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수의학박사 학위논문

Effects of high fat diet on hippocampal plasticity in relation with aging and exercise

해마에서 고지방식이와 노화 및 유산소 운동이 신경가소성에 미치는 영향

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Thesis for the Degree of Doctor of Philosophy

Effects of high fat diet on hippocampal plasticity in relation with aging and exercise

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Effects of high fat diet on hippocampal plasticity in relation with aging and exercise

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Abstract

The brain is the regulatory center of the body; it responds to the environment and continuously adapts *via* plasticity, the structural reconstruction of neuronal cells. Together with dendritic branching and synaptic formation in existing neurons, new neurons are generated in the dentate gyrus and subventricular zone of the hippocampus and lateral ventricle, respectively. Anatomical hippocampal plasticity is associated with learning and memory. Mild cognitive impairment in neurodegenerative diseases, including Alzheimer's disease, is caused by a reduction in neural plasticity. Understanding the mechanism of continuous neurogenesis and the replacement of old granule cells will shed light on neurodegenerative diseases.

Proliferating and differentiating cells are susceptible to endogenous and exogenous factors, such as hormones, minerals, stress, diet, toxic materials, and drugs. Diets with a high fat content and a sedentary lifestyle are primary risk factors for obesity and subsequent metabolic syndrome. Dietary control and exercise are commonly prescribed lifestyle therapies to ameliorate the harmful effects of obesity and metabolic syndrome. Exercise is an effective intervention and considered as a polypill with multiple functions, such as anti-obesity, anti-aging, and anti-inflammation. To date, the effect of combining diet and exercise on the hippocampus in models of obesity has not been clearly established. In addition to lifestyle factors, aging is also risk factor for the development of obesity or metabolic syndrome; recent studies have noted that obesity and metabolic syndrome accelerate the senescence of brain.

Obesity- and aging-induced hippocampal functional deficits are related to

impairments in neurogenesis. In addition, synaptogenesis is important for proper hippocampal functioning. In the adult hippocampus, newly generated neurons integrate into neuronal circuits *via* synapse formation. Synaptophysin is located at the synapse in the presynaptic vesicular membrane and *N*-methyl-D-aspartate receptor (NMDAR) is inserted in the postsynaptic membrane. Synaptophysin is a marker of presynaptic plasticity and synaptogenesis. Glutamate is released from the presynaptic membrane and mediates the activation of NMDARs. NMDARs participate in new synapse formation and the maturation of newly generated neurons.

The aim of the present study was to investigate the effect of combined dietary change and exercise intervention on hippocampal neurogenesis and synaptogenesis in high fat diet (HFD)-fed obese mice. Additionally, we used a chemical-induced brain aging model to establish the coexistence of aging and obesity. D-galactose (D-gal) generates reactive oxygen species (ROS) and chronic treatment of D-gal induces a brain aging-associated phenotype. We used immunohistochemistry and western blot analysis to reveal changes in neurogenic and synaptic marker proteins.

In the first experiment, we investigated the effects of dietary change and/or exercise on hippocampal structural plasticity of HFD-fed obese mice. First, we confirmed the negative effects of HFD-feeding on adult neurogenesis in the dentate gyrus: eight weeks of HFD-feeding decreased the expression of nestin, Ki67, and doublecortin (DCX) positive cells. Interestingly, synaptophysin and NMDAR expression in the hippocampus was not significantly altered by a HFD. In addition, exercise was more effective at increasing neurogenic and synaptic proteins; however, a simple dietary change to normal chow diet did improve neurogenesis in the dentate gyrus. The positive effects of exercise are widely recognized but we

found that combining dietary change and exercise synergistically improved hippocampal neurogenesis. We confirmed that both dietary change and exercise increased the expression of brain-derived neurotrophic factor (BDNF) and phosphorylated cAMF-response element binding protein (pCREB) and these interventions had a synergistic effect when combined. In addition, we investigated changes in oxidative stress-related markers. Both dietary change and exercise reduced the accumulation of 4-hydoxynonenal (4-HNE) *via* an increase in defensive antioxidant superoxide dismutase1 (SOD1).

In the second experiment, we investigated the effect of D-gal-induced aging on hippocampal neural plasticity in HFD-fed obese mice. Both HFD-feeding and D-gal-treatment reduced nestin, Ki67, and DCX positive cells. In the combined model of aging and obesity, these parameters were significantly reduced and synaptic marker proteins showed similar pattern of change. We further investigated the effect of exercise on the combined model of aging and obesity. Treadmill exercise attenuated the negative effects of HFD-feeding and D-gal-induced aging. In addition, exercise increased nestin-positive neural stem cells, their proliferation, and neuronal differentiation in the dentate gyrus. Furthermore, exercise increased synaptic proteins in the whole hippocampus. The proposed mechanism for this change was changes in BDNF-pCREB signaling and 4-HNE-SOD1 production. We confirmed that D-gal-induced aging and HFD-feeding reduced BDNF-pCREB signaling and increased the accumulation of 4-HNE *via* SOD1 exhaustion. Exercise increased BDNF-pCREB signaling and reduced 4-HNE production by SOD1 induction in both models.

In summary, HFD-feeding aggravates hippocampal structural plasticity and dietary change was effective at ameliorating this impairment. Combined dietary

change and exercise treatment synergistically improved HFD-induced impairments. Furthermore, D-gal-induced aging in HFD-fed obese mice aggravated the impairment in hippocampal plasticity and exercise showed anti-aging and anti-obese effects in the hippocampus. We suggest that changes in the number of neural stem cells, their proliferation, and the differentiation of neuroblasts is correlated with an upregulation in BDNF-pCREB signaling and SOD1 with a concurrent downregulation of 4-HNE. Furthermore, dietary change and exercise treatment attenuated the reduction in neurogenic and synaptic proteins by inducing BDNF-pCREB signaling and reducing 4-HNE *via* SOD1.

Keywords: High fat diet, exercise, dietary change, D-galactose, hippocampus, neural plasticity

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List of abbreviations

DG	**dentate gyrus**
DCX	**doublecortin**
CD	**chow diet**
HFD	**high fat diet**
D-gal	**D-galactose**
BDNF	**brain-derived neurotrophic factor**
CREB	**cAMP-response element binding protein**
pCREB	**phosphorylated cAMP-response element binding protein**
NMDAR	***N*-methyl-D-aspartate receptor**
4-HNE	**4-hydroxynonenal**
SOD	**superoxide dismutase**
ML	**molecular layer**
GCL	**granule cell layer**
PL	**polymorphic layer**

Introduction

Hippocampal plasticity

The hippocampus is crucial for memory and learning (Deng et al. 2010, Zatorre et al. 2012). Hippocampal structural plasticity, including neurogenesis and synaptogenesis, is important for the proper functioning of the hippocampus. After brain development, the adult brain can continuously generate new neurons in restricted areas of the brain: the subgranular zone of the hippocampal dentate gyrus and subventricular zone of the lateral ventricle (Deng et al. 2010, Hack et al. 2005). Neural stem cells reside in the neurogenic region and their activation is followed by proliferation, differentiation, maturation, and integration into neuronal circuits. The generation of new neurons in the dentate gyrus of the adult hippocampus has been reported across many mammalian species, including rodents, dogs, primates, and humans (Eriksson et al. 1998, Gould et al. 1999, Kuhn et al. 1996, Siwak-Tapp et al. 2007). Activation, proliferation, and neuronal differentiation of neural stem cells are more susceptible to environmental factors when compared with the resistant mature granule cells in the dentate gyrus (Danzer 2008).

While their susceptibility makes newly generated cells more easily degenerate because of harmful factors, newly generated neurons have a lower threshold for the induction of long-term potentiation (LTP) and are suited for experience-dependent plasticity (Hernández-Rabaza et al. 2009). Along with neurogenesis, synaptogenesis or dendritic sprouting makes the hippocampus highly plastic and reactive to environmental stimuli and these processes are important for learning and memory (Deng et al. 2009, Fischer et al . 2004, Ramírez-Amaya et al. 2001,

Toni et al. 2007). Recently, much evidence has shown that obesity, and subsequent metabolic syndrome, are highly correlated with cognitive malfunction (Dinel et al. 2011, Farr et al. 2008, Kanoski and Davidson 2011). In particular, eating behaviors, such as preference for a diet with a high fat content, and a sedentary lifestyle are the primary causes of obesity and metabolic syndrome (Wagner et al. 2012). Therefore, anatomical plasticity, such as neurogenesis and synaptogenesis, should be studied in the context of obesity because of their importance in hippocampus-dependent memory and learning. The aim of the present study was to investigate the effect of high fat diet (HFD)-induced obesity on anatomical plasticity in the adult hippocampus.

