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Original Article

The effect of regular aerobic exercise on urinary brain-derived neurotrophic factor in children

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Abstract

Background Nervous system development in early life influences the quality of cognitive ability during adulthood. Neuronal development and neurogenesis are highly influenced by neurotrophins. The most active neurotrophin is brain-derived neurotrophic factor (BDNF). Physical activity has a positive effect on cognitive function. However, few experimental studies have been done on children to assess the effect of aerobic regular exercise on BDNF levels.

Objective To assess the effect of regular aerobic exercise on urinary BDNF levels in children.

Methods This clinical study was performed in 67 children aged 6-8 years in Palembang. The intervention group (n=34) engaged in aerobic gymnastics three times per week for 8 weeks, while the control group (n=33) engaged in gymnastic only once per week. Measurements of urinary BDNF were performed on both groups before and after intervention. Mann-Whitney and Wilcoxon rank tests were used to analyze the differences between groups.

Results There was no difference in urinary BDNF levels between the two groups prior to the intervention. After intervention, the mean urinary BDNF levels were significantly higher in the intervention group than in the control group, 230.2 (SD 264.4) pg/mL vs. 88.0 (SD 35.4) pg/mL, respectively (P=0.027). We also found that engaging in aerobic gymnastics significantly increased urinary BDNF levels from baseline in both groups (P=0.001).

Conclusion Regular aerobic exercise can increase urinary BDNF levels and potentially improve cognitive function. Aerobic exercise should be a routine activity in school curriculums in combination with the learning process to improve children's cognitive ability. **[Paediatr Indones. 2014;54:351-7.]**.

Keywords: *aerobic gymnastics, urinary BDNF, children*

Cognitive ability is influenced by neuronal system maturation in histological structure and function in the brain. The development of the neuronal system in children affects the quality of cognitive function in adulthood.[1] Various factors influence the development of cognitive abilities, such as genetics, nutrition, stress, aging, and experience or learning stimulation.[2] Neuronal growth and development through precursor cells is called neurogenesis.[3] Neurogenesis relies on a group of proteins, known as neurotrophins, that stimulate neuronal growth, development, plasticity, and durability. Brain-derived neurotrophic factor (BDNF) is the most active neurotrophin. In humans, BDNF is mainly expressed in the hippocampus, the hypothalamus, and the cerebral cortex.[4]

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Physical activity has been shown to have a positive effect on cognitive function.[5] Several studies in animals and humans have shown that exercise stimulates an increase of BDNF expression in the brain, resulting in the growth of new neurons, increased neuronal differentiation and synaptic enhancement between neurons.[6--8] Exercise improves cognitive function through three mechanisms: exercise spurs neurogenesis by improving neuroplasticity;[9-12] exercise improves neuron survival,[11-13] and exercise improves brain vascularization which supports neurogenesis and neuronal survival.[7,10,11]

Exercise has been shown to stimulate neurogenesis, but few studies have looked at the effect of physical exercise on the neuroplasticity process in children, as influenced by BDNF. A limited number of studies have been done to assess the effect of a regular aerobic exercise program on neuronal development, using a biological approach. The aim of this study was to assess the effect of a regular aerobic exercise program on urinary BDNF levels in children.

Methods

This clinical study was carried out in children aged 6 to 8 years who attended the Madrasah Ibtidayah (MI) Najahiyah, Palembang, in the first semester of 2013. This age bracket was chosen because these children are still undergoing growth and development, and are able to cooperate, but have had limited exposure to a regular exercise program. Of 90 students, 76 students met the inclusion criteria of age and parental consent. We excluded students who were already in a regular exercise program outside of school and those with acquired or congenital disabilities. Subjects were randomized into intervention and control groups.

After intervention, 9 subjects dropped out because they either did not attend the gymnastics sessions more than 5 times or they did not provide urine specimens. A total of 67 subjects were analyzed, consisting of 34 subjects in the intervention group and 33 subjects in the control group (**Figure 1**).

