ISSN : 1225-9918
Journal of Life Science **2012** Vol. 22. No. 5. 569~574 DOI : http://dx.doi.org/10.5352/JLS.2012.22.5.569

The Effects of Exercise on Neurotrophins, Hepatocyte Growth Factor (HGF), and Oxidative Stress in Obese Children

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Received March 23, 2012 / Revised April 18, 2012 / Accepted May 3, 2012

This study was conducted to investigate the effect of exercise on oxidative stress, nerve growth, and hepatocyte growth factors in obese children. After 12 weeks of aerobic exercise training, the aforementioned parameters before and after the training were compared. As a result, the nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) were shown to be lower in the OT than in the NT before and after the training, respectively (p<0.05). The NGF was shown to have increased in both groups after the training (p<0.05). The hepatocyte growth factor (HGF) was shown to be higher in the OT than in the NT before the training (p<0.05), with no difference found afterwards. The malondialdehyde (MDA), ox-LDL, and 8-OHdG (Oxo-2'-deoxyguanosine) were shown to be higher in the OT than in the NT (p<0.05). For ox-LDL, a difference was found between before and after the training (p<0.05). The results of this study showed that obesity induced oxidative stress and caused the abnormalities of nerve and HGF secretion in obese children, and that the 12 weeks of aerobic exercise increased NGF levels, thereby promoting the development of neurogenesis in children.

Key words : Exercise training, neurotrophins, hepatocyte growth factor, oxidative stress, obese children

Introduction

NGF is a protein that is secreted by the order of the neuron, and plays an important role in the maintaining of the sympathetic nervous system and sensory neuron, and biological activities including cell growth [17]. Previous studies on NGF related to obesity reported that plasma NGF level was low in patients with morbid obesity, and that it was correlated with inflammatory factors, BMI, %fat and waist circumference [2]. In addition, NGF was reported to be higher in obese animal models than in lean animal models in terms of genetic perspective as it is synthesized and secreted from brown adipocytes, but its level was reported to vary depending on age [13]. In a previous study on NGF and exercise, Radak reported that NGF increased and memory function was improved in rats when the rats underwent swimming training for 8 weeks [14]. Schulz reported that NGF level and memory function significantly increased in the exercise group when an eight-week bicycle training was conducted on patients with multiple sclerosis [16].

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BDNF, which is another factor of nerve growth, is a protein found in the peripheral system as well as in brain. It is activated in a specific neuron of central and peripheral nervous systems, and plays a role in supporting the survival of neurons. In addition, it promotes the growth and differentiation of new neurons and synapses, thereby supporting the activation of learning, memory function, and intelligence. Several studies have been conducted to investigate a relationship between exercise and BDNF. Previous studies conducted on rats reported that BDNF expression increased after aerobic exercise [12]. However, a previous study conducted on human reported that no correlation of BDNF with exercise was found [11].

On the other hand, HGF promotes the regeneration of various tissues, has biological activities of mitosis promotion, motility promotion, morphogenesis, strengthens kidney regeneration, and inhibits hypertension. Thus, it has been reported to be closely associated with cardiovascular diseases such as hypertension and atherosclerosis [10]. Yasuda reported that HGF level was significantly lower in 20 patients with cardiovascular diseases than in the normal group, but that the HGF level significantly increased in the pulmonary vein and aorta after sub-maximal intensity exercise

training [20]. The aforementioned result indicated that there was a positive relationship between exercise and HGF in cardiovascular diseases. Obesity was reported to cause lipid profiles imbalance [6]. In addition, it was reported to cause immune system abnormalities, resulting in high inflammatory responses [15].

In fact, tissue oxidative stress is higher in obese group than in normal weight group. Furthermore, lipid profiles imbalance and lipid damage caused by obesity might increase a risk of cardiovascular disease, and negatively affect HGF secretion in children. There is a hypothesis that a regular exercise could positively affect neurogenesis process by improving obesity. However, few studies about the aforementioned hypothesis have been conducted to date. In particular, no study has been conducted to investigate the effect of increased oxidative stress caused by obesity on growth factors such as neurotrophins and HGF, and the effect of a regular exercise on the improvement of oxidative stress caused by obesity.

