Journal of Exercise Physiologyonline

February 2019
Volume 22 Number 1

Official Research Journal of the American Society of Exercise Physiologists

ISSN 1097-9751

JEPonline

# Levels of Hippocampal, Liver, and Plasma Insulin-like Growth Factor 1 (IGF-1) in Male Adult Rats Treated with Combination of Aerobic Exercise and Continuous Environmental Enrichment

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## ABSTRACT

**Yolanda S, Mailani R, Prasety SR, Widyani NMS, Juffry SK**. Levels of Hippocampal, Liver, and Plasma Insulin-like Growth Factor 1 (IGF-1) in Male Adult Rats Treated with Combination of Aerobic Exercise and Continuous Environmental Enrichment. **JEPonline** 2019;22(1):11-20. The purpose of this study was to determine the effect of the combination treatment of both aerobic exercise and environmental enrichment (EE) on the levels of hippocampal, liver, and plasma Insulin-like growth factor 1 (IGF- 1). IGF-1 has been recently shown to play a role in neuroplasticity including memory function. Both aerobic exercise and EE on their own have been proven to increase neuroplasticity. Twenty 7-month-old male Wistar rats were divided randomly into 4 groups: (a) control (C); (b) aerobic (A); (c) environmental enrichment (EE); and (d) combination of aerobic exercise and EE (A-EE) for 8 wks. There were no significant differences in the plasma and hippocampal IGF-1 levels between the groups (P = 0.65 and P = 0.13, respectively). There were significant differences in the liver IGF-1 levels between the A and the EE groups (P = 0.013) and between the A and the A-EE groups (P = 0.02). No increase of IGF-1 level in rats treated with a combination of aerobic exercise and continuous EE may be due to IGF-1's non-neural expression in the periphery and its synergistic effect with other growth factors.

**Key Words:** Aerobic Exercise, Environmental Enrichment, IGF-1

## INTRODUCTION

Insulin-like growth factor 1 (IGF-1) is an important mediator of growth hormone effects in body growth and tissue remodelling (5). It plays a major role in the regulation of cellular processes in tissues throughout the body including, but not limited to protein synthesis, maintenance of bone mass, and both immune and cardiovascular function (2). In the nervous system, it contributes to modulate synaptic plasticity, synapse density, neurotransmission, adult neurogenesis, and even promotes brain amyloid beta clearance (14,22). IGF-1 also has neurotrophic mechanisms, and is neuroprotective in pro-neuroinflammatory conditions (24).

In adults, IGF-1 is mainly synthesized in the liver via a process regulated by the growth hormone (GH). IGF-1 is also produced in gonad, bone, muscle, gut, adipose, and is present in plasma (11). Systemic IGF-1 can cross the blood-brain barrier by binding to the IGF-1R present in endothelial cells and later picked up either by astrocytes to be transferred to neurons or directly by neurons. Liver-produced IGF-1 is responsible for 95% of the brain IGF-1 (2). Besides the circulatory IGF-1, the brain can also synthesize IGF-1 independently of GH action during exercise, stress, inflammation or injury, as neuroprotective agent. There is a small local production in some brain regions, such as the subventricular zone (SVZ), the olfactory bulb (OB), the hippocampus (HP), and the cerebellum (13).

IGF-1 has been observed to promote neuroprotection in response to brain injury and neurodegeneration (23). Age-related reductions in IGF-I have been associated with a decrease in cerebral vascular density and blood flow (14), age-related decline in memory function (6,18), and low levels of IGF-1 has even been proposed as an early biomarker of Alzheimer's disease (22). Thus, increasing IGF-1 levels is an attractive method to prevent and reverse the age-related decline in memory function.

Exercise-dependent stimulation of angiogenesis and neurogenesis seems to be regulated by IGF-1; whereas, a peripheral increase in IGF-1 appears to be required for exercise-induced neurogenesis in the brain (17). Exercise has also been shown to increase neurotrophins, such as the brain-derived neurotrophic factor (BDNF) and IGF-1 (19). The blood IGF-1 level and its uptake to the brain is increased by regular exercise in adult rats (6,16,25). Also, the hippocampal expression of IGF-1 is increased during exercise (25).

