Exercise Intensity May affect Bdnf Level in the Hippocampus of Fructose-Induced Mice

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Abstract

Introduction – Several studies show that there is a negative correlation between obesity and cognitive function. Exercise can reverse the bad effect of obesity through mediation of BDNF. Research about the effect of exercise intensity to BNDF levels in hippocampus of mice is ever be done, but research about the effect of exercise intensity to BNDF levels in hippocampus of fructose-induced mice is never be done.

Objective – To prove the effect of different exercise intensity to BDNF level in hippocampus of fructose-induced mice.

Methods – A total of 32 mice were divided into four groups: Control group which was a CON and treatment groups which were low-intensity exercise (LIE), moderate-intensity exercise (MIE), and high-intensity exercise (HIE). The duration of treatment was 4 weeks. Swimming to exercise held 3 times a week and fructose solution dose was 270 grams for 9 days.

Results – This research revealed that there was significantly difference on body weight before and after administration of fructose solution for 9 days (p=0.000). The lowest BDNF levels after treatment was group C and the highest of BDNF levels was group MI. There was no significantly difference in BDNF levels among groups after treatment (p=0.378), but there was tendency for BDNF levels to increase with exercise intensity.

Conclusion – Swimming exercise with low-intensity, moderate-intensity, and high intensity has no effect on BDNF level in the hippocampus of fructose-induced mice.

Keywords: *fructose-induced mice, exercise intensity, BDNF level, hippocampus*

Introduction

It is generally known that unhealthy lifestyle can cause several bad effects. One of those effects is obesity. Obesity is a multifactorial disease which is indicated by excessive fat accumulation and body mass index more than normal.[1] There are more than 1.6 billion people in the world suffer from overweight in 2016. Six hundred millions of them are obese.[1] In long term, obesity can cause several diseases and pathological conditions.

The development of obesity is associated with high-caloric diet and sedentary life styles. Recently, fructose consumption has become more frequent than before. Fructose can be found in sweetened foods, soft drink, and other beverages. Fructose is more lipogenic than glucose that high consumption of fructose can lead to overweight and obesity.[2]

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Obesity can increase morbidity and mortality. Several studies show that there is a negative correlation between obesity and cognitive function.[3,4,5] Obesity can induce inflammatory response which lead to metabolic changes and impaired cognitive functions. Increased adipose tissue in obesity can cause insulin and leptin resistance, which eventually impair cognitive function. Rats that had been treated with high caloric diet showed memory deficit.[5]

Obesity can be treated by diet restriction, physical exercise, and, for special cases, pharmacological or surgery treatment.[6] Physical exercises have benefit in reducing body weight, improving mood and cognitive function.[7] Physical exercise could inhibit memory deficit in interferon α-induced cognitive-dysfunction rats. This effects of exercise is mediated by Brain-Derived Neurotrophic Factor (BNDF) in hippocampus.[7]

BDNF is one of neurotrophin which has important role in development, survival, and plasticity of peripheral and central nervous systems.[8] BDNF levels increase with physical exercise and diet restriction.[9,10] Research about the effect of exercise intensity to BNDF levels in hippocampus of mice is ever be done, but research about the effect of exercise intensity to BNDF levels in hippocampus of fructose-induced mice is never be done.[11]

This objective of this research is to prove the effect of different exercise intensity to BDNF level in hippocampus of fructose-induced mice.

Material and Methods

This research was randomized post-test only control group design, which conducted at Animal Model Laboratory of the Department of Biochemistry, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia. The study was approved by the Health Research Ethics Committee, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia number 277/EC/KEPK/FKUA/2019. The present study followed animal welfare principles in experimental science published by the European Convention for the Protection of Vertebrate Animals.

Eight week-old mice, weighing 20-30 grams, were acclimatized for 2 week. The sample size was eight rats for each group, and the number of study groups was four groups. The group consisted of a control group (CON) which had given no exercise, low-intensity group (LIE) which had given exercise with load 3% of body weight, moderate-intensity group (MIE) which had given exercise with load 6% of body weight, and high-intensity group (HIE) which had given exercise with load 3% of body weight.