Both groups engaged in gymnastics exercise. The

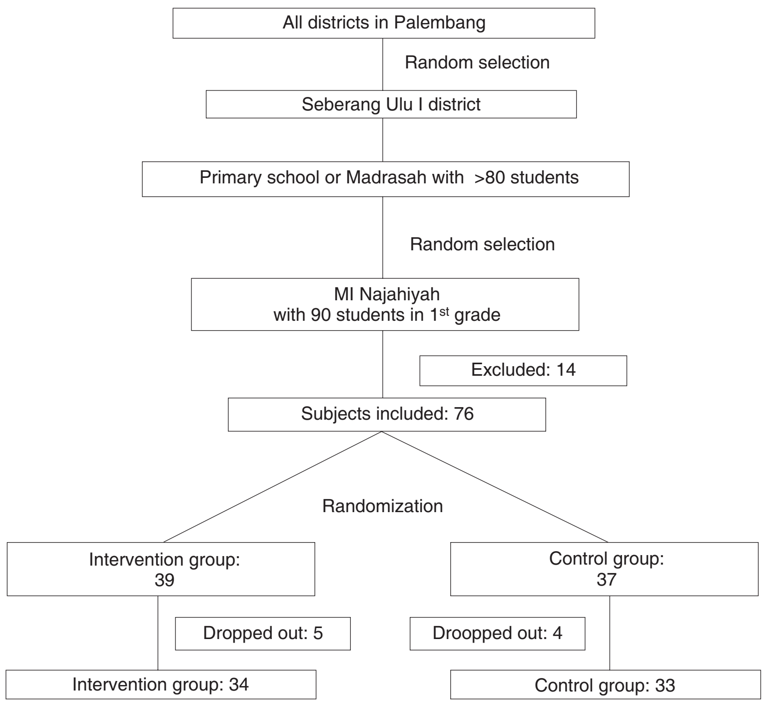

Figure 1. Subject collection and randomization

intervention group exercised 3 sessions per week for 8 weeks (24 sessions). Each exercise session was guided by a trained instructor, and conducted for 40-45 minutes in three stages: 5-minute warm up, 30-minute core exercise, and 5-10-minute cool down. During the core exercise, subjects performed imaginative and fun gymnastics movements, such as combinations of fun animal movements, worship motion exercises, and work activity movements. When undergoing exercise, subjects aimed to reach an aerobic exercise intensity target pulse rate of approximately 125-150 beats per minute. The control group exercised 1 session per week for 8 weeks (8 sessions). Control group subjects were guided by a local schoolteacher, with similar movement to intervention group but without intensity and time targets.

Based on ethical consideration, the authors could not measure BDNF levels from blood sample, so that, BDNF levels were measured from morning urine specimens collected just after subjects woke up. Urine specimens were collected twice: at 1-2 days before and 2-3 days after the 8-week training program. Laboratory officers waited at the school to collect specimens from subjects. Urine specimens were centrifuged to remove particulates and supernatants were stored temporarily at -80°C in the laboratory. Specimens were sent to the Makmal Terpadu Laboratory of Immunoendocrinology, University of Indonesia in Jakarta for analysis. Measurements of BDNF were performed by an enzyme-linked immunosorbent assay (ELISA) method using an *Abcam®* anti-human BDNF antibody kit, following manufacturer's instructions. Laboratory personnel were blinded to the identity of the specimens. Prior to the measurement of subjects' urinary BDNF levels, we calibrated the ELISA Kit for *Thermo® Multi-scan* ELISA reader. **Figure 2** shows that the ELISA Kit had high validity at various dilutions (r = 0.999), suggesting that laboratory measurement bias was low.

All data were analyzed using the *Microsoft ® Office Excel 2007* and *Statistical Package for the Social Sciences* (*SPSS*) version 13.0 software. Differences in BDNF levels were analyzed with the Wilcoxon-signed rank test and Mann-Whitney U test. The study was reviewed and approved by the Faculty of Medicine Unit for Bioethics and Humanities at the University of Sriwijaya.

Results

Table 1 shows that median age and nutritional status in the intervention group were not different with control group.

Subjects' patterns of physical activity and additional lessons outside school hours were traced in order to minimize bias. All subjects in both groups had similar patterns of physical activity. Most subjects were moderately active for 2-3 hours per day, engaging in ball games or cycling. The rest of their time was spent on low level activities, such as watching television, playing video games, studying, and sleeping. Their modes of transport to school were also similar, with 95.5% of subjects walking and 3 subjects (4.5%) cycling. None of the subjects in either group were engaged in regular exercise programs nor had additional lessons outside school hours.