Furthermore, environmental enrichment (EE), a method of raising animals in a large cage containing novel objects, running wheels and social interaction, can affect neural plasticity via the overexpression of neurotrophic growth factors such as BDNF, a vascular endothelial growth factor (VEGF), fibroblast growth factor-2 (FGF-2), IGF-1, and synaptic activity-regulating genes (25). EE stimulates neurogenesis and cognitive function in relation to the hippocampus via IGF-1 (8). EE has been shown to up-regulate the IGF-1 receptor gene in the adult rats hippocampus and sensorimotor cortex (8).

Aerobic exercise and environmental enrichment also stimulate IGF-1 expression through growth hormone releasing hormone (GHRH) and GH expression, which stimulates liver to express IGF-1 that enters the circulation to various target organs including the hippocampus (12,18). Both aerobic exercise and EE on their own have been shown to improve the neuroplasticity and IGF-1 expression, but the effect of the combination of both treatments toward IGF-1 expression has not been examined. Thus, the purpose of this study was to

examine the liver, plasma, and hippocampal IGF-1 levels in adult rats treated with combination of aerobic exercise and continuous environmental enrichment.

## METHODS

### Animals

The use of animals in this study was approved by the Health Research Ethics Committee, Faculty of Medicine, Universitas Indonesia no.1018/UN2.F1/ETIK/2017. Male Wistar rats were obtained from health research and development's animal laboratories at age 7 months, weighing 300 to 400 gm. Animals were given access to food and water ad libitum.

Twenty adult male Wistar rats were divided randomly into 4 groups: (a) control group (C); (b) group treated with aerobic exercise (A); (c) group treated with environmental enrichment (EE); and (d) group treated with combination of aerobic exercise and environmental enrichment (A-EE). The C group and the A group animals were housed in standard cages except during exercise treatment for the A group.

Groups treated with environmental enrichment were placed in the environmental enrichment cage (Marlau's cage) continuously for 24 hrs. The animals were maintained on a 12-hr light and dark cycle that was controlled for temperature and humidity. The acclimatization period was performed prior to the treatment to introduce the animals to the environmental enrichment cage, treadmill, and other research conditions.

### Aerobic Exercise

The aerobic exercise was performed on an animal treadmill with 4 tracks. Exercise-relevant groups were given 5 $d \cdot wk^{-1}$ (Monday to Friday) treatment for a period of 8 wks. The type of exercise performed was aerobic exercise with 20 $m \cdot min^{-1}$ treadmill speed at a duration of 30 min. The speed for warming up and cooling down was 10 $m \cdot min^{-1}$ with a duration of 5 min.

### Environmental Enrichment (EE)

The EE was given using a larger cage (cage size: 800 x 600 x 510 mm consisting of 2 floors) containing many different objects besides food and water, including running wheels, a tunnel, stairs, maze, and many other engaging objects (1). The EE and A-EE groups were put inside the EE cage continuously for 24 $hrs \cdot d^{-1}$, 7 $d \cdot wk^{-1}$ for 8 wks. In order to feed themselves, the rats had to climb from the lower floor to the top floor, through a maze and go down through the sliding tunnel.

Physical activity was encouraged by the large exploration area and free access to running wheels. Cognitive stimulation was obtained by changing the maze configuration 3 $times \cdot wk^{-1}$ on every Monday, Wednesday, and Friday using 6 mazes (A-F) that consisted of a total of 12 maze configurations, A1-F1 and A2-F2 (see Figure 1).

