before the restriction phase. Each mouse was given 50 mg of food. The remaining food that was not consumed by mice was weighed again after 24 hours. The average amount of food consumed by 12 mice (CR and AE + CR groups) in ad libitum for 7 days was 6.41 ± 1.15 gr per day. Each mouse then was given 70% of the amount of food. Mice that received restriction calorie treatment were placed alone in a small cage, to anticipate aggressive behavior during the experiment.

2.2. Aerobic exercise

The exercise was carried out on 12 mice (AE and a combination of AE + CR groups), by placing mice in running wheels. Adaptation to running speed was carried out for 7 days. In the early stages of adaptation, mice ran at speeds of 3m / minute. The speed was increased gradually until they were able to run according to the target of aerobic at speed of 10 m/min [9]. Aerobic exercise was carried out every day for 8 weeks, with a duration of 40 minutes per day. The exercise begins with running speed of 5 m / min for 5 minutes, followed by running at a speed of 10m / minute for 30 minutes and ending with running at a speed of 6 m / min for 5 minutes for the cooling phase.

2.3. Morris' Water Maze Test (MWMT)

The test was carried out in the cylinder fiber diameter of 60 cm, plain water with dark food coloring was filled into the cylinder until the water level was reached a depth of 10 cm from the bottom. Darkly hidden footing was placed at a fixed position under 1 cm from the water surface. Mice were let to swim in the cylinder for 1 min every day for 1 week for adaptation to MWMT test. The MWMT procedure was then performed for 3 days. Mice were released from the starting place, and they were

allowed to swim to find a flat footing. If after 1-minute mice did not find it, they were manually placed on the flat and stayed on it for 15 seconds. The procedure was carried out 2 times. The third swam activity was the test procedure. Mice were released into the water from early positions and left to find a flat footing. Latent time procedure tests were noted [10].

2.4. ELISA Measurement of PGC-1 α

Isolation of brain tissue immediately after the mice was sacrificed by cervical dislocation. All parts of the brain were taken to be weighed and placed in PBS 1%. Tissue homogenization and sandwich ELISA for PGC-1 α was guided using a POS 1B ELISA manual Mouse Kit Sunlong Biotech Co., Ltd. Brain tissue was rinsed in 0.01 M PBS at a ratio of 0.1 ml of PBS to 10 mg of tissue. The tissue was homogenized and stored overnight at -20 ° C. Two freeze-thaw cycles were performed to break the cell membranes. The homogenates were centrifuged for 5 min at 3000 rpm, at 4 ° C. The supernatant was taken and assayed. The supernatant was taken for assay and was measured by optical density reader (V-max multiple readers) at 450 nm. PGC-1 α level measurement is based on a standard curve with the equation $y = 0.002x + 0.192$, $R^2 = 0.963$.

3. Results

PGC-1**α** levels in all intervention groups were higher than in the control group. Combination AE+CR group had PGC-1**α** levels that were not different from the AE group. PGC-1**α** levels in both groups were higher than the CR group (figure 1).

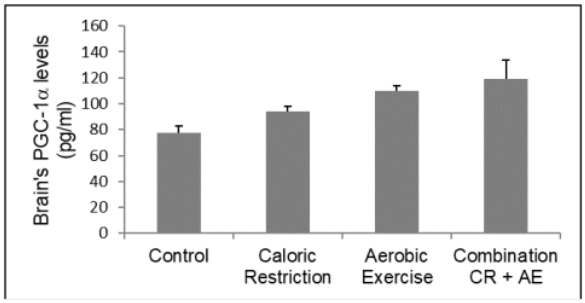

Figure 1. PGC-1α levels in Control and Treatment Groups. The one-way ANOVA test was significant [F3, 20=30.327, P<0.001]. The post hoc Bonferroni test showed significant difference between all groups (control vs CR, control vs AE, control vs combination CR + AE, CR vs AE, and CR vs combination CR+AE; p<0.05), except, the post hoc Bonferroni test between AE vs combination CR+AE, was not significant (p>0.05).

All groups of mice have similar spatial memory on the first day of MWMT. All mice were able to find footing flat faster in the second and third days. All experimental groups have a shorter latency than the control group. The decrease in latent time to find footing flat were greater in the AE + CR combination group and the AE group compared to CR group. The latent time to find footing flat between the AE + CR combination group and AE group was no difference. This showed that the AE + CR combination group and the AE group have the same spatial memory ability, and both groups have better spatial memory abilities than the only CR group.

