Effect of resistance training at different intensities on hippocampal neurotrophic factors and peripheral CCL11 levels in obese mice

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Abstract : We investigated the effect of moderate- and high-intensity resistance training on hippocampal neurotrophic factors and peripheral CCL11 levels in high-fat diet (HFD)-induced obese mice. C57/black male mice received a 4 weeks diet of normal (control, CON; n = 9) or a high-fat diet (HF; n = 27) to induce obesity. Thereafter, the HF group was subdivided equally into the HF, HF + moderate-intensity exercise (HFME), and HF + high-intensity exercise (HFHE) groups (n = 9, respectively), and mice were subjected to ladder-climbing exercise for 8 weeks. The hippocampal brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) levels were significantly lower in the HF group than in the CON group (p < 0.05). In addition, in the HFME and HFHE groups were significantly higher than in the HF group (p < 0.05). The peripheral CCL11 levels were significantly higher in the HF group than in the CON group (p < 0.05). In addition, in the HFME and HFHE groups were significantly lower than in the HF group (p < 0.05). However, there was no significant difference according to the exercise intensity among the groups. Collectively, these results suggest that obesity can induce down-regulation of neurotrophic factors and inhibition of neurogenesis. In contrast, regardless of exercise intensity, resistance training may have a positive effect on improving brain function by inducing increased expression of neurotrophic factors.

Keywords : resistance training, exercise intensity, obesity, neurotrophin, eotaxin-1

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1. Introduction

Obesity is a major cause of chronic diseases and has a negative impact on the structure and functions of the brain [1,2]. Obesity can cause down-regulation of the neurotrophic factors (NTFs), which play an important role in neuroplasticity and neurogenesis, and weakening or impairment of the permeability of the blood-brain barrier, which protects the brain [3,4].

Improvement in physical activity through regular exercises is an effective method to prevent or treat obesity [5]. In addition, physical exercises are effective in maintaining and enhancing brain functions and alleviating symptoms of brain disease, with increase in NTFs, such as brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) [8,9]. Previous studies examined changes in the circulating NGFs with various exercises through an analysis of the blood BDNF and NGF concentrations in humans. The results suggested that NTFs are elevated with chronic and acute exercises as well as aerobic and resistance exercises [10,11]. Additionally, studies suggest that NTF levels may depend on the intensity of exercises [12]. However, most previous studies were limited to aerobic exercises, and conflicting results were reported in studies with various animal models. In particular, Lee et al. (2009) reported that BDNF and tyrosine receptor kinase B levels significantly increased in the hippocampus of rats after running at a low or moderate intensity but not after running at a high intensity [13]. Recently, Ghodrati-Jaldbakhan et al. (2017) reported that the hippocampal BDNF levels that were reduced with morphine administration did not increase after treadmill exercises and decreased after high-intensity exercises [14].

CCL11 (eotaxin-1), which is a chemokine secreted from the airways, epithelial cells, monocytes, and fibroblasts, induces accumulation of eosinophils in the lungs [15].

However, increased blood CCL11 levels are significantly correlated with inhibition of neurogenesis [16,17]. Vasudevan et al. (2006) reported a significant increase in serum eotaxin levels in obese mice with a high-fat diet (HFD) [18].

Obesity can cause down-regulation of NTFs. Although there is a possibility of increase in blood CCL11 levels inhibiting neurogenesis, related studies are limited, and no studies have confirmed changes induced by interventions with resistance training at various intensities. Therefore, the purpose of this study was to examine the effects of moderate- and high-intensity resistance training on hippocampal NTFs and peripheral CCL11 levels in obese mice on a HFD.