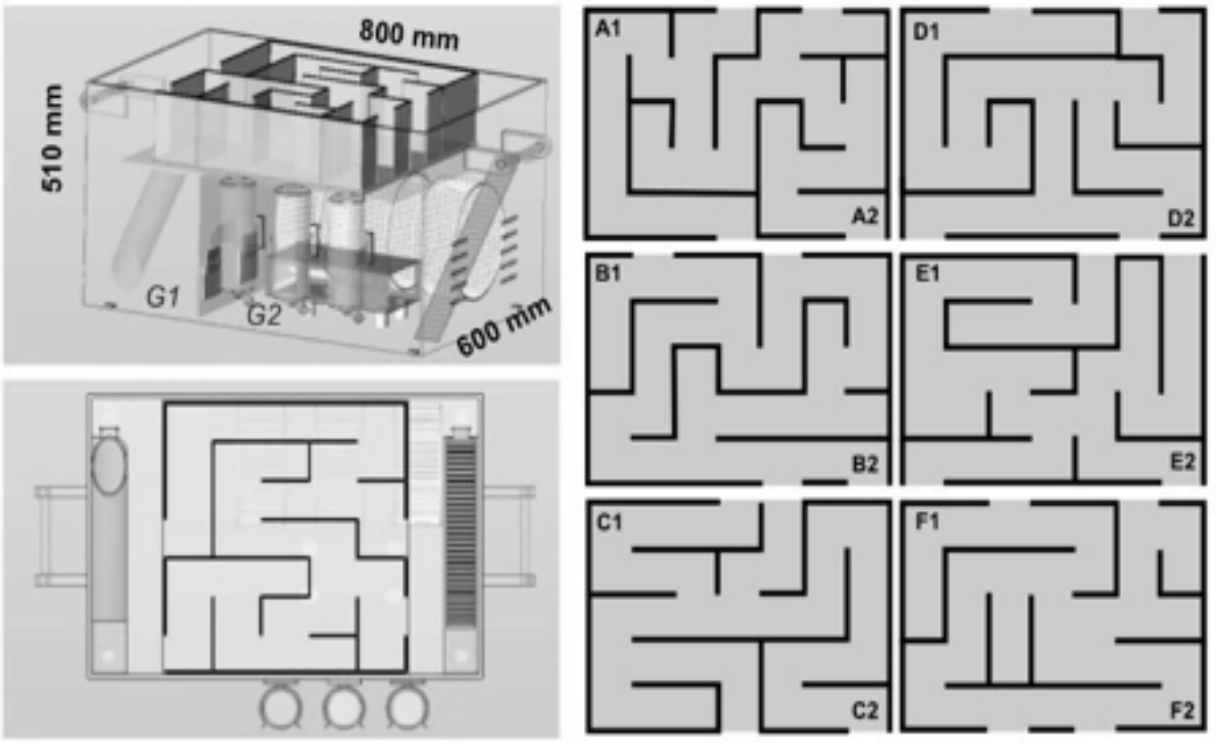



**Figure 1. Marlau's Cage and Maze Configuration (1).**

**IGF-1 Levels**

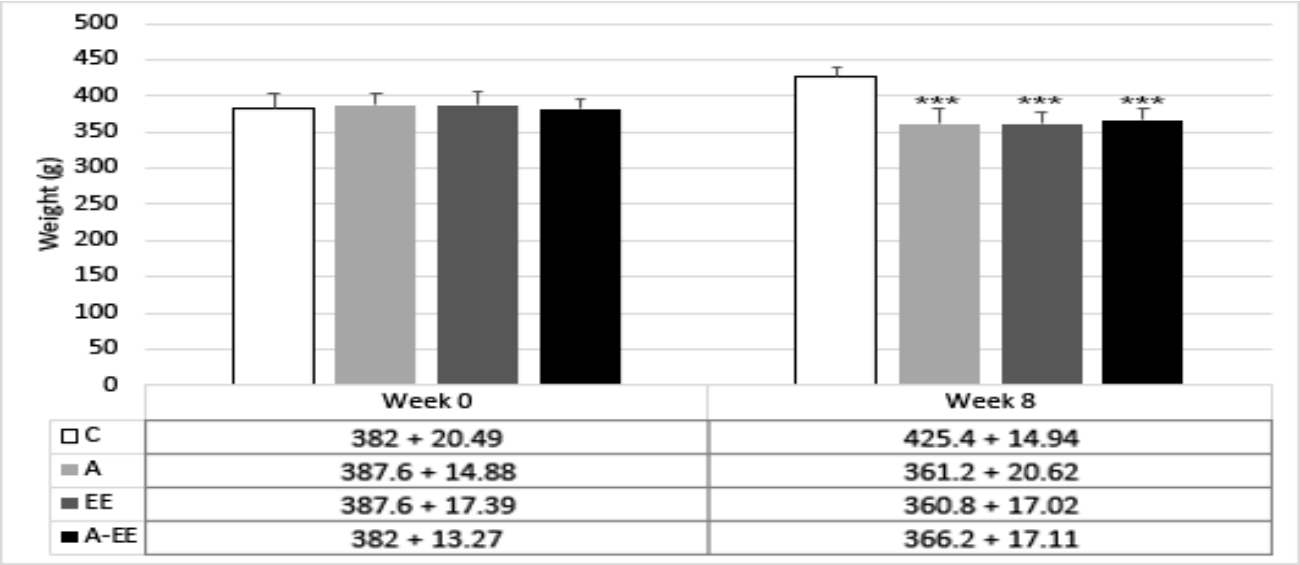
At the end of the 8 wks, the rats were sacrificed. The blood was taken and the liver and hippocampus were isolated. The blood sample was centrifuged into plasma, and homogenization was performed on the liver and hippocampal tissue. The Bradford test was performed to assess total protein in the plasma, liver, and hippocampus. The ELISA examination was performed to assess plasma, liver, and hippocampal IGF-1 levels. The ELISA examination used was sandwich ELISA technique for the detection of antigen-antibody reaction of IGF-1 with the Qayeebio Elisa kit (QY-E10935).