Weight Body weight was measured before and after the intervention to assess the effect of behavior modification on nutritional status. All groups of mice experienced weight gain. The highest increase was found in the control group, followed by aerobic exercise and calorie restriction groups. AE + CR combination group gained weight only 0.2 grams for 8 weeks of treatment.

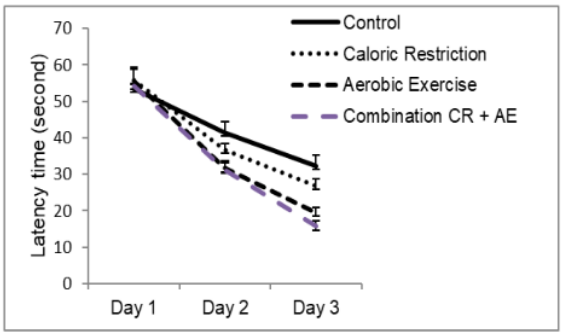

Figure 2. Time needed the mice to reach footing. On day 1, all three groups showed equivalent latency time. Control (53.5±5.8 second), CR (55.8±3.4 second), AE (55.5±3.8 second), and combination CR+AE (54.2±4.7 second). The one way ANOVA test showed no significant (NS): [F3, 20= 0.351,P>0.05]. On the 3rd day, the latency time of control group (32.3±2.9 second) was longer than CR group (26.8±1.9), a shorter time than control and CR groups was AE group (19.5±1.4), the shortest time was the combination CR+AE group (15.7±1.6 second). The one-way ANCOVA test for the 3rd day showed significantly: (F3, 19= 86.18, P<0.001). The post Bonferroni test for 3rd Day: control vs CR, P<0.01; control vs AE, P<0.001, control vs combination CR+AE, P<0.001, CR vs AE, P<0.001, CR vs combination CR+AE, P<0.001, while there were differences between AE vs combination CR+AE, p>0.05.

4. Discussion

This study has measured the effects of aerobic exercise and calorie restriction on PGC-1 α levels in brain tissue. PGC-1α is a key indicator of mitochondrial biogenesis[8]. Aerobic exercise and calorie restriction are lifestyle modifications that directly affects mitochondria because these two interventions are related to the energy balance mechanism [11,12]. Degeneration is highly dependent on mitochondrial function in regulating cell death and cell survival [11] This study has shown that aerobic exercise will increase the levels of PGC-1α in brain tissue. Previous studies also proved the same thing. Exercise increases the level of PGC-1α, which effects on increasing the synapses' plasticity and neurogenesis in the dentate gyrus [13]. Exercise increases cell demand for oxygen, so cells experience hypoxia preconditioning at a tolerable level. This condition stimulates the pathway sequence for the cell survival mechanism[14]. Hypoxia preconditioning increases the production of ROS and Reactive Nitrogen Species (RNS), which stimulate TNFα production. TNFA stimulates the synthesis of AMP-dependent kinases which will trigger transcription and synthesis of PGC-1α proteins[15–17]. This study also proved that calorie restriction has a positive impact on increasing levels of PGC-1α. CR causes a decrease in blood glucose levels, which stimulates insulin-like growth factor-1 (IGF-1), AMP-dependent kinase (AMPK), sirtuins, NADH, and FOXO. These proteins synergize to synthesize PGC-1α which will stimulate mitochondrial biogenesis and cell proliferation[17–19]. Sirtuins stimulation aims to create an adaptation response to gluconeogenic/glycolytic pathways in response to PGC-1α through transcription co-activator. [20]. Calorie restriction (CR) also induces the expression of endothelial NO synthetase (eNOS) which increases the production of nitric oxide (NO) and finally mitochondrial biogenesis involving PGC-1α. This autophagy leads to an increase, recycling damaged components. This process is modulated, in part, by the target of rapamycin (mTOR) mammals and forkhead boxes containing protein O (FOXO) transcription factors [11]. CR has a positive effect on mitochondrial survival. Mitochondria under CR

conditions, show less oxygen consumption, reduce membrane potential, and produce oxygen species that are less reactive, but still able to maintain critical ATP production. As a result, CR can induce PGC-1α to reduce oxidative stress and attenuate age-dependent oxidative damage [21].