Mice were doing swimming exercise for 80% of their maximum swimming time, 3 times a week, in 28-32^{o}C water for 4 weeks. Swimming duration was determined by swimming ability test. Mice swam with load in their tail and then masured the duration they could swim until they drown. Before exercise began, mice had been administered by fructose solution via intragastric tube to increase fat mass. The dose of fructose solution was 270 grams for 9 days.[12]

Mice were terminated after the treatment had finished, and then the hippocampus tissue was incised. The hippoccampus tissues were minced to small pieces and rinsed in ice-cold Phosphate-buffered saline (PBS) to eliminate excess blood entirely. Tissue pieces were weighed and then homogenized in PBS. To further break the cells, the suspension was sonicated with an ultrasonic cell disrupter. The homogenates were then centrifuged for 5 min at 5000xg to get the supernatant.

ELISA method was used to determine mouse BDNF levels in the brain homogenate. All procedures and calculations were performed using a commercial kit according to the manufacturer's instructions (Elabscience Biotechnology, China). The optical density of each well was determined using a microplate reader set to 450 nm. BDNF concentrations (pg/mL) were calculated using standard curves.

Data were analyzed by Statistic Package for Social Science (SPSS) Statistics for Windows, version 16 (SPSS Inc., Chicago, IL, USA). Test of Normality was conducted using Shapiro-Wilk test, whereas, test of Homogeneity was conducted test using Levene test. Statistical difference was tested by using Independent Sample T-Test and One way-ANOVA. All data were

presented in mean±SD. All statistical analysis was conducted using level of significance (P<0.05).

Results

1. Animal characteristics

The initial body weight of animals in this research was, group CON 23.87±3.23 grams, group LIE 26.00±3.11 grams, group MIE 24.17±3.82 grams, and group HIE 26.43±2.82 grams. The body weight before treatment was not significantly different among groups (p=0.360).

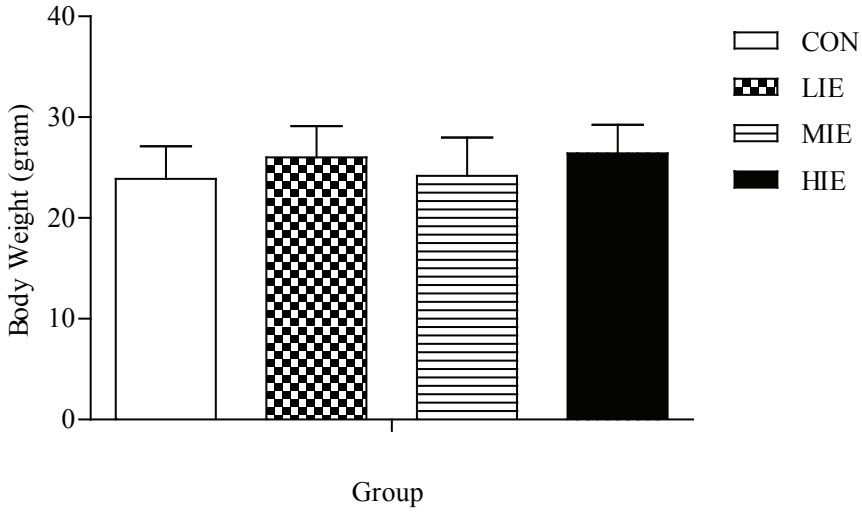

Fig. 1 The initial body weight of animals in all group

2. Increasing weight by fructose administration

The mean body weight of the mice before fructose administration was 26.21±3.80 grams, and the mean body weight of the mice after fructose administration was 33.25±4.10 grams. There was a 26.86% increase in body weight after fructose administration. This research revealed that there was a significant difference in body weight before and after administration of fructose solution for 9 days (p=0.000).