Table 2 and **Figure 3** show that there was a significant increase in urinary BDNF levels in both groups after routine exercise. The control group had

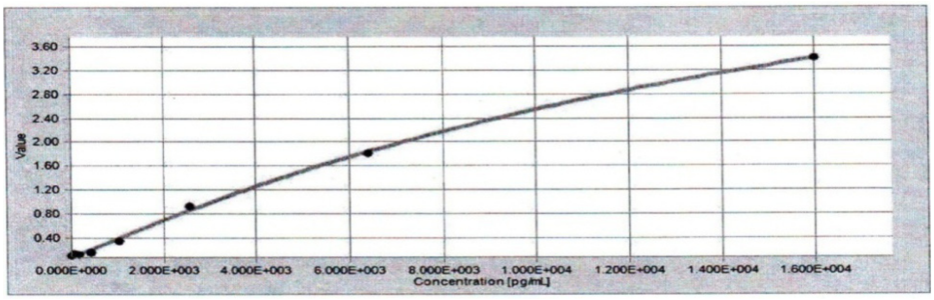

Figure 2. Quantitative curve of *ab99978 Human BDNF ELISA Kit*

Table 1. Baseline characteristics of subjects

Characteristics	Intervention group n=34	Control group n=33
Median age (range), years	6.8 (5.8–7.3)	7.0 (5.7–7.8)
Median weight (range), kg	17.3 (13.0–27.0)	18.0 (13.5–29.0)
Median nutritional status (range), %	90.5 (74.3–122.0)	93.0 (80.0–123.8)
Nutritional status		
Undernourished, n (%)	17 (50.0)	10 (30.3)
Well-nourished, n (%)	16 (47.1)	21 (63.6)
Overweight, n (%)	1 (2.9)	2 (6.1)
Gender		
Female, n (%)	20 (58.8)	17 (51.5)
Male, n(%)	14 (41.2)	16 (48.9)

significantly increased levels of BDNF after they exercised once per week. For the intervention group, a regular gymnastics exercise program was undertaken 3 times per week, following a recommended program.[14] After the exercise program, the intervention group had significantly increased BDNF levels far exceeding that of the control group (P=0.027). **Table 3** shows the median BDNF levels by gender. Statistical analysis revealed no significant difference in BDNF levels between genders in both groups, suggesting that BDNF levels are not influenced by gender.

Discussion

Our results suggest that regular physical activity increases BDNF production. There are several factors that affect the production of BDNF, such as

Table 2. Urinary BDNF level before and after exercise program

BDNF levels	Intervention group n=34	Control group n=33	Mann-Whitney U test
Mean BDNF (SD), pg/mL			
Before	30.9 (22.9)	48.6 (43.9)	-
After	230.2 (264.4)	88.0 (35.4)	
Median BDNF (range), pg/mL			
Before	27.9 (7.6–141.9)	27.9 (2.5–152.8)	[B]P=0.589
After	95.6 (43.4–1190.0)	89.9 (19.0–193.0)	[B]P=0.027
Wilcoxon rank test	[A]P=0.001	[A]P=0.001	

[A]Differences before and after in same group

[B]Differences before and after intervention between groups

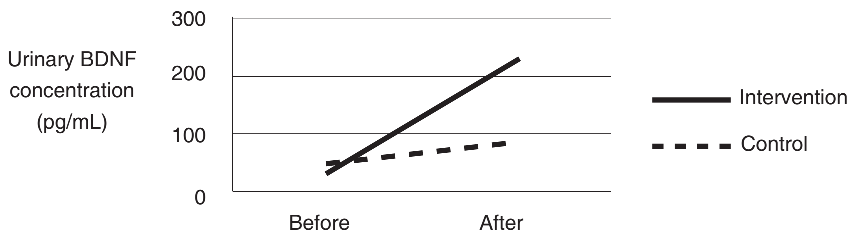

Figure 3. The mean urinary BDNF levels before and after exercise program

Table 3. Urinary BDNF levels before and after exercise program by gender, regardless of group