**Statistical Analyses**

The Shapiro-Wilk test was used to verify data normality. One-way ANOVA test was used to analyze body weight, plasma, and hippocampal total protein levels, and also for liver, plasma, and hippocampal IGF-1 levels followed by the Tukey *post hoc* test. The Kruskal-Wallis test was used to analyze liver total protein levels.

**RESULTS**

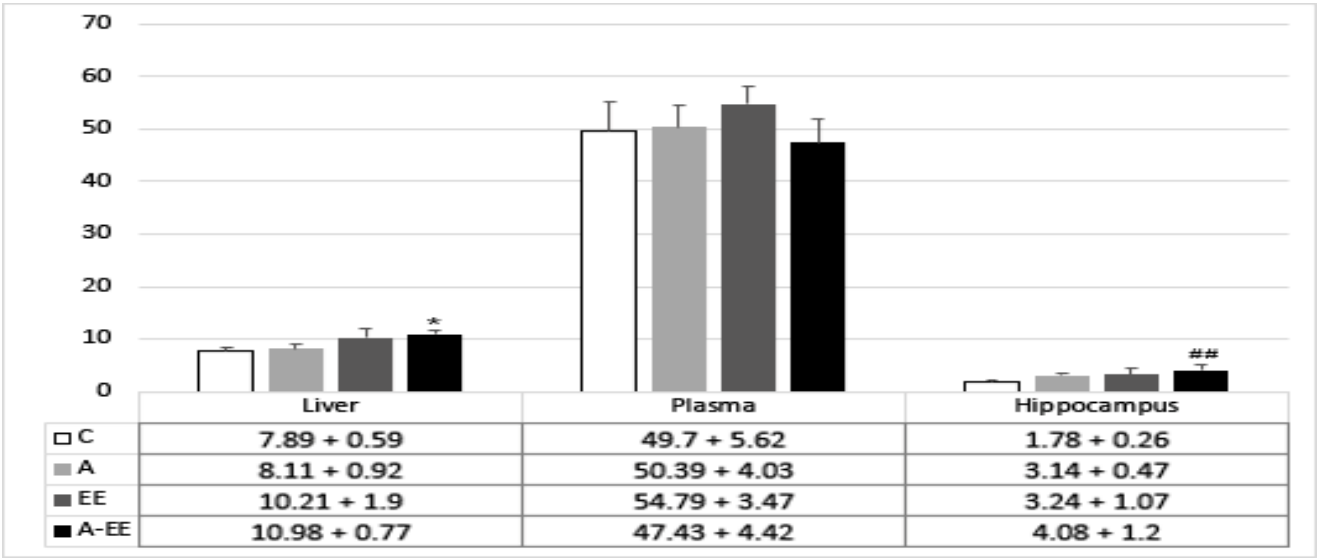
The body weight of each group is presented in Figure 2. The body weight of all the rats at the start of the study was homogenous. The highest week 8 body weight was observed in the control (C) group, with body weight in C, A, EE, and A-EE groups were 425.4 ± 14.94, 361.2 ± 20.62, 360.8 ± 17.02, and 366.2 ± 12.11, respectively. There were significant differences in body weight during week 8 between all treatment groups with the control group (P=0.000).




| | Week 0 | Week 8 |
|---|---|---|
| C | 382 + 20.49 | 425.4 + 14.94 |
| A | 387.6 + 14.88 | 361.2 + 20.62 |
| EE | 387.6 + 17.39 | 360.8 + 17.02 |
| A-EE | 382 + 13.27 | 366.2 + 17.11 |