Neurogenic markers

We used nestin, Ki67, and doublecortin (DCX) as neurogenic markers. Nestin is a class IV intermediate filament protein that is expressed in neural stem cells and progenitor cells of the central nervous system (Wiese et al. 2004). Ki67 is nuclear protein that is expressed in proliferating cells during all active phases of the cell cycle except G_0 resting phase (Kee et al. 2002). DCX is a microtubule-associated protein expressed in migrating neuroblasts or immature neurons (Brown et al. 2003). Nestin is expressed in type-1, 2a, and 2b stem/progenitor cells. Type 2a and 2b cells are negative and positive for DCX, respectively. Putative transiently amplifying progenitors proceed from type-2 to type-3 cells and lose nestin expression. On the other hand, type-3 cells express DCX and polysialated form of neural cell adhesion molecule (PSA-NCAM) (Kempermann et al. 2004). DCX expression persists in the next postmitotic stage. With maturation, immature

neurons lose DCX expression and begin to express NeuN, which initiates the terminal differentiation of the neuron (Mullen et al. 1992).

Synaptic proteins

We selected synaptophysin and *N*-methyl-D-aspartate receptor (NMDAR) as markers for synaptic plasticity. Synaptophysin is located in the presynaptic vesicular membrane and regulates activity-dependent synapse formation (Tarsa and Goda 2002). NMDAR is a glutamatergic ionotropic receptor expressed in the postsynaptic membrane. When activated, NMDAR allows calcium ion translocation and the subsequent generation of synaptic plasticity (LTP and long-term depression). The NMDAR consists of heterodimers that differ in subunit composition dependent upon brain region and developmental period (Paoletti et al. 2013). The subunit composition affects the activity of NMDAR channels and synaptic plasticity is controlled by the composition (Barria and Malinow 2005). There are seven documented different subunits; NMDAR1, NMDAR2A, and NMDAR2B are predominantly detected in adult hippocampus (Kim et al. 2005). NMDAR1 is an essential component of the NMDAR complex and NMDAR2A/NAMDR2B determines the function of the receptor complex (Paoletti et al. 2013).

BDNF-pCREB signaling

As mediator of neural plasticity, brain-derived neurotrophic factor (BDNF) acts as key molecule in synaptic plasticity, neuroprotection, and neuronal survival (Beck et al. 1994, Leal et al. 2014). BDNF signaling induces phosphorylation of

transcription factor cAMP-response element binding protein (CREB). pCREB binds to the CRE site in DNA, which results in the binding of the CREB-binding protein and subsequent transcription of target genes (Wood et al. 2006).

Oxidative stress

We investigated changes in the expression of the oxidative stress-related markers, 4-hydroxynonenal (4-HNE) and superoxide dismutase 1 (SOD1), as an additional mechanism of neural plasticity changes caused by HFD-induced obesity and/or D-galactose (D-gal) treatment-induced aging. During the development of obesity and aging, oxidative stress accumulates and generates by-products *via* lipid peroxidation, protein carbonylation, and the oxidization of DNA nucleotides (Radak et al. 2011). We focused on lipid peroxidation because the brain contains a large proportion of lipids and is susceptible because it has a shortage of antioxidants (Butterfield et al. 2002). The superoxide is the most abundant free radical and initiates biological membrane destruction *via* lipid peroxidation (Mićić et al. 2003, Southorn et al 1988). 4-HNE is the end-product of lipid peroxidation and its accumulation is involved in structural and functional impairments in the hippocampus. Superoxides are catalyzed to hydrogen peroxide by SOD. Cytoplasmically located SOD1 contains a Cu/Zn catalytic center and its deficiency is related with age-related muscle loss, cataract, hepatocellular carcinoma, and a shortened lifespan (Muller et al. 2007).

Few studies have investigated the effect of a combined intervention, such as dietary change and exercise, on hippocampal neurogenesis and synaptogenesis. In

addition, to the best of our knowledge, no study has reported the effect of D-gal-induced aging on these parameters in the HFD-induced obesity model. Moreover, the alterations in BDNF-pCREB and 4-HNE-SOD1 expression need to be investigated to elucidate the mechanism of the present interventions.

In present study, we first established HFD-induced obese mice with/without dietary change and/or exercise treatment. Following this, a D-gal-induced chemical aging model was established with/without HFD-feeding and/or exercise treatment. To investigate the expression of neurogenic markers, synaptic proteins, BDNF, and 4-HNE in the hippocampus, we performed immunohistochemistry and immunoblot analysis.

In the first experiment, we focused on lifestyle interventions, such as dietary control and exercise, which are prescribed to ameliorate the onset or harmful effects of metabolic syndrome. Both dietary control and exercise are effective in reversing an obesity induced phenotype, for example increased body weight, glucose level, and insulin resistance (Pan et al. 1997, Ross et al. 2004). Regular exercise is regarded as a polypill as it targets multiple organs with numerous beneficial effects, such as anti-aging, anti-obesity, and anti-inflammation (Fiuza-Luces et al. 2013). Natural materials that mimic the beneficial effects of exercise are currently being investigated. Synthetic materials, such as AICAR, that target a signal pathway affected by exercise, have been developed and studied (Kobilo et al. 2014, Momken et al. 2011). In recent animal and human studies, obesity-induced insulin resistance is related with increased susceptibility to Alzheimer's disease (AD) (Talbot et al. 2013). HFD-feeding aggravates AD-related brain pathology in a transgenic mouse model of AD (Julien et al. 2010). Exercise and/or dietary replacements ameliorate amyloid deposition and the metabolic index in this model

(Maesako et al. 2012). Furthermore, exercise is more effective than dietary change at controlling amyloid deposition and memory impairments (Maesako et al. 2012).

Diabesity has been the focus of scientific researchers as risk factor for AD and vascular dementia. Aging is the important causative factor of dementia and cognitive malfunction; however, the diabesity pandemic is prevalent and its development rises with increasing age. Age-dependent increases in the rate of metabolic syndrome are peripherally related to decreased glucose tolerance, insulin secretion, insulin insensitivity, and sarcopenic obesity (Lim et al. 2010, Stevic et al. 2007). In addition, obesity promotes age-related cognitive decline (Elias et al. 2005).

In the second experiment, we focused on brain aging. Many studies have assessed the effect of aging or obesity alone; however, the combined effects of aging and obesity on hippocampal neurogenesis and synaptogenesis are yet to be elucidated. Polyphagia and physical inactivity cause overweight, which may result in a progressive reduction in physical activity (Butte et al. 2007), and aging is related to a progressive reduction in physical activity. Forced treadmill exercise is an effective behavioral intervention to ameliorate obesity and the age-induced reduction of physical activity. The accumulation of oxidative stress has been targeted to understand the biology of aging and slow or reverse the aging process (Salmon et al. 2010). The brain is susceptible to oxidative stress-induced lipid peroxidation because it has a high content of polyunsaturated fatty acid, high metabolic rate, and low antioxidant level (Butterfield and Lauderback 2002, Reynolds et al. 2007). Subsequently, there has been a great deal of attention on physical exercise as a method of rejuvenation in the age-related brain phenotype.

Adult hippocampal neurogenesis exponentially decreases with increasing age; however, there is a controversy related to the age-related deforestation of the neural

stem cell pool. Aging negatively affects neural stem cell activation, proliferation, and neuronal differentiation (Gil-Mohapel et al. 2013, Lugert et al. 2010, van Praag et al. 2005). The rate of proliferation slows and gliogenesis increases in an age-dependent manner (van Praag et al. 2005). Naturally aged mice are the ideal model to study aging because all body systems exhibit age-related alterations together; however, aging animals is expensive and time consuming. Genetically engineered transgenic or chemical-induced models can be used as alternatives. We choose the D-gal-injected mouse as the candidate model of aging. D-gal-injected mice have been used as a chemical-induced aging model since their establishment by Gong Guoqing in 1991 (Guoqing and Fuben 1991). In brief, D-gal is a reducing sugar, and an overdose results in the accumulation of galactitol and the formation of reactive oxygen species (ROS) and advanced glycation end products (AGE) (Ho et al. 2003, Song et al. 1999). In brain, an aging phenotype is produced by chronic treatment of D-gal for 6–10 weeks (Lei et al. 2008, Xu et al. 2002). D-gal-elicits an aging phenotype, for example a reduction in adult neurogenesis (Cui et al. 2006, Nam et al. 2014c), learning and memory impairments (Cui et al. 2006, Nam et al. 2013), motor skill acquisition and locomotion impairments (Ma et al. 2014b), and impairments in inhibitory GABAergic neurons (Gu et al. 2013). Additionally, D-gal treatment shows progressive energy metabolism failure in the cortex and hippocampus (Zhang et al. 2008). To determine whether treatment of D-gal aggravated the brain phenotype in obese mice, we established a combined model of aging and obesity. The effect of treadmill exercise on hippocampal neural plasticity has not been demonstrated in this combined model; therefore, we sought to investigate the effect of forced physical exercise on hippocampal neurogenic and synaptic marker proteins.