2. Methods

2.1. Animals and diets

Thirty-six 32-week-old male C57BL/6 mice were used in this study. Four mice were housed per cage in the Dong-A University College of Medicine Animal Laboratory. The laboratory conditions were maintained constant: 55% relative humidity, 22 ± 2 ℃, and a 12-h dark-light cycle. After 1 week of adaptation maintenance and free access to food and water, the mice were randomly divided into two groups: control group (CON, $n = 9$) and high-fat diet group (HF, $n = 27$). To induce obesity, CON group was fed a normal diet (69.41% carbohydrate, 24.34% protein, and 6.25% fat), while HF group was fed a high-fat diet (35% carbohydrate, 20% protein, and 45% fat) for 4 weeks. After inducing obesity, the HF group was divided into three groups; HF ($n = 9$), HF + moderate-intensity resistance exercise (HFME, $n = 9$), and HF + high-intensity resistance exercise (HFHE, $n = 9$).

2.2. Resistance exercise intervention

Based on a previous study [19], mice were

made to perform a ladder-climbing exercise on a ladder with an 80° slope as resistance training 5 days a week for 8 weeks. In both HFME and HFHE groups, the tail of the mice was attached with a pendulum weighing 75% of the body mass before climbing up the ladder. Upon successfully climbing the ladder to the top, the one repetition maximum (1RM) value was assessed through gradual addition of weights of 15% of the body mass to the tail. A total of eight rounds of climbing were performed by mice of both groups in one set of exercises with loads equivalent to approximately 50% and 75% of 1RM.

2.3. Samples of blood and tissue

Animals were sacrificed at 48 h after the end of training to rule out the temporary effects of resistance exercise training. All mice were anesthetized by using ethyl ether. Next, blood was collected from the abdominal aorta, and the hippocampus was removed. Serum was obtained by centrifugation at 3000 rpm for 10 min. Blood and hippocampus were immediately stored at -80 ℃.

2.4. Blood and tissue analysis

Serum profiles were analyzed by using total cholesterol (TC), TG (Asan Pharmaceutical, Korea), and high-density lipoprotein cholesterol (HDL-c) kits (Shinyang Diagnostics, Korea). Low-density lipoprotein cholesterol (LDL-c) was calculated using a previously described formula [20]. The analysis of CCL11 level was carried out using an ELISA kit for a mouse CCL11/Eotaxin Duoset (DY420, R&D system Inc., USA).

The hippocampus tissues were lysed in 200 μl radioimmunoprecipitation assay (RIPA) buffer to extract protein from the samples. The tissue was homogenized and centrifuged for 30 min at 14,000 rpm. The protein concentration of the supernatant was measured using the BCA protein assay kit (PIERCE, USA). Samples of equal protein content were resolved by SDS-polyacrylamide gel electrophoresis on a 10 or 12% gel and transferred to a membrane. The membrane was blocked with 5% skim milk in phosphate-buffered saline (PBS), and subsequently incubated at 4 ℃ overnight with primary antibodies (1:1000 dilution) against BDNF (sc-65513, Santa Cruz Biotechnology, USA) and NGF (sc-365944, Santa Cruz Biotechnology, USA). The membrane was incubated with goat anti-mouse or anti-rabbit IgG conjugated secondary antibody for 1 h at room temperature. The signal was developed with an ECL solution (Amersham Pharmacia Biotech, USA) and visualized with ImageQuant™ LAS-4000 system (GE Healthcare, Sweden).

2.5. Statistical analysis

All statistical analysis was performed using the Statistical Package for Social Sciences (version 25.0), and values were reported as mean ± standard error (SE). One-way ANOVA was used to verify the inter-group differences in the blood components and the results of tissue analysis. When statistical significance was evident, Tukey's post hoc analysis was carried out. Statistical significance was set as α = 0.05.