**Figure 2. Comparison of Mean Body Weight between Groups. C** = Control, **A** = Aerobic, **EE** = Environmental Enrichment, **A-EE** = Combination of aerobic and EE. ***P<0.001 vs. Week 8 C

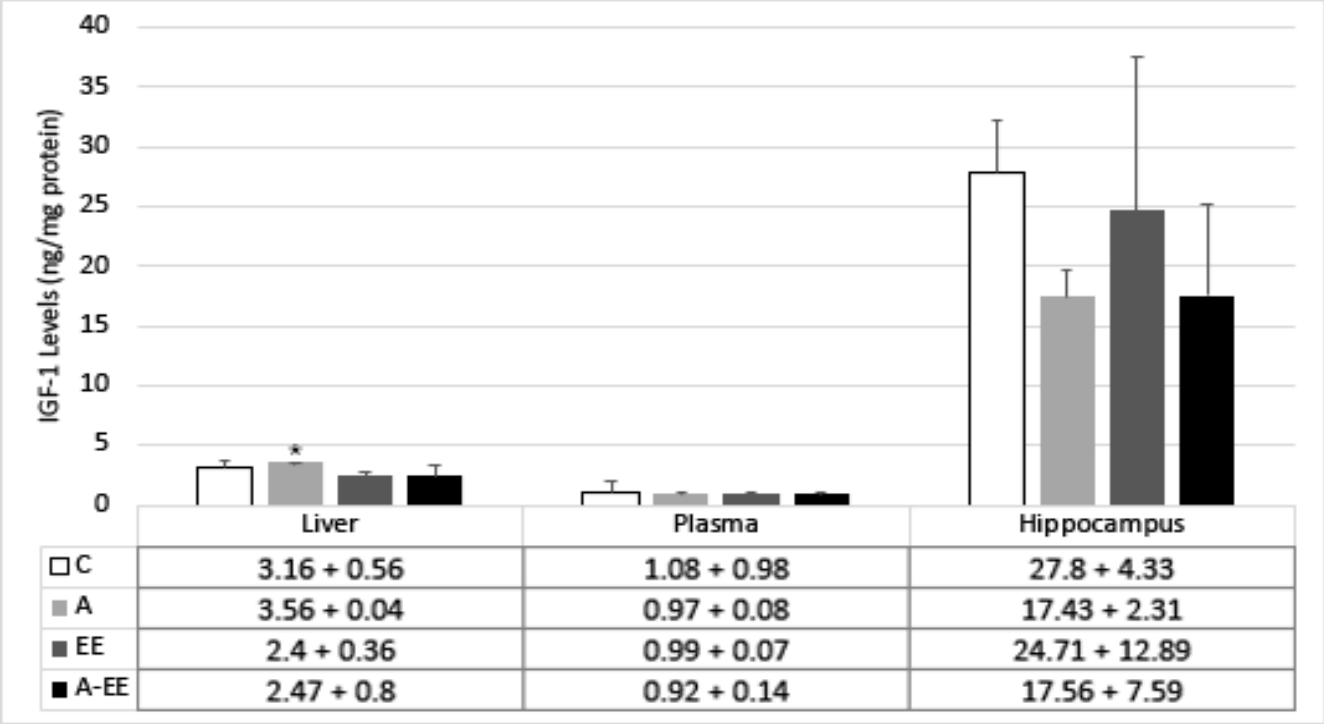
The total protein levels in the liver, plasma, and hippocampus (Bradford test) are presented in Figure 3. The liver total protein level was the highest in the A-EE group. The total liver protein in C, A, EE, and A-EE groups were 7.89 ± 0.59, 8.11 ± 0.92, 10.21 ± 1.9, and 10.98 ± 0.77, respectively. There were significant differences between C and the A-EE group (P=0.015), and between the A group and the A-EE group (P=0.015). There were no significant differences in plasma total protein levels between the groups (P=0.11). The plasma protein total in C, A, EE, and A-EE groups were 49.7 ± 5.62, 50.39 ± 4.03, 54.79 ± 3.47, and 47.43 ± 4.42, respectively. The highest total hippocampal protein was observed in the A-EE group, with the hippocampal protein total in C, A, EE, and A-EE groups that were 1.78 ± 0.26, 3.14 ± 1.07, 3.24 ± 0.47, and 4.08 ± 1.2, respectively. There was a significant difference between the C group and the A-EE group (P=0.003).




