

Effects of intermittent ladder-climbing training on neurobiological markers in mice with type 2 diabetes

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Abstract : This study aimed to investigate the effect of ladder-climbing exercise training on neurobiological markers in the hippocampus of mice with type 2 diabetes (T2DM). Twenty-one C57BL/6 male mice were randomly assigned to the non-diabetic control (NDC, $n = 7$), diabetic control (DC, $n = 7$), and diabetic training (DT, $n = 7$) groups. The DT group performed ladder-climbing training (LCT) five times a week for eight weeks. We measured the levels of hippocampal neurobiological markers (catalase [CAT], brain-derived neurotrophic factor [BDNF], nerve growth factor [NGF], amyloid-beta [$A\beta$], tau, and CC motif chemokine ligand 11 [CCL11]). The BDNF levels were significantly higher in the DT group than in the DC group ($p < 0.05$). The $A\beta$ and CCL11 levels were significantly higher in the DC group than in the NDC and DT groups ($p < 0.05$). The tau levels were significantly higher in the DC group than in the NDC group ($p < 0.05$). However, there was no significant difference in CAT and NGF levels among the groups ($p > 0.05$). These results suggest that while T2DM could induce neurodegeneration, LCT may be effective in alleviating neurodegeneration caused by T2DM.

Keywords : resistance exercise, neurotrophins, neurogenesis, neurodegeneration, type 2 diabetes

1. Introduction

Diabetes mellitus (DM) is a chronic disease, and its prevalence and incidence rate are increasing rapidly worldwide. According to a recent report of the International Diabetes

Federation (IDF), the population afflicted with DM increased by 88%, from 151 million in 2000 to 285 million in 2009; in 2019, 463 million or 9.3% of adults aged 20–79 were estimated to be patients with diabetes [1].

DM causes various complications not only in the peripheral nerves but also in the central nervous system. In particular, type 2 DM (T2DM), of which the prevalence is rapidly

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increasing in Asia, including Korea, was suggested to have more adverse effects on brain function, causing a markedly lower memory and learning ability than type 1 DM [2,3]. In addition, since DM is reported as an independent risk factor for developing vascular dementia and Alzheimer's disease (AD), AD is often referred to as brain diabetes and/or type 3 DM [4–6]. This is supported by the fact that insulin resistance (IR), the main symptom of T2DM, is associated with the accumulation of amyloid-beta ($A\beta$) and hyperphosphorylated tau that is observed in AD [7]. In several previous studies [8,9], it was reported that T2DM had a negative effect on the expression of neurotrophins, which determine the survival and differentiation of neurons, such as brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF). Bathina et al. (2017) reported that BDNF production was suppressed in the brain tissues in a streptozotocin (STZ)-induced T2DM model [8]; Gumuslu et al. (2018) reported a reduced hippocampal NGF gene expression as a result of inducing T2DM using STZ/nicotinamide [9]. It has been suggested that one of the mechanisms causing AD and impairing brain function in T2DM is the excessive production of reactive oxygen species (ROS) induced by hyperglycemia [2,8]. In other words, excessive production of ROS is associated with cytotoxicity caused by calcium metabolism abnormality and glutamate accumulation in neurons, leading to apoptosis of neurons and the accumulation of $A\beta$ [2].

On the other hand, regular participation in exercise is as essential as dietary therapy to control the level of blood glucose [10]. It has also been suggested that it could reduce the risk of developing DM-induced AD and oxidative stress, and could be effective in increasing the expression of neurotrophins [3,11]. However, despite previous studies [12] that showed that resistance training could be more effective in glycemic control because it significantly reduced the HbA_{1c} in comparison

to aerobic training in T2DM patients, there is a limitation that most of the previous studies proved the effectiveness of either aerobic training only or the combination of aerobic and resistance training. Therefore, the purpose of this study is to verify the effect of regular resistance training on hippocampal neurobiological markers that are responsible for neurodegeneration, using the intervention of ladder-climbing training (LCT) in T2DM mice.

2. Methods

2.1. Experimental animals

Twenty-one male C57BL/6 mice (aged 4 weeks) were obtained from Samtako (Osan, Korea), and four mice were housed per cage in the Dong-A University College of Medicine Animal Laboratory (relative humidity: $60 \pm 5\%$, temperature: 22 ± 2 °C, and light: 12 h dark/12 h light cycle). The animal experiments were approved by the Dong-A University Medical School Institutional Animal Care and Use Committee (DIACUC-16-22), and all procedures were conducted in accordance with the committee guidelines.