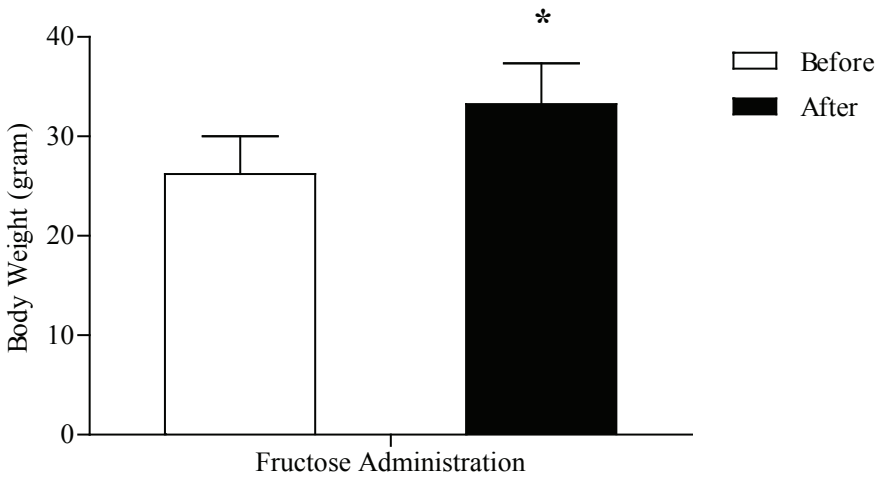

Fig. 2 The mean body weight of the mice before and after fructose administration

3. Hippocampal BDNF levels

The lowest BDNF levels after exercise treatment was group CON and the highest of BDNF levels was group MIE. The result of BDNF levels was illustrated in figure 1.

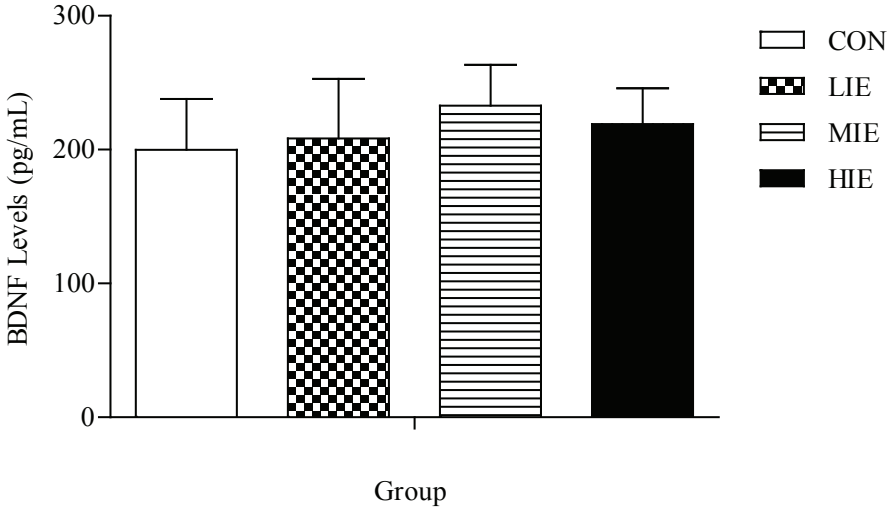

Fig. 3 Hippocampal BDNF levels after treatment in all group

There was no significantly difference in BDNF levels among groups after treatment (p=0.378). There was tendency for BDNF levels to increase with exercise intensity but BDNF levels decrease when exercise intensity increase to high.

Discussion

The absence of difference in body weight before treatment was consistent with homogeneity in this research so that the initial body weight did not have any effect on the results. However, there was significantly difference on body weight before and after administration of fructose solution. This result proved that fructose ingestion via intragastric tube could increase body weight of mice significantly. This results had the same pattern with the research conducted by Wulansari[12].

Fructose metabolism is different than glucose metabolism. The rate of fructolysis is not inhibited by its products.[12] High fructose in diet can serve as a relatively unregulated source of acetyl-CoA. Therefore, ingestion of high concentration of fructose can increase the rate of de novo lipogenesis.[2]

Fructose ingestion will make a positive energy balance that is energy intake is greater than energy expenditure. This excessive energy is stored in the form of fat accumulation. In long term, this can cause overweight and obesity.[6] Fructose does not stimulate insulin secretion from pancreatic β-cells. Thus, fructose consumption produce less postprandial insulin levels than glucose consumption does. On the other hand, fructose consumption reduce leptin levels in the circulation.[2] Leptin has effect of controlling appetite and body weight.[6] The combination effect of low insulin dan leptin can cause increase the likelihood of weight gain.[2]