| | Liver | Plasma | Hippocampus |
|---|---|---|---|
| C | 7.89 + 0.59 | 49.7 + 5.62 | 1.78 + 0.26 |
| A | 8.11 + 0.92 | 50.39 + 4.03 | 3.14 + 0.47 |
| EE | 10.21 + 1.9 | 54.79 + 3.47 | 3.24 + 1.07 |
| A-EE | 10.98 + 0.77 | 47.43 + 4.42 | 4.08 + 1.2 |

**Figure 3. Liver, Plasma, and Hippocampal Total Protein Level. C** = Control, **A** = Aerobic, **EE** = Environmental Enrichment, **A-EE** = Combination of aerobic and EE. *P<0.05 vs. Liver A and P<0.05 vs. Liver C; ##P<0.01 vs. Hippocampus C

The levels of IGF-1 in the liver, plasma, and hippocampus are presented in Figure 4. The findings indicate that the highest liver IGF-1 level was observed in the aerobic exercise (A) group, with the liver IGF-1 levels in C, A, EE, and A-EE groups (3.16 ± 0.56, 3.56 ± 0.04, 2.4 ± 0.36, 2.47 ± 0.8, respectively). There were significant differences in liver IGF-1 levels between the A group and the EE group (P = 0.013) and between the A group and the A-EE group (P = 0.02). The results of IGF-1 plasma showed that the IGF-1 plasma level in the control group was 1.08 ± 0.98, while the plasma IGF-1 levels in the A group was 0.97 ± 0.08, the EE group was 0.99 ± 0.07, and the A-EE group was 0.92 ± 0.14. Based on statistical test, it was concluded that there were no significant differences between the groups (P = 0.65). The hippocampal IGF-1 level of the control group was 27.8 ± 4.33, the A group was 17.43 ± 2.31, the EE group was 24.71 ± 12.89, and the A-EE group was 17.56 ± 7.59. Based on statistical test, there were no significant differences between the groups (P = 0.13).




| | Liver | Plasma | Hippocampus |
|---|---|---|---|
| C | 3.16 + 0.56 | 1.08 + 0.98 | 27.8 + 4.33 |
| A | 3.56 + 0.04 | 0.97 + 0.08 | 17.43 + 2.31 |
| EE | 2.4 + 0.36 | 0.99 + 0.07 | 24.71 + 12.89 |
| A-EE | 2.47 + 0.8 | 0.92 + 0.14 | 17.56 + 7.59 |

**Figure 4. Liver, Plasma, and Hippocampal IGF-1 Level. C** = Control; **A** = Aerobic; **EE** = Environmental Enrichment; **A-EE** = Combination of Aerobic Exercise and EE. *P<0.05 vs. Liver EE and P<0.05 vs. Liver A-EE

## DISCUSSION

The animals used in this study were 7-month-old Wistar male rats that represent humans in their 20s, which is the age when decline in memory function starts to occur (3). IGF-1 is an important growth factor for the central nervous system (CNS) to help with improvement in memory function. One of the mechanisms in which it exerts its effect is through the glutamatergic synapses (20), more specifically by the increase in the number and activity of N-methyl-D-aspartate (NMDA) type of glutamate receptors (2).

The primary source of IGF-1 in the CNS is peripheral IGF-1 that is primarily produced by the liver and crosses the blood-brain barrier. The findings in the present study indicated that the level of liver IGF-1 was the highest in the group treated with aerobic exercise. This finding is

in agreement with previous studies (10,16,21) in which aerobic exercise increased peripheral IGF-1. There were significant differences in the liver IGF-1 levels in the A group compared to the EE and the A-EE groups. Sensory, motor, and cognitive stimulation and interaction by the EE are important elements in enhancing and adapting the neuronal system for the normal brain function (25). The results may be explained in part due to IGF-1's synergistic action with other growth factors, such as BDNF that also improves memory function (9). Peripheral BDNF levels were examined in this study as part of a larger study (data not shown), and the results of the plasma BDNF levels support this explanation.