Accordingly, in this study, a comparative analysis of NGF, BDNF, HGF, and oxidative stress was conducted between obese and normal weight children who underwent 12–week aerobic exercise training with the exercise intensity set by the calculation of individual energy expenditure.

Materials and Methods

Subjects

Of obese children aged 10-12 years with body mass index (BMI) > 95th percentile or obesity index (%) > 120%, who were recruited via the interview with obesity specialists, and poster and internet, 15 children were randomly selected as subjects. The obesity index was calculated using a formula of [obesity index (%)=(actual body weight-standard body weight)×100/standard body weight] by setting 50th percentile of %body weight for height in Korean pediatrics and adolescence as the standard body weight [19]. In addition, 15 normal children with the same sex and age as the obese children were recruited as a control group. Both obese and normal children, and their parents signed an informed consent form, and the study was conducted after the approval of the institutional review board.

Training Program

The aerobic exercise program of this study was performed four durations a week for 12 weeks. For 1st~3rd week, HRR 40% intensity was set, and the total consumption calorie was calculated by multiplying a targeted consumption calorie of mean 3.50 kcal/kg and the individual body weight. The exercise duration was calculated by dividing the heart rate corresponding to the exercise intensity HRR 40% by energy expenditure/min. For 4th~12th week, HRR 60% intensity and a targeted consumption calorie of mean 4.38 kcal/kg were set. The heart rate and exercise duration were calculated using the method used in 1st~3rd week [19].

Maximal oxygen uptake test

Before and after the training, a maximal oxygen uptake test was conducted using a gas analyzer (Quark b2, Cosmed, Italy) and a treadmill (Intertrack 6025, Taeha, Korea). The modified Balke treadmill protocol, which was developed for children with low fitness level, was used, and the test was conducted under the supervision of pediatric specialists [19].

Biochemical analysis

The NGF was analyzed using antibody-immobilized beads 87-human NGF reagent and human serum adipokine (panel B) kit. The HGF was analyzed using Antibody-Immobilized beads 87-Human HGF reagent and human serum adipokine (panel B) kit in the same way used for NGF. The BDNF was measured via ELISA. The MDA was analyzed using a spectrophotometer and a BIOXYTECHLPO-586 kit (Oxis Co, USA). The ox-LDL level was measured via sandwich enzyme-linked immuno sorbent assay using murine mono clonal antibody mAb-4E6, and the ox-LDL attached to the solid phase was detected using peroxidase-conjugated anti apolipoprotein B antibody (Mercodia AB, Sweden). The 8-OHdG was measured using a spectrophotometer (Hewlette Packard Co, USA) and a DNA damage ELISA kit (Assay Designs, USA).

Data analysis

For all data obtained from this study, their mean and standard deviation were calculated using SPSS Windows Ver 18.0 statistics package. Two-way ANOVA by repeated measurement was conducted to test differences in the body composition, cardiorespiratory function, and blood biochemical elements according to group and exercise duration after the training. A statistical significance level of α=0.05 was used in this study.

Results

Changes in the blood biochemical elements are presented in Fig. 1, 2, 3, 4, 5, and 6. Before the training, the NGF and BDNF were shown to be significantly lower in the OT group than in the NT group (p<0.05), whereas the HGF was shown to be significantly higher in the OT group than in the NT group (p<0.05). After the training, the NGF and BDNF were shown to be significantly lower in the OT group than in the NT group, whereas no difference in the HGF was found. Compared to before the training, the NGF was only shown to have increased in the both groups after the training (p<0.05), but no difference in other factors was found. The MDA was shown to be higher in the OT group than in the NT group before and after the training, respectively (p<0.05), but no difference was found between the periods. The ox-LDL was shown to be higher in the OT group than in the NT group before and after the training, respectively (p<0.05). The ox-LDL level was shown to have increased in the both groups after the training (p<0.05). The 8-OHdG was shown to be higher in the OT group than in the NT group before the training (p<0.05), but no difference was found between the periods.