Median BDNF (range), pg/mL	Female n=37	Male n=30	Mann-Whitney U test
Before	29.9 (2.5–125.0)	22.8 (7.6–152.8)	P=0.277
After	95.0 (19.0-1190.0)	86.3 (31.6-323.0)	P=0.076

age, physical activity, body weight, body mass index, nutritional status, gender, and genetics.[2] To reduce bias, authors conducted the equivalency of age, sex, nutritional status, and physical activity levels between two groups by exclusion criteria. The age of less than 6 or more than 8, extreme nutritional status, and a child with history of regular exercise were excluded. Both groups were not matched because of difficulties to arrange exercise schedule, and some children were drop out from study. The study was conducted on students in the same school in order to avoid differences in curriculum. None of our subjects had additional lessons outside the school curriculum. Children i n both groups had similar patterns of physical activity and none followed regular exercise programs. Statistical analysis showed no differences in age, sex and nutritional status in both groups.

Subjects collected morning urine specimens upon waking up and these specimens were used to measure BDNF levels. The neurotrophin is an anabolic factor which is active in recovery period. Sleep is one of the recovery phases, during sleep there is rearrangement of proteins for rebuilding the body. There was activation of anabolic hormones in order to reconstruct the protein filaments that were damaged because of the stress. Activation of these anabolic hormones will stimulate the activation of growth factors like NGF and BDNF.[14-16] Early morning urine collection is also intended to reduce the variations in physical activity which may influence BDNF synthesis. Earlier studies have claimed that BDNF levels may increase 44-83% after high levels of physical activity.[17] Zoladz *et al*. found a six-fold increase of plasma BDNF in young adults after physical activity.[18] Previous human studies have been limited to measuring BDNF levels in young adult plasma after physical activity.[17-19] However, animal studies have reported increased BDNF expression in the brain.[20-22]

Gymnastics is an aerobic physical activity. During aerobic exercise, the body, including the brain, needs greater oxygen and calorie supplies, resulting in intermittent hypoxia and hypoglycemia. Intermittent hypoxia and hypoglycemia triggers the production of HIF-1α and sirtuin proteins. These gene transcription proteins stimulate the production of anabolic factors, such as BDNF and NGF, stimulate the synthesis of vasculo-endothelial growth factors (VEGF) for improving blood flow, and increase the production of various antioxidants to reduce inflammation.[1,23,24]

Brain-derived nerotrophic factor activates various metabolic circuits to stimulate neurogenesis and the neuronal apoptotic cycle,[17] and stimulates the growth of dopaminergic and cholinergic ganglion synapses, sensory neurons and motor neurons. This nerotrophic factor also activates signal transduction through dimerization and autophosphorylation of the TrkB receptor, as well as induces neuritin, that stimulates mitotic differentiation of neurons.[5,6,28] A stimulating lifestyle with improved physical activity and learning processes increase the expression of BDNF in the brain. Movement stimulation increases brain plasticity, characterized by accretion of synaptic connectivity, axon elongation, growth collateral ramifications, and remodeling. The addition of neuronal connectivity increases cognitive levels.[26]

Gymnastic exercises involve complex motions. In this study, subjects performed mild-moderately intense aerobic exercise with unique but simple movements. Most of the children were enthusiastic to engage in the exercise. The gymnastics movements mimicked those of animals, regular work, and worship. These movements evoked a child's imagination to build on the intellectual component. Gymnastics increase the formation of specific proteins and neuronal synapses to improve complex connectivity between synapses.[27] Gymnastics also regulates balance, due to allowing collaboration between the right and left brain without competition, a collaboration which is difficult to achieve with drugs, surgery, or other means. The longer the training, the more parts of the brain collaborate without inhibit the activities of other parts of the brain. This collaboration brings emotion and cognition to the same level, allowing the brain to give the same attention to both paths.[28]

The BDNF levels also increased in our control group. The control group also performed gymnastics, but only once per week included in the regular class curriculum. Even this limited amount of activity was associated with increased BDNF production of twice that of baseline, although this increase was not as high as in the intervention group.

In conclusion, children who engaged in a regular aerobic exercise program have increased BDNF levels, which may act to build a child's cognitive abilities. Therefore, routine stimulation with aerobic exercise should be added to school curriculums, in addition to the learning stimulation they received. Educational institutions may need to review the curriculum so that regular physical activity programs become part of intra-curriculum activities. Exercise should be regularly implemented 3-4 times per week and reach the recommended aerobic intensity.

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