Materials and Methods

Experimental design

In experimental design 1, we investigated the effect of dietary change and/or treadmill exercise in HFD-fed obese mice (Fig. 1A). In experimental design 2, we investigated the effect of D-gal-induced brain aging and/or treadmill exercise in the hippocampal dentate gyrus of HFD-fed obese mice (Fig. 1B).

Experimental animals

We choose C57BL/6J mice because they are most susceptible strain to HFD induced obesity among several strains of mice (Rossmeisl et al. 2003). Four-week to five-week-old male C57BL/6J mice were purchased from Japan SLC, Inc. (Shizuoka, Japan). The animals were placed in a mouse cage (5 mice/cage) in a conventional condition and they were maintained under controlled temperature (23℃) and humidity (60%) on a 12-h light-dark cycle. Food and water were freely available. The procedures for handling and caring of animals followed the Guide for the Care and Use of Laboratory Animals issued by Institute of Laboratory Animal Resources, USA, 1996, and the experimental protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of Seoul National University (approval no. SNU-120305-5 and SNU-131223-2). All experiments were conducted to minimize the number of animals used and the suffering caused by the procedures used in this study.

Diet and exercise treatment

Four-week-old mice were acclimated to laboratory conditions for 1 week and the animals (n = 30 in each group) were divided into sedentary (Sed) and exercised (Ex) groups. The Sed and Ex groups were further divided into 3 subgroups as CD-Sed/Ex, HFD-Sed/Ex, and HFD-CD-Sed/Ex group (n = 10 in each group), respectively. The animals were fed a commercially available CD (chow diet, 16.71% calorie from fat, Purina 5008 diet, Purina Korea, Korea) or HFD (60% calorie from fat, D12492i, Research Diets, NJ, USA) for 8 weeks. In the HFD-CD-fed groups, HFD were provided for 4 weeks, after then, diet was changed to CD until the end of the experiment. During the last 3 days of 4th week of dietary treatment, Ex and Sed groups were familiarized with treadmill (Model 1050 Exer 3/6; Columbus Instruments, Columbus, OH, USA) for 3 days. In order to facilitate the adaptation to treadmill running, speed and durations were 10 m/min, 20 min for the first day, with an increment of 20 min/day until reaching 60 min/day to fulfill the 70% of maximal oxygen consumption (Wu et al. 2008). After becoming familiarized with the treadmill, electrical shock to stimulate the mice to run was discontinued to avoid pain stress and gentle prodding with a soft brush was used. The running duration was 60 min/day, and the running speed was increased gradually from 10 to 11 m/min. The speed was accelerated 1 m/min every 2 weeks. The sedentary mice were put on the treadmill without running for 1 h/session/day for 4 weeks before sacrifice (Fig. 1A).

Additionally in experiment 2, five-week-old mice were acclimated for 1 week to laboratory conditions, the animals (n = 40 in each group) were divided into sedentary (Sed) and exercised (Ex) groups. The Sed and Ex groups were further

divided into 3 subgroups as CD-Sed/Ex, D-gal-Sed/Ex, HFD-Sed/Ex, and HFD-D-gal-Sed/Ex group (n = 10 in each group), respectively. The animals were fed a CD (chow diet, Purina 5008 diet) or HFD (D12492i, Research Diets) for 6 weeks. D-gal was subcutaneously administered to 6 week-old mice at concentration of 100mg/kg per day. Ex and Sed groups were familiarized with treadmill (Model 1050 Exer 3/6; Columbus Instruments) for 1 week at 6 week-old. In order to facilitate the adaptation to treadmill running, we carried out exercise according to the protocol used in previous study (Nam et al. 2014c). Speed and durations were 10 m/min, 20 min for the first day, with an increment of 10 min/day until reaching 60 min/day to fulfill the 70% of maximal oxygen consumption. After becoming familiarized with the exercise, electrical shock to stimulate the mice to run was discontinued to avoid pain stress and gentle prodding with a soft brush was used. The running duration was 60 min/day, and the running speed was increased gradually from 10 to 12 m/min. The speed was accelerated 1 m/min every 2 weeks. The sedentary mice were put on the treadmill without running for 1 h/session/day for 6 weeks before sacrifice (Fig. 1B).

Check for body weight, food intake, and non-fasting blood glucose level

Body weight was measured on Monday morning of every week and at the end of the experiment. Food intake was measured, and corrected for spillage by weighing the jars containing food every week between a.m. 9.00 to a.m. 10.00 h. Data are expressed as kilocalorie (kcal)/mouse/day. To measure non-fasting blood glucose level, blood was sampled on Monday morning for last 5 weeks in experiment 1 or last 3 weeks in experiment 2 before sacrifice by ‘‘tail nick’’ and analyzed by using

a glucometer device (ACCU-CHEK® GO; Roche, Mannheim, Germany).

Intraperitoneal glucose tolerance test (IPGTT)

To determine the effect of HFD-feeding, dietary change, and exercise on glucose tolerance, we conducted IPGTT. All mice were fasted for overnight (12 h) at the last day of the 8th week of the experiment 1. Glucose (2g per body weight kg) was intraperitoneally injected to each mouse with sterile syringe. Blood sample were collected from tail vein. Plasma glucose levels were measured immediately before and 15, 30, 60, and 120 min after glucose loading. To determine blood glucose levels, we used glucometer device (ACCU-CHEK® GO; Roche, Mannheim, Germany). Then, mice were fed *ad libitum* and exercised during another 3 days according to the experimental design.

Tissue processing

For histology, at the end of the experiment, the mice in all experimental groups (n = 5 per group) were anesthetized with 30 mg/kg Zoletil 50® (Virbac, Carros, France) and perfused transcardially with 0.1 M phosphate-buffered saline (PBS, pH 7.4) followed by 4% paraformaldehyde in 0.1 M phosphate-buffer (PB, pH 7.4). The brains were removed and post-fixed in the same fixative for 24 h. For immunohistochemical staining, brain tissues were cryoprotected by infiltration with 30% sucrose overnight. Thirty-µm thick brain sections were serially cut in the coronal plane using a cryostat (Leica, Wetzlar, Germany). The sections were collected in six-well plates containing PBS and stored in storage solution until

further processing.

Immunohistochemistry

In order to obtain accurate data for immunohistochemistry, free floating sections were carefully processed under the same conditions to obtain accurate data for immunohistochemistry. The tissue sections were selected between 1.46 mm and 2.46 mm posterior to the bregma for each animal with reference to a mouse atlas (Franklin and Paxinos 1997). Five sections in 180 μm apart from each other were sequentially treated with 0.3% hydrogen peroxide (H_2O_2) in 0.1 M PBS and 10% normal goat or rabbit serum in 0.1 M PBS. Then, they were incubated with diluted mouse anti-nestin antibody (1:200; Millipore, Temecula, CA, USA), rabbit anti-Ki67 antibody (1:1,000; Millipore, Temecula, CA), or goat anti-DCX antibody (1:50; Santa Cruz Biotechnology, Santa Cruz, CA) overnight, and subsequently exposed to FITC-conjugated anti-rat IgG (1:200; Vector Labs., Burlingame, CA), biotinylated goat anti-chicken, rabbit anti-goat, goat anti-rabbit or horse anti-mouse IgG (diluted 1:200; Vector Labs.) and streptavidin peroxidase complex (diluted 1:200, Vector Labs.). Then, the sections were visualized by reaction with 3,3'-diaminobenzidine tetrahydrochloride (Sigma, St. Louis, MO).

To elucidate the effects of dietary change and/or exercise, the corresponding areas of the dentate gyrus were measured from five sections per animal. The number of Ki67-positive cells in all groups was counted using an image analysis system equipped with a computer-based CCD camera (Optimas 6.5 software, CyberMetrics, Scottsdale, AZ).

The region of interest (ROI) in the dentate gyrus was analyzed using an image

analysis system. Images were calibrated into an array of 512 × 512 pixels corresponding to a total dentate gyrus (100× primary magnification). Pixel resolution was 256 gray levels. The intensity of nestin, DCX immunoreactivity was evaluated by means of a relative optical density (ROD), which was obtained after transforming the mean gray level using the formula: ROD = log (256/mean gray level). ROD of the background was determined in unlabeled portions using NIH Image 1.59 software and the value was subtracted for correction, yielding high ROD values in the presence of preserved structures and low. The ROD ratio of was calibrated as a percentage.