Discussion

Obesity causes antioxidant defense capability weakness and an easy exposure to oxidative stress. Reactive oxygen species (ROS), which is an inducer of oxidative stress, deforms various tissues, and negatively affect nerve cells, leading to abnormal nerve growth. Neurotrophin (NT), which has been known to be involved in the development and functions of neurons, is a life-sustaining protein. It consists of four factors (NGF, BDNF, neurotrophin-3 (NT-3), and neurotrophin-4 (NT-4)) in a structural point of view [8]. NGF and BDNF, the most well-known nerve growth factors that belong to NT family, play an important role in the differentiation, proliferation, and survival of neurons as well as the maintenance and development of sympathetic nervous system and sensory nervous system [5].

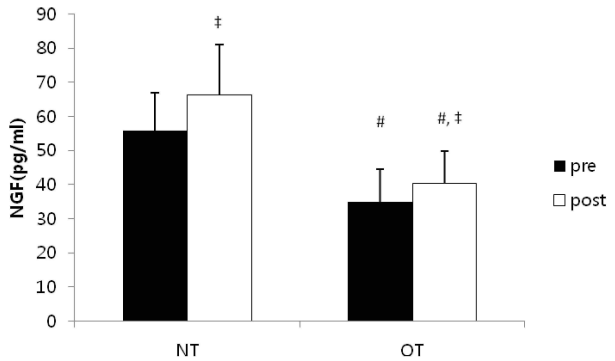

Fig. 1. Effect of exercise training on NGF. # within group pre vs post: p<0.05, ‡between group NT vs OT: p<0.05

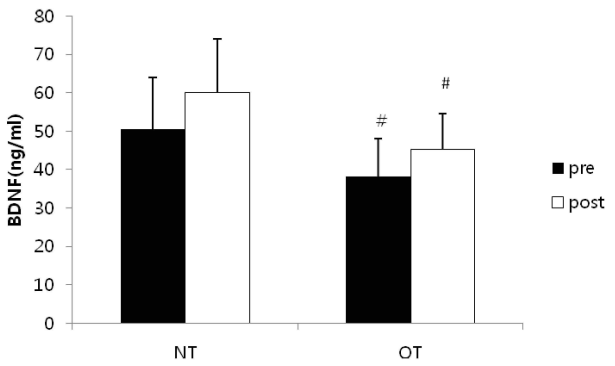

Fig. 2. Effect of exercise training on BDNF. # within group pre vs post: p<0.05, ‡between group NT vs OT: p<0.05

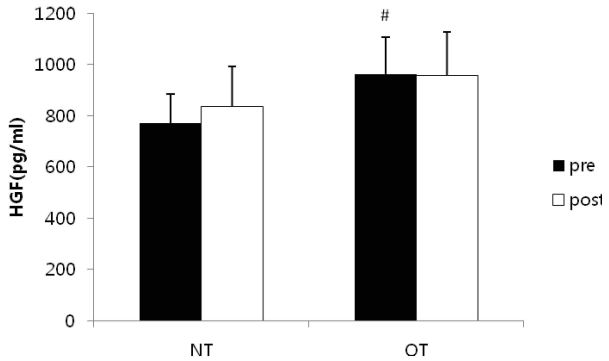

Fig. 3. Effect of exercise training on HGF. # within group pre vs post: p<0.05, ‡between group NT vs OT: p<0.05