As reported by Anne Maass and colleagues (14), exercise induced an increase in the peripheral neurotrophic factor and the angiogenic factor of which both are thought to be critical to hippocampal plasticity and improvements in memory. Thus, we investigated the effects of plasma, liver, and hippocampal IGF-1 levels in adult male rats treated with a combination of aerobic exercise and continuous environmental enrichment. The combination of aerobic exercise and the EE (A-EE) group showed the lowest plasma IGF-1 level. In that the liver IGF-1 level in the A group was higher than the other groups, the lower plasma levels of IGF-1 in the treatment groups may be caused by the intake of IGF-1 by its target organs, decreasing the plasma levels. Another cause of the low plasma IGF-1 levels in the treatment groups may be circadian rhythm disruption. The group treated with exercise (treadmill) experienced a disturbed circadian rhythm due to the exposure to light in the treadmill room, while the control group was always kept at a 12-hr light/dark cycle in their cage without disturbing the circadian rhythm. This is one of the limitations of our study.

The plasma IGF-1 level changes during the day, suggesting some circadian control. The circadian clock generates rhythms in physiology and behavior known as circadian rhythm and synchronizes the processes in organisms with the environment (7). Cryptocome (CRYs) is essential for circadian clock function, and is also an essential player in regulation of IGF-1 production and signalling (7). In line with this, Chaudary et al. (7) observed that in the liver and other tissues of mice lacking CRYs, the expression of IGF-1 was reduced in both the mRNA and protein levels. This finding was correlated with the reduced levels of circulating IGF-1 in the mice.

The highest plasma IGF-1 levels in the control group may also be explained in part due to food metabolism process. Gastrointestinal tissue expresses IGF-1 for metabolism of glucose, carbohydrate, and protein (4). Adipose tissue also expresses IGF-1 for the regulation of fat metabolism and to prevent weight gain. The control group, in which the body weight increases the highest may have the fat accumulation in adipose tissue, thus may be expressing higher IGF-1 for fat metabolism.

The total protein hippocampus in the group treated with the combination of aerobic exercise and continuous EE (A-EE) was the highest versus the other groups. From this result, we may also assume that A-EE improves memory function through increased expression of other growth factors, such as FGF-2, BDNF, and NGF. This explanation will need further study for a better understanding on the production and regulation of plasma IGF-1.

The hippocampal IGF-1 level in the control group has the highest concentration compared to the other groups, similar to the results obtained in the plasma. Though circulating levels of IGF-1 contribute, at least in part, to the level of IGF-1 in the brain, the level of IGF-1 in the

brain is not always correlated with the level in the peripheral tissues (15). It has been shown that regulation of the IGF-1 level in the brain is regulated in a different manner than the peripheral IGF-1 level (2). The IGF-1 level is also influenced by factors of bodily health that are not directly related to exercise, such as nutrition and glucose metabolism (14). As mentioned earlier, IGF-1 in the peripheral tissue has many functions and its production may be induced by non-neural factors, thus contributing to the difference in liver, plasma, and hippocampal IGF-1 levels.

Hippocampal IGF-1 level was higher compared to liver and plasma IGF-1 levels by 7- to 25-fold. Hence, it may be assumed that the high hippocampal IGF-1 level is related to its function to regulate brain activity especially memory function. The source of the hippocampal IGF-1 needs to be assessed further, by observing IGF-1 expression in the genetic level in the hippocampus.

As part of the larger study, spatial memory function was examined (data not shown) and the A-EE group performed the best compared to the other groups. This supports our hypothesis that a combination of aerobic exercise and environmental environment increases memory function. Although the primary mechanism might not be through IGF-1, it is likely to be due to its synergistic function with other growth factors.

## CONCLUSIONS

There was no increase in the IGF-1 level in the liver, plasma, and hippocampal IGF-1 in the rats treated with the combination of aerobic exercise and continuous environmental enrichment. This may be due to the difference in regulation of brain and peripheral IGF-1 levels. IGF-1 in the peripheral tissue has many functions and its production may be induced by non-neural factors, thus contributing to the difference in liver, plasma, and hippocampal IGF-1 levels.

## ACKNOWLEDGMENTS

This study was funded by Hibah Publikasi Terindeks Internasional Untuk Tugas Akhir Mahasiswa UI (PITTA) Universitas Indonesia 2018 no. 2132/UN2.R3.1/HKP.05.00/2018.

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