Western blot analysis

To confirm the expression pattern of nestin, DCX, BDNF, NMDAR1, NMDAR2A, NMDAR2B, synaptophysin and 4-HNE change in the hippocampus, 5 mice from each group were killed and used for western blot analysis. After euthanizing the mice and removing their brains, the hippocampi were dissected out with a surgical blade. Tissues were homogenized in 50 mM PBS (pH 7.4) containing 0.1 mM ethylene glycol bis (2-aminoethyl Ether)-*N,N,N′,N′* tetraacetic acid (EGTA) (pH 8.0), 0.2% Nonidet P-40, 10 mM ethylendiamine-tetraacetic acid (EDTA) (pH 8.0), 15 mM sodium pyrophosphate, 100 mM β-glycerophosphate, 50 mM NaF, 150 mM NaCl, 2 mM sodium orthovanadate, 1 mM phenylmethylsulfonyl fluoride (PMSF) and 1 mM dithiothreitol (DTT). After centrifugation, the protein level was determined in the supernatants using a Micro BCA protein assay kit using bovine serum albumin as the standard (Pierce Chemical, Rockford, IL). Aliquots containing 50 μg of total protein were boiled in a loading buffer that contained 150 mM Tris

(pH 6.8), 3 mM DTT, 6% SDS, 0.3% bromophenol blue and 30% glycerol. The aliquots were then loaded onto a polyacrylamide gel. After electrophoresis, the proteins were transferred from the gel to nitrocellulose transfer membranes (Pall Corp., East Hills, NY). To reduce background staining, the membranes were incubated with 5% non-fat dry milk in PBS containing 0.1% Tween 20 for 45 min, followed by incubation with mouse anti-nestin antibody (1:200; Millipore, Temecula, CA, USA), goat anti-DCX antibody (1:50; Santa Cruz Biotechnology, Santa Cruz, CA), rabbit anti-BDNF (1:1,000; Novus, Littleton, CO), anti-NMDAR1 (1:1,000; Millipore, Temecula, CA), anti-NMDAR2A (1:1,000; Thermo Fisher Scientific, Rockford, IL), anti-NMDAR2B (1:1,000; Millipore, Temecula, CA), anti-pCREB (1:10,000; Millipore, Temecula, CA), anti-SOD1 (1:1,000; StressMarq Biosciences Inc, Victoria, BC, Canada), anti-synaptophysin (1:10,000; Abcam, Cambridge, UK), and mouse anti-4-HNE (1:1,000; Alpha Diagnostic International, San Antonio, TX), peroxidase-conjugated anti-rabbit IgG or anti-mouse IgG and an enhanced luminol-based chemiluminescent (ECL) kit (Pierce Chemical). The blot was densitometrically scanned for the quantification of ROD of each band using NIH Image 1.59 software.

Statistical analysis

Data represent the means of experiments performed for each experimental area. Differences among the means were statistically analyzed by two-way analysis of variance followed by Bonferroni's post-tests using GraphPad Prism 5.0 software (GraphPad Software Inc., La Jolla, CA) in order to elucidate differences between dietary change × exercise. A $p < 0.05$ was considered significant.

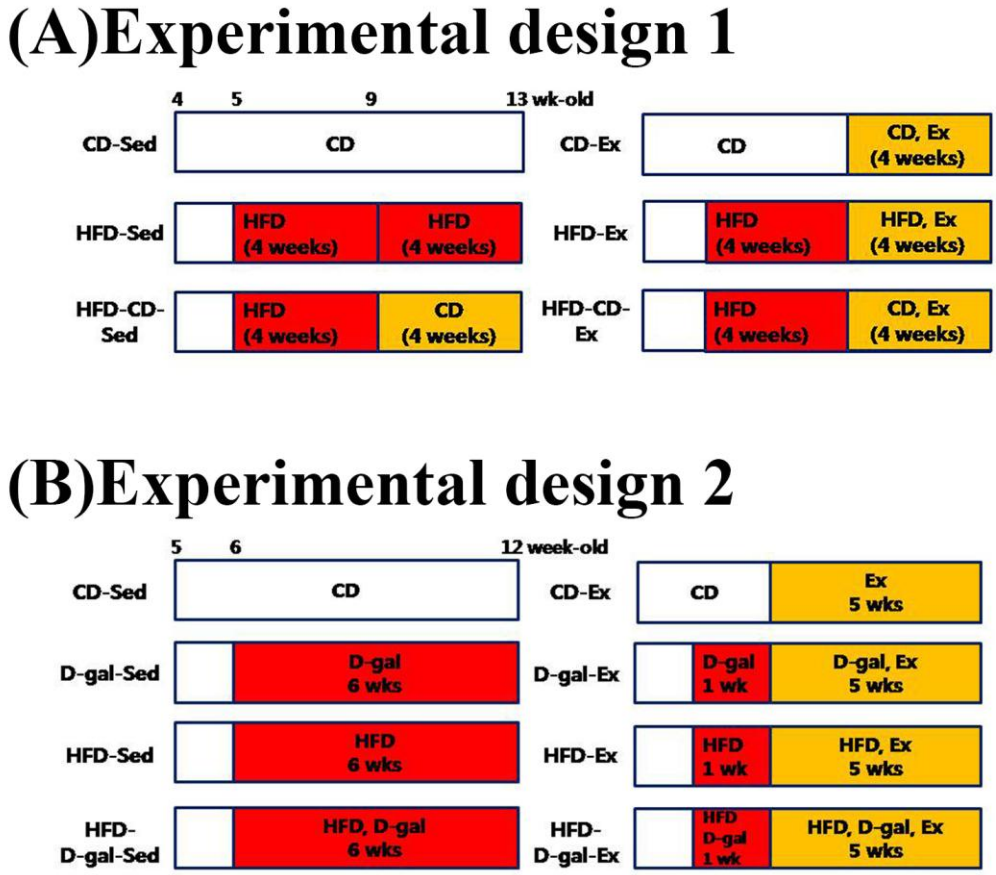


Fig. 1. Schematic presentation of the experimental design. (A) Effect of dietary change and/or treadmill exercise in HFD-fed obese mice, (B) effect of aging and/or treadmill exercise in the HFD-fed obese mice. N = 10 mice per group, 5 mice used for immunohistochemistry and immunoblot analyses, respectively. CD, chow diet; HFD, high fat diet; D-gal, D-galactose; Ex, treadmill exercise.

Results

Experimental design 1

Effects of dietary change and/or exercise on body weight, food intake, non-fasting blood glucose level and glucose tolerance

The results showed differential changes in physiological parameters that were dependent on dietary change or exercise. Body weight and food intake tended to be higher in the HFD group than in the CD or HFD- CD groups, and these differences persisted until the end of the experiment. Body weight and food intake in the HFD-CD group was reduced after dietary intervention to CD and this decreased food intake was restored to the level of the CD group over time (Fig. 2A and 2B). Non fasting-blood glucose levels showed a similar pattern to body weight. Blood glucose increased in the HFD-fed groups, while the glucose level in the HFD-CD groups continuously decreased to the level of the CD group (Fig. 2C). In addition, the glucose tolerance test showed that diet intervention from HFD to CD was effective at enhancing the body's glucose tolerance (Fig. 2D).

After exercise, there was a significant difference between exercise-induced reductions in body weight, non-fasting blood glucose, and enhancement of glucose tolerance in the HFD-Ex group and the sedentary group (HFD-Sed) (Fig. 2). In CD-Ex or HFD-CD-Ex groups, exercise-induced changes were not significant from those in their respective sedentary groups (Fig. 2). Interestingly, a combination of dietary change and exercise was more effective at restoring these parameters than

exercise alone in the continuous HFD-feeding (Fig. 2).