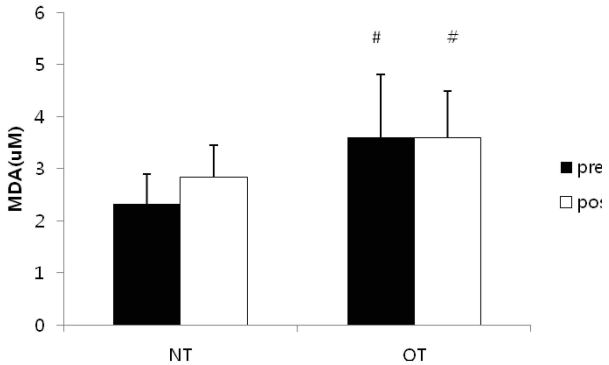

Fig. 4. Effect of exercise training on MDA. # within group pre vs post: p<0.05, ‡between group NT vs OT: p<0.05

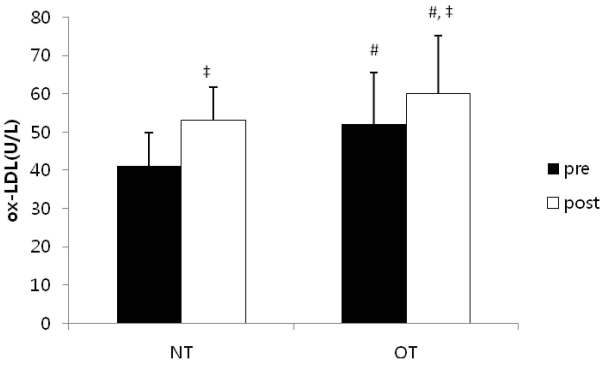

Fig. 5. Effect of exercise training on ox-LDL. # within group pre vs post: $p<0.05$, ‡between group NT vs OT: $p<0.05$

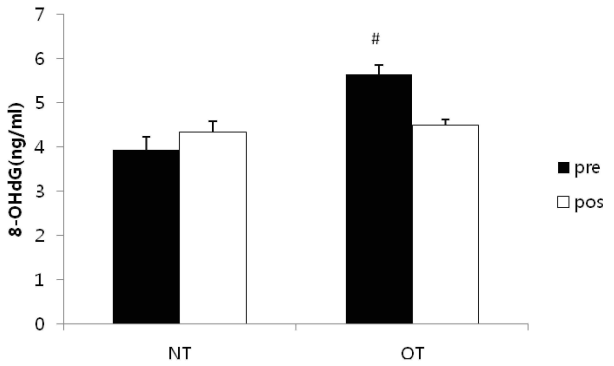

Fig. 6. Effect of exercise training on 8-OHdG. # within group pre vs post: $p<0.05$, ‡between group NT vs OT: $p<0.05$

In this study, the MDA, a marker of lipid peroxidation, the ox-LDL, a marker of oxidative LDL-c, the 8-OHdG, a marker of DNA damage, were shown to be higher in obsess children than in normal children, which was consistent with results obtained from adults. ROS, which causes oxidative stress, causes the damage and neurodegeneration of cells, and deforms the functions of protein, lipid and DNA. Furthermore, it could affect the apoptosis of neuronal cells [3]. NT has been recently regarded as a potential adipokine associated with metabolic syndrome and inflammatory diseases [9]. Tissue damages caused by oxidative stress have been known to cause abnormal adipocytokine secretion in fat tissues [7]. It has been suggested that NGF should be regarded as an adipokine as it is directly secreted from adipocytes [18]. The abnormal secretion of adipokine could cause abnormal neurogenesis, which, in turn, negatively affects neuron growth. The reason for the association of NGF with obesity is that NT is sensitive to inflammatory responses. That is, NGF has a positive relationship with inflammatory markers, and it is negatively affected by metabolic syndrome factors such as obesity. As a result of this study, the blood NGF and BDNF levels were shown to be lower in obese children than in normal children before and after the training, respectively, which was opposite to the results obtained from the markers of blood oxidative stress. This result suggests that obesity, profiles imbalance, and increased oxidative stress reduce the secretion level of nerve growth factors.