3. Results

Changes in body weight are shown in Fig 1. The body weight was significantly increased in the HF group ($p < 0.05$), and significantly higher than in the CON group ($p < 0.05$), indicating that 4 weeks of HFD induced obesity (Fig 1A). Following 8 weeks of intervention (Fig 1B), body weight was significantly higher in the HF group than in the CON, HFME and HFHE groups ($p < 0.05$). In addition, in the HFME and HFHE groups were significantly lower than in the HF group ($p < 0.05$).
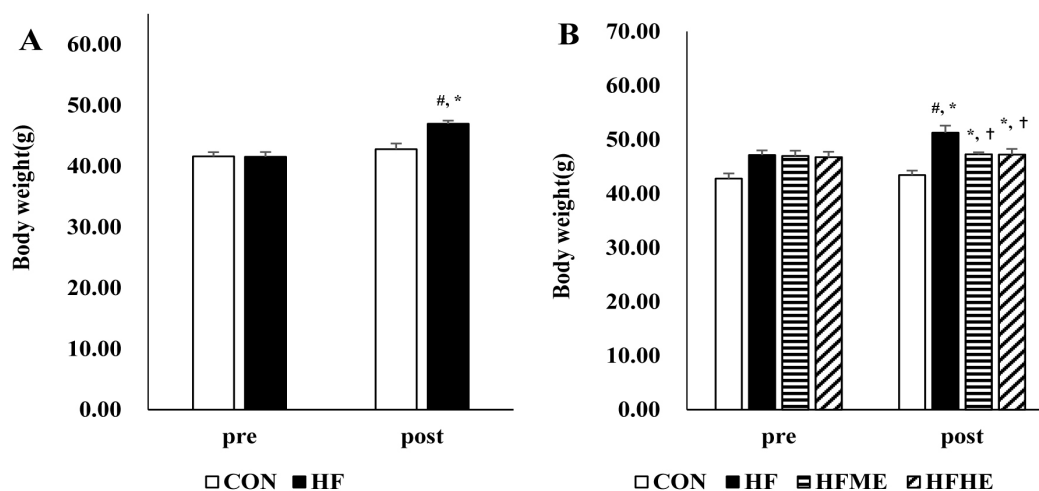
Fig. 1. Changes in body weight after intervention. Data are presented as mean ± SE. CON: control; HF: high-fat diet; HFME: HF + moderate-intensity resistance exercise; HFHE: HF + high-intensity resistance exercise; [#]versus Pre ($p < 0.05$); [*]versus CON group ($p < 0.05$); [†]versus HF group ($p < 0.05$).

Table 1. Changes in lipid profiles after intervention

Variables	CON	HF	HFME	HFHE
TC (mg/dL)	146.75 ± 7.00	263.78 ± 9.37[*]	213.49 ± 5.69[*,†]	216.07 ± 7.93[*,†]
TG (mg/dL)	51.89 ± 2.09[†]	63.74 ± 3.33	52.79 ± 4.23	55.20 ± 2.18
HDL-c (mg/dL)	58.19 ± 4.63	51.74 ± 1.81	73.60 ± 4.54[†]	72.39 ± 5.54[†]
LDL-c (mg/dL)	78,18 ± 5.91	199.29 ± 10.55[*]	129.34 ± 9.24[*,†]	132.64 ± 7.72[*,†]

Data are presented as mean ± SE. CON: control; HF: high-fat diet; HFME: HF + moderate-intensity resistance exercise; HFHE: HF + high-intensity resistance exercise; TC: total cholesterol; TG: triglyceride; HDL-c: high-density lipoprotein cholesterol; LDL-c: low-density lipoprotein cholesterol; [*]versus CON group ($p < 0.05$); [†]versus HF group ($p < 0.05$).

Changes in blood lipid profiles are shown in Table 1. The TC and LDL-c levels were significantly lower in the HF, HFME, and HFHE groups than in the CON group ($p < 0.05$). In addition, in the HFME and HFHE groups were significantly lower than in the HF group ($p < 0.05$). The TG levels were significantly higher in the HF group than in the CON group ($p < 0.05$). The HDL-C levels were significantly higher in the HFME, and HFHE groups than in the HF group ($p < 0.05$).