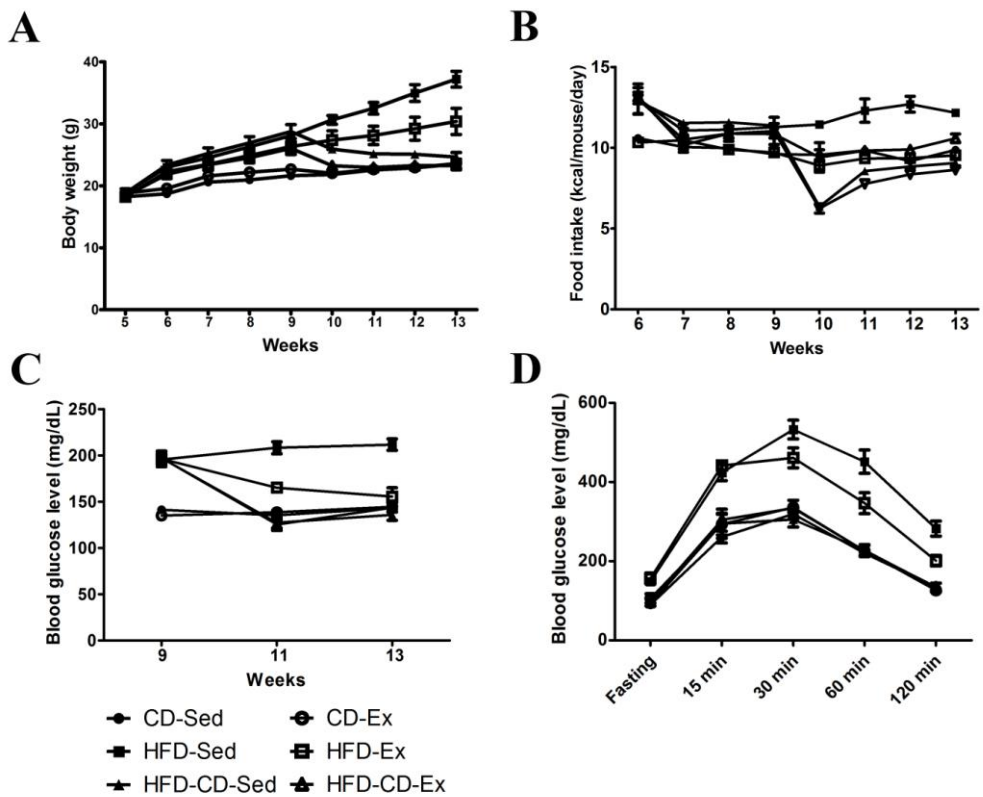
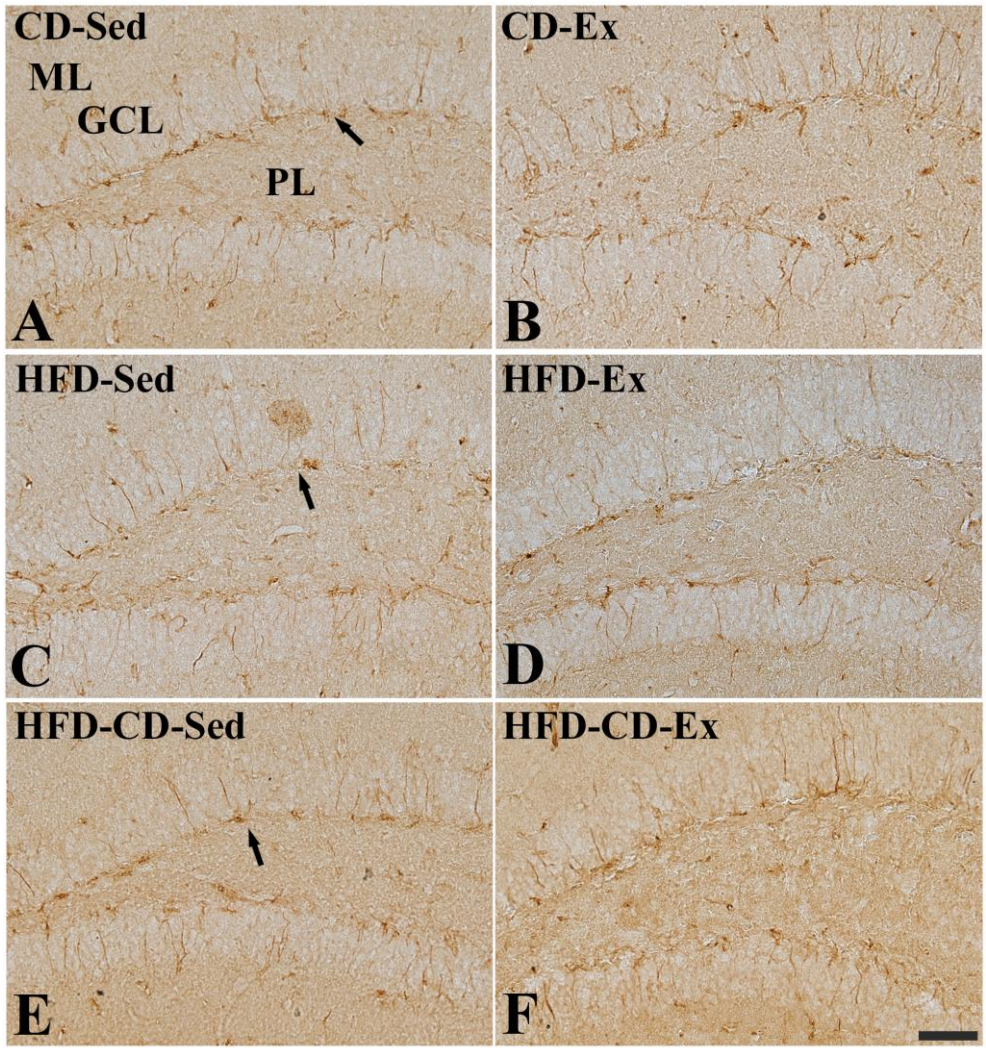


Fig. 2. Changes in body weight (A), food intake (B), non-fasting blood glucose level (C), and blood glucose during glucose tolerance test of the CD-Sed/Ex, HFD-Sed/Ex, and HFD-CD-Sed/Ex groups. Data are presented as the mean ± the standard error of the mean (SEM).

Effects of dietary change and/or exercise on neural stem cells

In the CD-Sed group, nestin immunoreactive cells and fibers were detected in the subgranular zone and granule cell layer of the dentate gyrus (Fig. 3A). Nestin immunoreactivity and protein levels in this group were used as the standard value. In the CD-Ex group, the number nestin-immunoreactive cells and fibers was prominently increased when compared with that in the CD-Sed group (Fig. 3B). There was also a significant increase to 133.63% ($P < 0.05$) and 160.79% ($P < 0.01$) in nestin immunoreactivity and protein levels, respectively, of the CD-Sed group (Fig. 2G, 5A, and 5B). In HFD-Sed group, nestin immunoreactive cells and fibers were decreased but this did not reach statistical significance (Fig. 3C). In this group, nestin immunoreactivity and protein levels were decreased to 61.40% ($P < 0.05$) and 84.95% ($P > 0.05$), respectively, of the CD-Sed group (Fig. 3G, 6A, and 6B). In the HFD-Ex group, nestin immunoreactive cells and fibers increased when compared with that in the HFD-Sed group (Fig. 3D). In this group, nestin immunoreactivity and protein levels were increased significantly to 176.65% ($P < 0.05$) and 198.15% ($P < 0.01$), respectively, of the HFD-Sed group. Interestingly, there was no significant difference between the HFD-Ex and CD-Ex groups ($P > 0.05$; Fig. 3G, 6A, and 6B). In the HFD-CD-Sed group, dietary change increased the number of nestin-immunoreactive cells and fibers to 159.22% ($P < 0.01$) of the HFD-Sed group (97.77% of the CD-Sed group; Fig. 3E and 3G). Hippocampal nestin levels were not significantly different between the HFD-CD-Sed and HFD-Sed (HFD-CD-Sed: 121.86% of HFD-Sed; $P > 0.05$) or CD-Sed (103.47%; Fig. 6A and 6B) groups. However, nestin immunoreactivity and protein levels were significantly increased in the HFD-CD-Ex group to 127.90% ($P < 0.05$) and

165.98% ($P < 0.01$) of the HFD-CD-Sed group, respectively (Fig. 3G, 6A, and 6B). There was no statistically significant difference in nestin-related parameters between the exercise groups ($P < 0.01$; Fig. 3G, 6A, 6B, and Table 1).