A regular exercise has been known to enhance antioxidant capacity. In addition, it has been suggested that the regular exercise positively affects neurogenesis. In studies using animals [14] and humans [16], an eight-week aerobic exercise was reported to have increased NGF level and improved memory function. Thus, it has been speculated that exercise promotes normal NGF secretion by reducing oxidative stress. This phenomenon has been also observed for BDNF. Neeper reported that BDNF mRNA expression increased in rats after three-day running training [12]. Aderbal reported that a regular exercise increased the expression of neurotrophic factors such as NGF and BDNF in rats, thereby improving brain function [1]. This result indicates that a regular exercise positively affects an increase in neurotrophic factors. Meanwhile, in a recent study conducted on middle aged men and women, Levinger reported that a ten-week weight training increased muscle strength and lean body mass, but did not affect BDNF [11]. They suggested that a further study was required to investigate a relationship between aerobic training and dietary therapy. In this study, changes in the NGF and BDNF were compared according to exercise duration and subject group in obese children after 12-week training. As a result, as for BDNF, a difference in the BDNF was observed between the groups. Meanwhile, as for NGF, a difference in the NGF was observed between the groups, and the 12-week training increased the NGF level. Based on the results of this study, a regular exercise is likely to stimulate neurogenesis, and induce the activity of nerve growth factors, thereby strengthening learning and cognitive functions [4], as well as to play a positive role as a major regulator of cell growth.

HGF has been known to be the most potent factor that promotes the growth of hepatic cells. It has been also known to promote the growth of various cells such as melanocytes and proximal tubular epithelial cells besides hepatic cells. In addition, as HGF is synthesized from human adipocytes, serum HGF in obese patients was shown to be three times higher than that in normal persons. Obesity is closely associated with a risk of cardiovascular diseases. Thus, it is important to study the mechanism of serum HGF elevation in

obese patients. Studies on serum HGF have been conducted on obese adults in most cases, and to investigate its association with various chronic diseases. However, it is unclear whether HGF is important with respect to hepatocyte growth or potential risk factor of cardiovascular disease in children. In this study, the serum HGF level was not affected by the training, and was shown to be significantly higher in the OT group than in the NT group. This result is opposite to the result that the levels of nerve growth factors such as NGF and BDNF were lower in obese children. It is reasonable to consider HGF as one of risk factors of cardiovascular diseases caused by obesity rather than as a cell growth factor in obese children as shown in obese adults. This study showed that it was difficult to change HGF level via 12-week exercise training.

In conclusion, the results of this study showed that obesity caused oxidative stress, and negatively affected the secretion of nerve growth factors in children, but that the 12-week aerobic exercise increased the NGF level, thereby promoting the development of neurogenesis in children.

Acknowledgement

This work was supported by the National Research Foundation of Korea Grant funded by the Korean Government (KRF-2009-332-G00093).

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초록 : 운동 트레이닝이 비만 어린이의 neurotrophins, HGF (hepatocyte growth factor)와 산화스트레스에 미치는 영향

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본 연구는 운동 트레이닝이 비만 어린이의 산화적 스트레스, 신경성장 및 간 세포성장 인자에 미치는 영향을 알아보기 위하여 12주간 유산소 운동을 실시한 후 트레이닝 전과 후의 농도 수준을 비교하였다. 연구결과, NGF와 BDNF는 트레이닝 전과 후 모두 OT군이 NT군 보다 낮았으며, NGF는 트레이닝에 따라 두 그룹 모두 증가되었다. HGF는 트레이닝 전 OT군이 NT군 보다 높게 나타났지만, 트레이닝 후 차이는 없었다. MDA, ox-LDL, 8-OHdG 모두 OT군이 NT군 보다 높은 수치를 나타냈고, 트레이닝에 따른 차이는 ox-LDL에서만 발견되었다. 이상의 결과, 비만은 성장기 어린이에게 산화적 스트레스를 유발하고, 신경과 간 성장인자의 분비 이상을 초래한다는 것을 알 수 있었고, 12주 유산소 운동은 이들 인자들의 개선에 긍정적인 영향을 주고 인지기능 향상에도 도움을 줄 수 있을 것으로 본다.