Changes in hippocampal neurotrophic factors levels are shown in Fig 2. The BDNF levels were significantly lower in the HF, HFME, and HFHE groups than in the CON group ($p < 0.05$). In addition, in the HFME and HFHE groups were significantly higher than in the HF group ($p < 0.05$). The NGF levels were significantly lower in the HF group than in the CON group ($p < 0.05$). In addition, in the HFME and HFHE groups were significantly higher than in the HF group ($p < 0.05$). In both BDNF and NGF levels, there
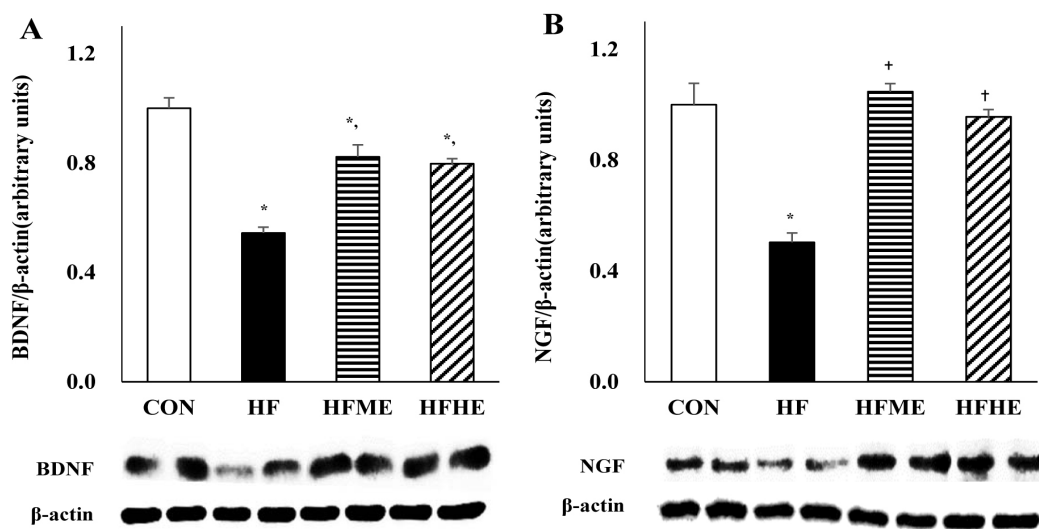
A

B

Fig. 2. Changes in hippocampal neurotrophic factors levels after intervention. Data are presented as mean ± SE. CON: control; HF: high-fat diet; HFME: HF + moderate-intensity resistance exercise; HFHE: HF + high-intensity resistance exercise; *versus CON group ($p < 0.05$); †versus HF group ($p < 0.05$).

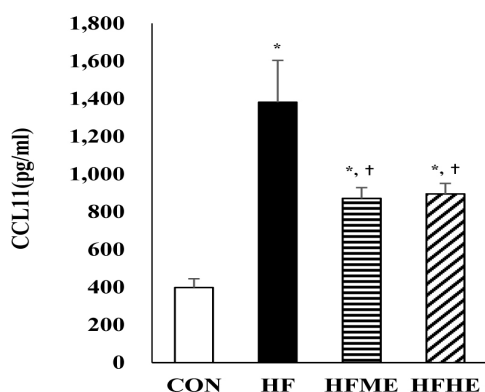was no significant difference between HFME and HFHE groups.

Fig. 3. Changes in peripheral CCL11 levels after intervention. Data are presented as mean ± SE. CON: control; HF: high-fat diet; HFME: HF + moderate-intensity resistance exercise; HFHE: HF + high-intensity resistance exercise; *versus CON group ($p < 0.05$); †versus HF group ($p < 0.05$).

Changes in peripheral CCL11 levels are shown in Fig 3. The CCL11 levels were significantly higher in the HF group than in the CON group ($p < 0.05$). In addition, in the HFME and HFHE groups were significantly lower than in the HF group ($p < 0.05$). There was no significant difference between in the HFME and HFHE groups.