2.2. T2DM induction and ladder-climbing training (LCT) protocol

At 32 weeks of age, mice were randomly divided into non-diabetic control (NDC, $n = 7$) and diabetic control (DC, $n = 14$) groups. The NDC group consumed a standard diet (fat 6.3%) for four weeks. The DC group consumed a 45% fat diet for four weeks; T2DM was induced at 36 weeks of age by injecting a 40 mg/kg solution (made by dissolving STZ [Sigma Chemical, USA] in 0.1 M sodium citrate solution [pH 4.5]) four times in the lower abdomen, after fasting the mice for 6 h. To confirm T2DM induction, blood extracted from the tail vein was measured using a glucometer (GlucoDr, All Medicus, Korea); mice with a fasting blood

glucose of 250 mg/dL or more were defined as having T2DM. After inducing T2DM, the DC group was divided into a DC group ($n = 7$) not participating in the exercise training and a diabetic training (DT, $n = 7$) group participating in the LCT. The LCT protocol was adapted from a previous study [13]; mice were made to perform a ladder-climbing exercise on a ladder with an 80° slope as resistance training five days a week for eight weeks. For the maximal load test, the mice were prompted to climb up a ladder with a weight pendulum of 75% of their weight on their tails. If the mouse could climb to the top, the weight pendulum of 15% of its weight was increased each time to evaluate 1-repetition maximum (1-RM). Based on the results, exercise intensity at approximately 75% of 1-RM was used for each round of exercise for a total of 8 repetitions of climbing.

2.3. Blood and tissue sampling

To exclude the transient effects of exercise, blood and tissue samples were obtained 48 h after the end of exercise training. All mice were anesthetized with ethyl ether, and the blood samples were obtained from the abdominal vena cava via syringes. After blood sampling, the hippocampus was extracted. Blood samples and the hippocampus were immediately stored at $-80\text{ }^{\circ}\text{C}$ until analysis.

2.4. Blood and tissue analysis

Serum CCL11 levels were determined with an enzyme-linked immunosorbent assay (ELISA) using the mouse CCL11/Eotaxin DuoSet (DY420, R&D system, USA) according to the manufacturer's instructions. As previously described [13], 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 with 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 $4\text{ }^{\circ}\text{C}$ overnight with primary antibodies (1:1000 dilution) against brain-derived neurotrophic factor (BDNF; sc-65514), nerve growth factor (NGF; sc-365944), amyloid-beta ($A\beta$; sc-28365), tau (sc-32274), and catalase (CAT; sc-271803) (all antibodies were purchased from 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 the ImageQuant™ LAS-4000 system (GE Healthcare, Sweden).

2.5. Statistical analysis

Statistical analysis was carried out with SPSS 25.0 statistical package (IBM Corp., USA). Results are expressed as means \pm standard error. The data were analyzed with one-way ANOVA, and significance was set at $p < 0.05$.

3. Results

The neurobiological markers of the three groups after eight weeks of LCT are shown in Fig. 1. The BDNF levels were significantly higher in the DT group than in the DC group ($p < 0.05$). The $A\beta$ levels were significantly higher in the DC group than in the NDC and DT groups ($p < 0.05$). The tau protein levels were significantly higher in the DC group than in the NDC group ($p < 0.05$). The CCL11 levels were significantly higher in the DC group than in the NDC and DT groups ($p < 0.05$). However, there was no significant difference in CAT and NGF levels ($p > 0.05$).