CD-Sed
ML
GCL
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CD-Ex
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C
HFD-Ex
D
HFD-CD-Sed
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HFD-CD-Ex
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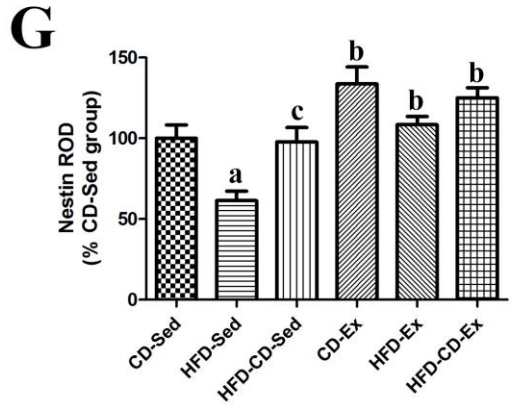
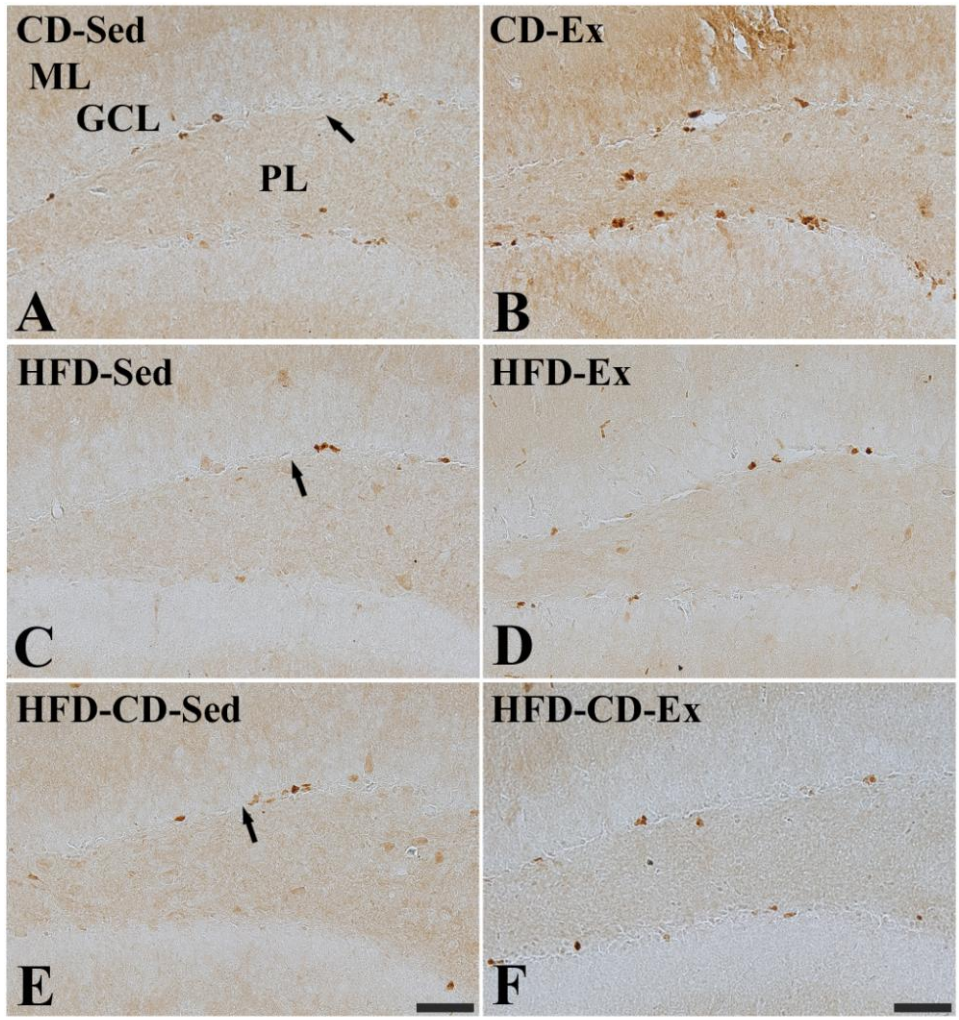

G
Nestin ROD
(% CD-Sed group)
150
100
50
0
a
c
b
b
b
CD-Sed
HFD-Sed
HFD-CD-Sed
CD-Ex
HFD-Ex
HFD-CD-Ex

Fig. 3. Immunohistochemistry for nestin in the dentate gyrus of the CD-Sed (A) CD-Ex (B), HFD-Sed (C), HFD-Ex (D), HFD-CD-Sed (E), and HFD-CD-Ex (F) groups. Nestin-immunoreactive cell bodies are located in the subgranular zone and their fibers are found in the granule cell layer. Note that nestin-immunoreactivity is significantly decreased in the HFD-Sed group and dietary change ameliorated the reduction in the HFD-CD-Sed group. However, physical exercise was effective in improving the nestin immunoreactive cells and fibers in all exercised groups compared to their respective sedentary groups. GCL, granule cell layer; PoL, polymorphic layer; ML, molecular layer. Bar = 50 μm. (G) Relative optical density (ROD) demonstrated as percentages of CD-Sed in nestin-immunoreactivity per section in each groups ($^{a}P < 0.05$, between CD vs. HFD groups, $^{b}P < 0.05$, between sedentary vs. exercise groups, $^{c}P < 0.05$, between HFD vs. HFD-CD groups). All data are shown as the mean ± SEM.

Effects of dietary change and/or exercise on cell proliferation

In the CD-Sed group, In the CD-Sed group, Ki67-immunoreactive nuclei were located mainly in the subgranular zone of the dentate gyrus (Fig. 4A). The mean number of Ki67-immunoreactive nuclei per section in this group was used as the standard value. After exercise, Ki67-immunoreactive nuclei were significantly increased in the CD-Ex group to 129.72% of the CD-Sed group ($P < 0.01$; Fig. 4B and 4G). Ki67-immunoreactive nuclei were dramatically decreased in the HFD-Sed group to 58.04% of CD-Sed group ($P < 0.01$; Fig. 4C and 4G). Ki67-immunoreactive nuclei were increased in the HFD-Ex group to 141.57% of the HFD-Sed group ($P < 0.05$); however, this was 63.34% lower than the CD-Ex group ($P < 0.01$; Fig. 4D and 4G). In the HFD-CD-Sed group, Ki67-immunoreactive nuclei were increased to 142.77% of the HFD-Sed group ($P < 0.05$; Fig. 4E and 4G). In this group, the average number was 82.87% lower than the CD-Sed group. In addition, Ki67-immunoreactive cells were increased in HFD-CD-Ex group to 118.57% of the HFD-CD-Sed group ($P < 0.05$; Fig. 4F and 4G). The average number of Ki67-immunoreactive nuclei in this group was similar to the HFD-Ex group ($P > 0.05$) and this number was still low when compared with that in the CD-Ex group (Fig. 4F, 4G, and Table 1).


CD-Sed
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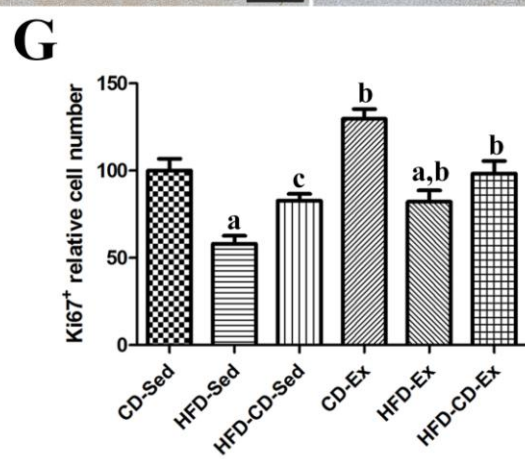
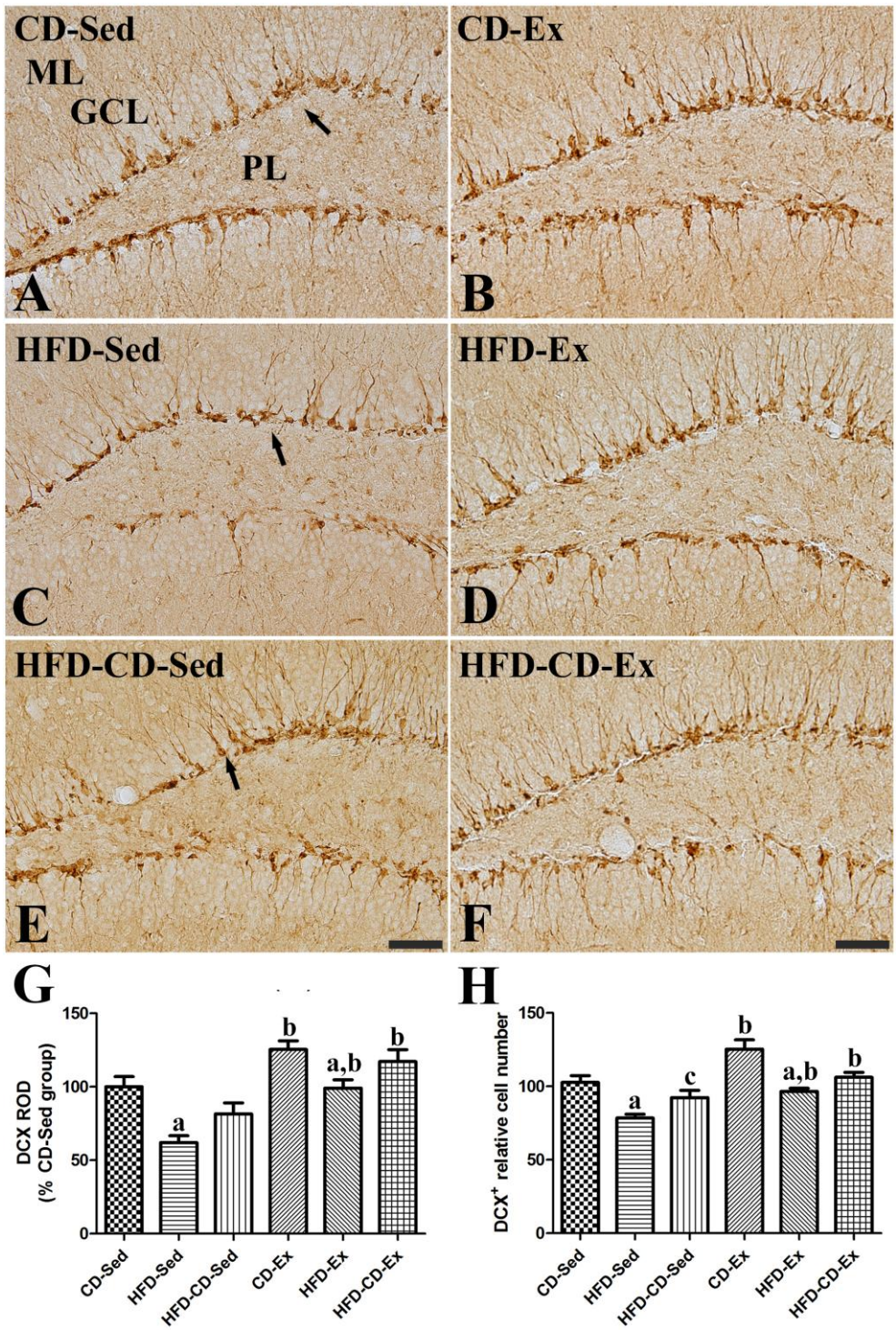