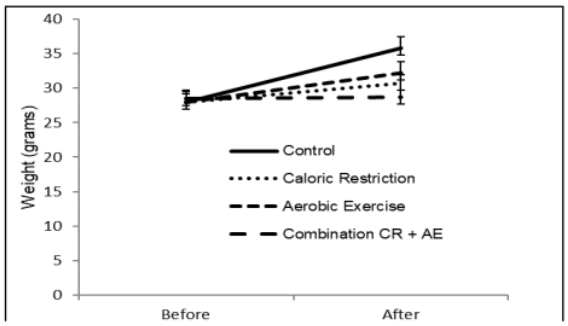

Figure 3. Weights of mice before and after 8 weeks intervention. All of the groups showed similar weights on before intervention. Control (28.0 ± 1.7 gr), CR (28.0 ± 1.1 gr), AE (28.0 ± 1.7 gr), and CR + AE combination (28.5 ± 1.2 gr). The one-way ANOVA test showed no significant: [F3.20 = 0.181, P> 0.05]. After the intervention, weights of the control group (32.3 ± 2.9 gr) was biggest than others, CR group (30.7 ± 1.4 gr), AE group (32.2 ± 1.6 gr), the lightest group was the CR + AE combination group (28.7 ± 0.8 gr). The one way ANCOVA test for after intervention showed significant: (F3,19 = 86.18, P <0.001). The post Benferroni hoc test for after intervention, control group was differed from others (control vs. CR, P <0.01; control vs. AE, P <0.05, control vs. CR + AE combination, P <0.001), CR group was not differenced with AE group, P> 0.05, and CR + AE combination, P> 0.05, while the AE group was difference with CR + AE combination group, P <0.05.

Aerobic exercise and calorie restriction also have a positive impact on improving cognitive function. Previous research has proven that CR and exercise increased the resistance of animals to exte[illegible]ating activities and improved motor coordination[2]. This may be related to an increase in PGC-1α. PGC-1α is involved in the formation and maintenance of dendritic spines in a brain that teaches the brain to perform neuroplasticity[13]. PGC-1α overexpression increases dendritic spines and enhances the molecular differentiation of synapses. Mitochondrial biogenesis plays important roles in the formation and maintenance of hippocampal dendritic spines and synapses. PGC-1α and mitochondrial biogenesis stimulate gene transcriptor C-AMP element Binding Protein (CREB) [13,16]. CREB plays a central role in the growth of dendritic spines. CREB is a gene transcriptor that activates transcription and synthesis of various protein growth factors such as neurotrophic factors and vascular growth factors to stimulate neurogenesis[22].

This study has revealed that AE has a better effect than CR, and there is no difference between AE and a combination of AE + CR. Exercise may have a better effect on the brain than dieting because exercise has a more complex impact. Caloric restriction and exercise improve cognitive function with the same two mechanisms. Repair of neuronal cells due to the adaptation of oxidative stress and the adaptation of nutrition balance. However, exercise has a positive impact through two other mechanisms that CR does not have. Muscle movement during exercise is a form of motor learning stimulation that enhances communication between nerve cholinergic synapses. This stimulation

directly stimulates the proliferation of neurons[23]. In addition, exercise also leads to adaptation to improved vascularity of the brain to increase oxygen supply and nutrition[21].

This study also revealed that a combination of aerobic exercise and caloric restriction did not have a different effect with the only exercise on PGC-1α and cognitive function. The combination of exercise and caloric restriction is very useful if it aims to lose weight while maintaining the function of neurons. However, caloric restriction has a risk when applied to the elderly who need a nutrient-rich diet. During the fasting period, there is a potential risk of renal reduction and an increase of glycemia function [24]. Clinicians are often reluctant to prescribe weight loss in older individuals or those with low bone mineral density [25]. Exercise that is carried out properly and correctly may be sufficient for elderly people who need the ability to maintain cognitive function.

5. Conclusion

Aerobic exercise and caloric restriction have positive effects to improve the ability of mitochondrial biogenesis and cognitive function in the brain. However, the combination of exercise and caloric restriction was not different with the only exercise, and both of them have better effects compare to only caloric restriction.

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