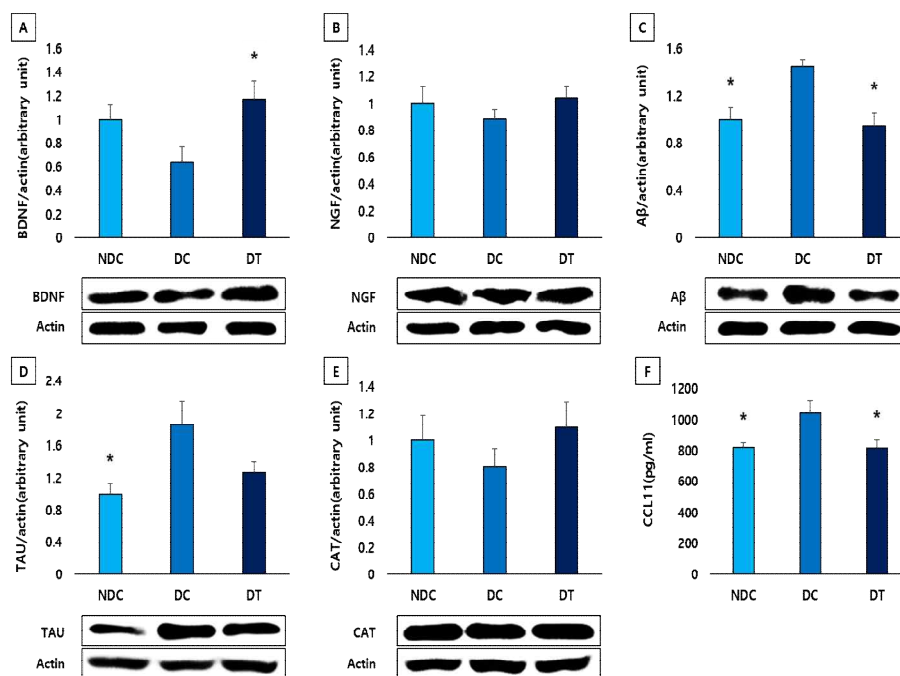


Fig. 1. The neurobiological markers of the three groups after eight weeks of LCT. Data are expressed as means \pm standard error. (A) BDNF; (B) NGF; (C) A β ; (D) Tau; (E) CAT; (F) CCL11; NDC, non-diabetic control group; DC, diabetic control group; DT, diabetic training group; *versus DC group ($p < 0.05$).

4. Discussion

The hippocampus plays a fundamental role in learning and memory, is responsible for the maintenance and enhancement of brain function through continued neurogenesis, and has a higher metabolic requirement than neurons elsewhere [14]. Therefore, in chronic metabolic disorders such as T2DM, the neuronal activity of the hippocampus is acutely affected, and the expressed BDNF and NGF in the hippocampus are the typical neurotrophins that regulate neurogenesis [14,15]. In this study, the BDNF and NGF levels in the hippocampus were studied to verify the effect of T2DM and resistance training on the expression of neurotrophin. As a result, NGF was not found to be significantly different; however, BDNF was found to be significantly higher in the DT group when compared to

that in the DC group. These results suggest that resistance training in T2DM may be effective in increasing the expression of hippocampal BDNF. Although the mechanism is unclear, the energy expenditure from resistance training may have played a major role. Stranahan et al. (2009) reported a significant increase in the hippocampal BDNF expression with voluntary exercise and/or caloric restriction (60% of the mean food intake) in the leptin receptor mutant (*db/db*) mice [16]. In addition, Jamali et al. (2020) suggested that because of the systematic review, the reduction in energy intake and the increased energy expenditure during training could modulate the level of BDNF in T2DM patients [17].

Glucose dysregulation, caused by insulin resistance (IR) and decreased insulin secretion, is one of the characteristics of T2DM that is

associated with the elevated risk of cognitive impairment and dementia; IR has been reported as a leading cause of exacerbating the risk of developing AD and pathological progression [7,17]. In other words, insulin not only plays a major role in regulating the peripheral glucose metabolism and energy homeostasis but also binds to the insulin receptors located in the main brain regions, such as the hippocampus, cerebral cortex, and hypothalamus, which activates the insulin signaling that would stimulate cell growth, survival and synaptic plasticity, and inhibit $A\beta$ peptide deposition and the phosphorylation of tau protein [7,17,18]. Meanwhile, CCL11, a type of chemokine, has been reported as a blood-borne factor associated with the inhibition of neurogenesis and cognitive decline [19]. Kumar et al. (2014) reported significantly high serum levels of CCL11 in subjects with diabetes [20], suggesting that a high level of CCL11 was associated with neurodegeneration caused by DM. In this study, the hippocampal levels of $A\beta$, tau, and serum CCL11 were obtained to verify the effect of T2DM and resistance training on neurodegeneration. As a result, the level of $A\beta$ and CCL11 was found to be higher in the DC group than in the NDC and DT groups; the level of tau protein was significantly higher in the DC group than in the NDC group. These results suggest that while T2DM could accelerate neurodegeneration, this could be inhibited by resistance training. While there was a limitation that this study could not directly study neurodegeneration, glycemic control from resistance training and improvement of insulin action may have played a major role. Tang et al. (2014) showed that IR was significantly reduced after eight weeks of LCT in obese rats [21], and Park et al. (2019) reported that the blood insulin levels significantly increased in T2DM rats after 12 weeks of LCT [22]. In addition, Kochetova et al. (2019) reported, as a result of regression analysis, a significant correlation between CCL11 and HbA_{1c} levels.

Alternately, although there is a debate about the decrease in antioxidant capacity caused by DM, in several T2DM animal models and previous studies conducted on patients, a reduction in antioxidant enzymes, such as CAT, superoxide dismutase, glutathione, and ascorbic acid was reported [23,24]. However, there was no significant difference in the level of hippocampal CAT in this study. Therefore, in future studies, it may be necessary to verify the reduction in antioxidant capacity through analyses that include other antioxidant enzymes in addition to CAT. In addition, in future studies, it is considered necessary to verify the variables related to glycemic control in blood and skeletal muscle.

5. Conclusion

Summarizing the results above, LCT can induce an increase in hippocampal BDNF expression, a decrease in $A\beta$ and tau protein, and a decrease in the level of circulating CCL11 in T2DM mice, suggesting that resistance training may be effective in alleviating the neurodegeneration caused by T2DM.

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