G
Ki67+ relative cell number
150
100
50
0
a
c
b
a,b
b
CD-Sed
HFD-Sed
HFD-CD-Sed
CD-Ex
HFD-Ex
HFD-CD-Ex

Fig. 4. Immunohistochemistry for Ki67 in the dentate gyrus of the CD-Sed (A) CD-Ex (B), HFD-Sed (C), HFD-Ex (D), HFD-CD-Sed (E), and HFD-CD-Ex (F) groups. Ki67-immunoreactive cells are located in the subgranular zone. Note that Ki67-immunoreactive cells are significantly decreased in the HFD-Sed group and dietary change ameliorated the reduction in the HFD-CD-Sed group. However, physical exercise is effective in improving the cell proliferation in all exercised groups compared to their respective sedentary groups. GCL, granule cell layer; PoL, polymorphic layer; ML, molecular layer. Bar = 50 μm. (G) The mean number of Ki67-immunoreactive cells per section in each groups ($^{a}P < 0.05$, between CD vs. HFD groups, $^{b}P < 0.05$, between sedentary vs. exercise groups, $^{c}P < 0.05$, between HFD vs. HFD-CD groups). All data are shown as the mean ± SEM.

Effects of dietary change and/or exercise on neuroblast differentiation

In the CD-Sed group, the cell bodies of DCX-immunoreactive neuroblasts were detected in the subgranular zone of the dentate gyrus and their dendrites extended into to the molecular layer of the dentate gyrus (Fig. 5A). In the CD-Ex group, DCX immunoreactivity and the number of DCX-immunoreactive neuroblasts were highest when compared to the other groups (125.44% and 115.89%, respectively, of the CD-Sed group; $P < 0.01$; Fig. 5G and 5H). The dendrites of neuroblasts were highly developed in this group when compared to the CD-Sed group (Fig. 5B). DCX protein levels were significantly increased in the CD-Ex group (162.48% of the CD-Sed group; $P < 0.01$; Fig. 6A and 6C). In the HFD-Sed group, DCX immunoreactivity and the number of DCX-immunoreactive neuroblasts were reduced to 62.11% and 76.13%, respectively, when compared with that in the CD-Sed group ($P < 0.01$; Fig. 5G and 5H). In this group, dendritic branching showed an uncomplicated shape when compared with that in the CD-Sed group (Fig. 5C). DCX protein levels in the HFD-Sed group were significantly decreased to 59.88% of the CD-Sed group ($P < 0.01$; Fig. 6A and 6C). In the HFD-Ex group, DCX immunoreactivity and the average number of DCX-immunoreactive neuroblasts were higher than that in the HFD-Sed group (159.54%, $P < 0.01$; 123.42%, $P < 0.05$, respectively; Fig. 5G and 5H). DCX protein levels in this group were 210.95% of the HFD-Sed group ($P < 0.01$; Fig. 6A and 6C). DCX immunoreactivity in the HFD-CD-Sed group was increased to 139.22% of the HFD-Sed group ($P > 0.05$; 81.50% of the CD-Sed group; Fig. 5G). In the HFD-CD-Sed group, the number of DCX-immunoreactive neuroblasts was increased to 116.81% of the HFD-Sed group ($P < 0.05$). In addition, the complexity of dendrites

was enhanced (Fig. 5E and 5H). DCX protein levels were increased to 151.64% of the HFD-Sed group ($P < 0.05$; 90.80% of the CD-Sed group; Fig. 6A and 6C). While we observed an increase in DCX-related parameters, their levels were lower than observed in the CD-Sed group. Finally, DCX immunoreactivity and the number of DCX-immunoreactive neuroblasts in the HFD-CD-Ex group were higher than that in the HFD-CD-Sed group (Fig. 5G and 5H). DCX immunoreactivity in the HFD-CD-Ex group was increased to 134.77% ($P < 0.01$) and 110.84% ($P > 0.05$) of the HFD-CD-Sed or HFD-Ex groups, respectively (109.84% of the CD-Sed group; Fig. 5G). In the HFD-CD-Ex group, the number of DCX-immunoreactive neuroblasts was increased to 115.68% ($P < 0.05$) and 109.41% ($P > 0.05$) of the HFD-CD-Sed and HFD-Ex groups, respectively, with enhanced dendritic complexity (Fig. 5F and 5H). Hippocampal DCX protein levels were significantly enhanced in this group: 178.40% and 128.26% of the HFD-CD-Sed and HFD-Ex groups ($P < 0.01$), respectively (Fig. 6A and 6C). These results indicate that the combined intervention of dietary change and exercise can enhance neuronal differentiation (Table 1).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DCX ROD
(% CD-Sed group)
150
100
50
0
a
b
a,b
b
CD-Sed
HFD-Sed
HFD-CD-Sed
CD-Ex
HFD-Ex
HFD-CD-Ex
H
DCX+ relative cell number
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b
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b
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HFD-Sed
HFD-CD-Sed
CD-Ex
HFD-Ex
HFD-CD-Ex

Fig. 5. Immunohistochemistry for doublecortin (DCX) in the dentate gyrus of the CD-Sed (A) CD-Ex (B), HFD-Sed (C), HFD-Ex (D), HFD-CD-Sed (E), and HFD-CD-Ex (F) groups. DCX-immunoreactive neuroblasts are detected in the subgranular zone of the dentate gyrus. Note that DCX-immunoreactive neuroblasts are significantly reduced in the HFD-Sed group and dietary change ameliorates the reduction in the HFD-CD-Sed group. DCX-immunoreactive neuroblasts increase in the CD-Ex, HFD-Ex, and HFD-CD-Ex groups compared to those in their respective sedentary groups. GCL, granule cell layer; PoL, polymorphic layer; ML, molecular layer. Bar = 50 μm. (G) Relative optical density (ROD) demonstrated as percentages of CD-Sed in DCX immunoreactivity per section in all groups. (H) The mean number of DCX-immunoreactive neuroblasts per section in each groups ($^{a}P < 0.05$, between CD vs. HFD groups, $^{b}P < 0.05$, between sedentary vs. exercise groups, $^{c}P < 0.05$, between HFD vs. HFD-CD groups). All data are shown as the mean ± SEM.