Fructose consumption can decrease BDNF level. Administration of fructose in mice during gestation and weaning period caused the offspring to have lower hippocampal BDN levels compared to the control.[13] The reason of this is because administration of fructose up-regulate the methylation process of DNA in the BDNF promoter region. This can suppress the transcription of BDNF gene. Administration of glucose also decrease activity of demethylation process which decrease BDNF levels further.[13]

BDNF is a crucial molecule in controlling body weight and a key regulator of energy balance.[14] Mutation of the gene of BDNF and its receptor (TrkB) caused obesity, whereas deletion of BDNF gene in paraventricular hypothalamus caused hyperphagia, thermogenesis disturbance and severe obesity.[14] This study indicated that high fructose diet could cause body weight gain by de novo lipogenesis as well as decreased BDNF levels which further caused more weight gain.

Mice which exercised (LIE, MIE, and HIE) had higher BDNF levels than control group (CON), though it was not significant. The increase of BDNF levels caused by exercise is mediated by peripheral and central mediators, involving IGF-1, FNDC 5/irisin, cathepsin B, and activation of CREB gene.[15,16,17] Fibronectin type III domain-containing protein *5* (FNDC5) is a myokine secreted by myocites during exercise. The secreted form of FNDC5 is called irisin.[18] Physical exercise increase FNDC5 levels in skeletal muscles and hippocampus. This will lead to the increasing of BDNF gene expression.[17,18]

This study showed that BDNF levels in MIE is greater than HIE, and BDNF levels in HIE is greater than in LIE. This indicated that there was not always a positive correlation between exercise intensity and subsequent health benefits. Exercise will consume an amount of oxygen. This will lead to stress to the body metabolic system and result in increasing of oxidative stress. In moderate-intensity exercise, this will enforce the body defense system to produce more anti-oxidant. However, in high-intensity exercise, the anti-oxidant produced by the body cannot encounter the oxidative stress. This lead to accumulation in oxidative stress.[15] This oxidative stress reduce BDNF levels in hippocampus.[9,15]

The increase of BDNF levels in this results had difference pattern with the other research. Previous researches showed that exercise, treadmill for rats and aerobic for human, increased BDNF expression through regulation of BDNF gene in hippocampus.[11,19] Mice which exercised with running wheel had an increase in BDNF mRNA levels in the dentate gyrus and this increase was maintained several weeks after exercise.[15]

There is a dose-dependent relationship between duration, exercise intensity and the result of increasing BDNF levels in exercise.[20] This indicated the existence of minimal doses (threshold) for exercise needed to increase BDNF levels. We assumed that to get the expected result, the right dose of exercise is needed.

In term of exercise duration, long-term exercise had greater increase in BDNF levels compared to short-term exercise. In the study of Griffin *et al.*[21], 3 weeks of exercise had not increased BDNF levels yet. However, when the exercise continued for 5 weeks, there was an increase in BDNF levels.[21] Intensity of exercise had a role for the effect exercise in increasing BDNF levels. Study which used low and high intensity exercise for rats showed that high-intensity exercise had significantly greater increase in hippocampal BDNF level compared to the low-intensity exercise.[15]

Type of exercise could determine the increase in BDNF levels too. Several studies used running exercise because running had been proved to increased BDNF levels and cognitive function.[8,18] Running exercise showed lower threshold for BDNF-mediated long term potentiation in dentate gyrus.[22] In other studies, high-intensity exercise with interval-method had a greater increase in BDNF levels than continue-method.[23,24]

In addition, the result of BDNF level measurement is affected by the time of examination. BDNF levels which was measured 60 minutes after exercise showed moderate increase. Examination of resting BDNF levels in human subject with programmed routine exercise had a little increase in BDNF level.[25] In this research, the examination was done in resting condition so that the increase of BDNF levels was a little.

Conclusion

Based on the results of this study, fructose administration can be used to increase deposit of adipose tissue and body weight. The good effect of exercise can be seen in the increased of BDNF levels. However, exercise intensity has no effect on BDNF level in the hippocampus of fructose-induced mice. Further research needs to explore the certain dose of exercise such as longer exercise duration and interval method.

Conflict of Interest: The authors declared that there is no conflict of interest.

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