4. Discussion

In studies including animal models, weight change determines whether or not obesity was induced by HFD. In addition, obesity induces an increase in total cholesterol (TC), triglyceride (TG), and low-density lipoprotein cholesterol (LDL-c) levels and a decrease in high-density lipoprotein cholesterol (HDL-c) level, resulting in dyslipidemia, which is a risk factor for cardiovascular diseases. In this study, weight changes and blood lipid profiles were measured to assess the effects of HFD and resistance training on obesity. The results

showed that 4 weeks of HFD significantly increased the body mass and the TC, TG, and LDL-c levels in blood, while 8 weeks of resistance training, regardless of the intensity, significant decreased the body mass and increased the blood HDL-c levels. These results were consistent with the results of previous studies that HFD increased the body mass of mice after 3 weeks more significantly compared to the normal diet [21] and that resistance training was effective for weight loss and improvement in the blood lipid profile in obese animal models.

NTFs are the key determinants of neuronal survival and differentiation, regulating the axonal and dendritic growth, neurotransmitter secretion, and synaptic plasticity in the nervous system [22]. Previous studies suggested that obesity may be involved in the regulation of NTFs and that NTF levels may play a major role in the enhancement of brain function through exercise [23,24,25]. In particular, Bullo et al. (2007) reported that circulating NGF levels may be low in morbidly obese people [23], and Roh and So (2017) reported that serum BDNF levels were significantly lower in the obese group compared to the non-obese group [24]. In addition, a longitudinal analysis by Arvidsson et al. (2018) confirmed that body fat content and vigorous physical activity were associated with serum NGF and BDNF levels [25]. In this study, BDNF and NGF levels in the hippocampus were analyzed to verify the changes associated with obesity and resistance training at different intensities. The results showed that serum BDNF and NGF levels were significantly lower in the HF group than in the CON group and significantly higher in HFME and HFHE groups than in the HF group. However, no significant differences were found between the HFME and HFHE groups. These results suggest that obesity down-regulates NTFs in the hippocampus, while resistance training, regardless of the intensity, can up-regulate NTFs, which are reduced by obesity;

considering the changes in body weight and blood lipid profile levels in this study, exercise can reduce obesity. Woo et al. (2013) found that BDNF and NGF levels significantly decreased in the hippocampus of obese mice on HFD and that resistance training for 8 weeks in mice on normal diet and exercise significantly increased BDNF and NGF levels [26]. In another study [27], 8 weeks of exercise in obese mice improved the blood lipid profile and increased c-Jun and BDNF levels in the hippocampus, suggesting that regular exercise has a positive effect on neuronal cell production.

Villeda et al. (2011) reported that blood transfusion using a connection between the abdominal cavities of young and old mice improved neurogenesis in the old mice but reduced it in the young mice, and an analysis of 66 blood proteins showed that CCL11 was associated with neurogenesis [16]. In this study, peripheral CCL11 levels were analyzed to assess the effects of obesity and resistance training at different intensities on neurogenesis. The results showed that the serum CCL11 level was significantly higher in the HF group than in the CON, HFME, or HFHE group, contrary to the hippocampal BDNF and NGF levels. These results are consistent with the results of previous studies that blood CCL11 levels increased in obesity and decreased with intervention through exercises [18, 24]. Considering that an increase in blood CCL11 levels is directly associated with suppression of neurogenesis [16], the results of this study suggest that resistance training may be effective in improving neurogenesis, regardless of exercise intensity. Vasudevan et al. (2006) reported that circulating eotaxin levels increased in obesity in both animals (mice) and humans but decreased after weight loss through dietary restrictions [18]. In addition, Cho and Roh (2017) reported that exercise may increase serum BDNF levels and decrease plasma CCL11 levels in obese men [28].

5. Conclusion

In conclusion, these results suggest that obesity can induce down-regulation of neurotrophic factors and inhibition of neurogenesis. In contrast, regardless of exercise intensity, resistance training may have a positive effect on improving brain function by inducing increased expression of neurotrophic factors.

Acknowledgment

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