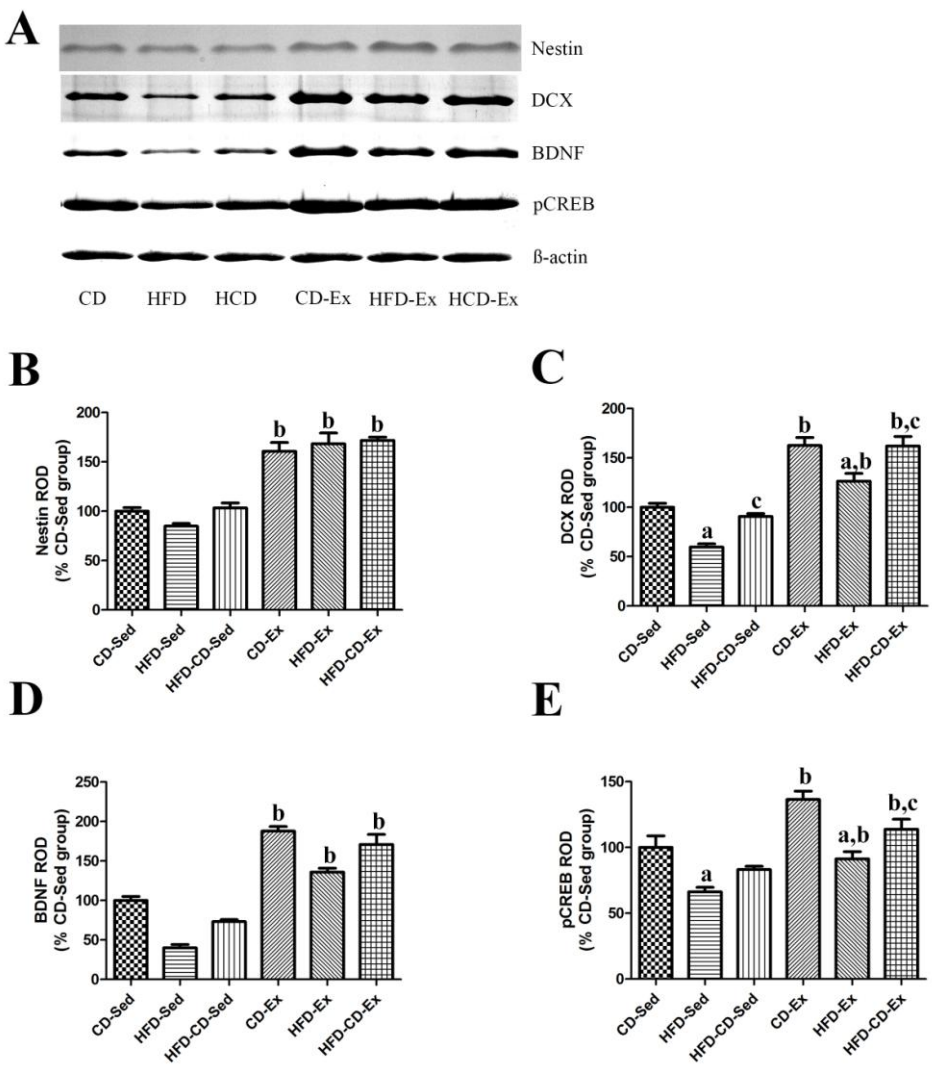
Effects of dietary change and/or exercise on BDNF expression

The mean BDNF protein level in the CD-Sed group was used as the standard value. In the CD-Ex group, there was significantly increased level, to 187.63% of the CD-Sed group ($P < 0.01$; Fig. 6A and 6D). In the HFD-Sed group, BDNF was significantly decreased to 40.10 % of the CD-Sed group ($P < 0.01$; Fig. 6A and 6D). In the HFD-Ex group, BDNF was significantly increased to 338.27% of the HFD-Sed group ($P < 0.01$; Fig. 6A and 6D). BDNF in the HFD-Ex group was lower (72.29%) than the CD-Ex group ($P < 0.01$; Fig. 6A and 6D). Hippocampal BDNF in the HFD-CD-Sed group was increased to 182.56% of the HFD-Sed group ($P < 0.05$) and its level was 73.20% of the CD-Sed group (Fig. 6A and 6D). However, BDNF in the HFD-CD-Ex group was increased to a similar level to the CD-Ex group, which was significantly higher than that in the HFD-CD-Sed (233.78%, $P < 0.01$) and HFD-Ex (125.89%, $P < 0.05$) groups (Fig. 6A, 6D, and Table 1).

Effects of dietary change and/or exercise on pCREB expression

The mean pCREB protein level in the CD-Sed group was used as the standard value. In the CD-Ex group, pCREB level was increased to 136.47% of the CD-Sed group ($P < 0.01$; Fig. 6A and 6E). In the HFD-Sed group, pCREB was decreased to 66.28% of the CD-Sed group ($P < 0.05$; Fig. 6A and 6D); however, hippocampal pCREB in the HFD-Ex group was increased to 137.69% of the HFD-Sed group ($P < 0.05$) and reduced to 66.87% of the CD-Ex group ($P < 0.01$; 91.26% of the CD-Sed group; Fig. 6A and 6D). In the HFD-CD-Sed group, pCREB was enhanced to 125.43% of the HFD-Sed group ($P > 0.05$) and reduced to 83.13% of the CD-Sed

group, although this did not reach significance (Fig. 6A and 6D). Furthermore, pCREB levels were increased in the HFD-CD-Ex group to 136.85% ($P < 0.01$) and 124.65% ($P < 0.05$) of the HFD-CD-Sed and HFD-Ex groups, respectively (Fig. 6A and 6D). pCREB level in the HFD-CD-Ex group was 83.36% of the CD-Ex group (Fig. 6A, 6D, and Table 1).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BDNF
pCREB
ß-actin
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HFD
HCD
CD-Ex
HFD-Ex
HCD-Ex
B
Nestin ROD (% CD-Sed group)
C
DCX ROD (% CD-Sed group)
D
BDNF ROD (% CD-Sed group)
E
pCREB ROD (% CD-Sed group)
CD-Sed
HFD-Sed
HFD-CD-Sed
CD-Ex
HFD-Ex
HFD-CD-Ex

Fig. 6. Western blot analysis of Nestin, DCX, BDNF, and pCREB in the hippocampus of the CD-Sed/Ex, HFD-Sed/Ex, HFD-CD-Sed/Ex groups (A). The relative optical density (ROD) of the immunoblot bands is demonstrated as percent values of CD-Sed in nestin (B), DCX (C), BDNF (D), and pCREB (E) in the whole hippocampus of the CD-Sed/Ex, HFD-Sed/Ex, HFD-CD-Sed/Ex groups ($^{a}P < 0.05$, between CD vs. HFD groups, $^{b}P < 0.05$, between sedentary vs. exercise groups, $^{c}P < 0.05$, between HFD vs. HFD-CD groups). All data are shown as the mean ± SE.

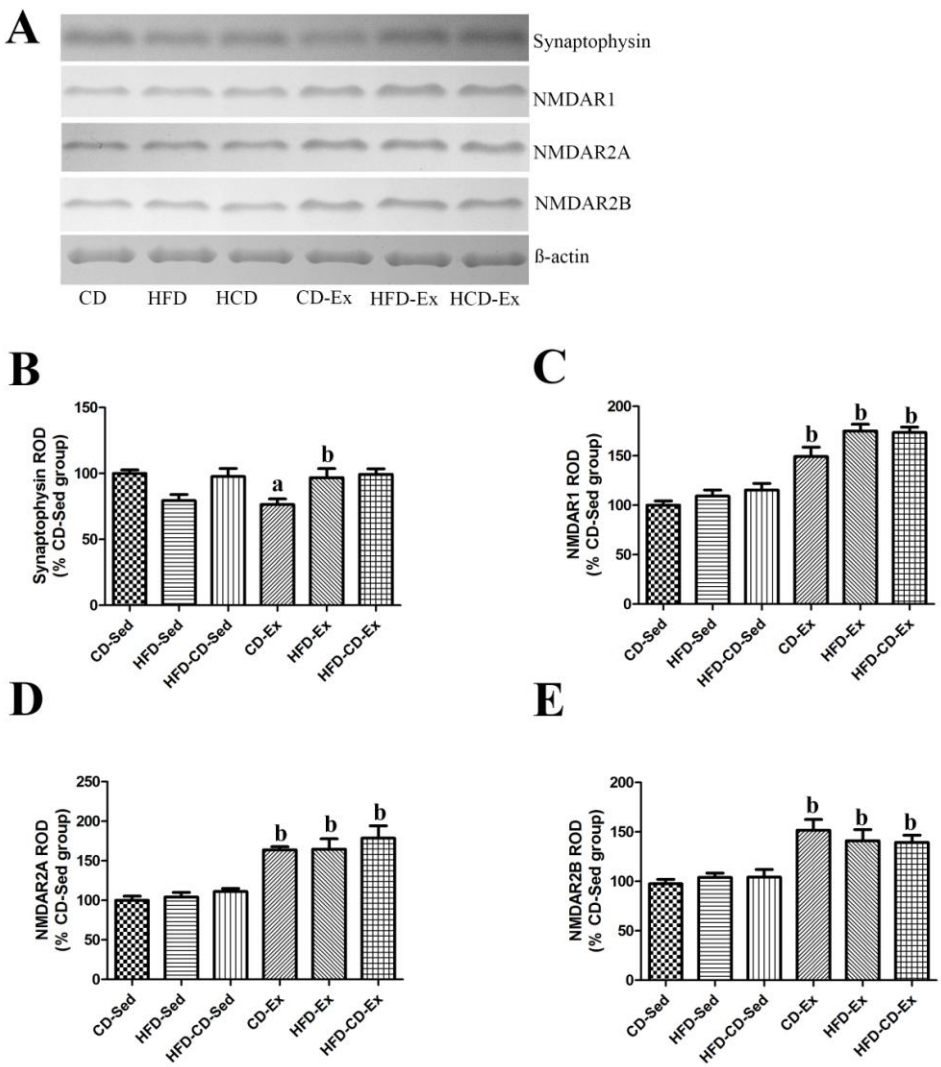
Effects of dietary change and/or exercise on synaptophysin expression

The mean synaptophysin protein level in the CD-Sed group was used as the standard value. In the CD-Ex group, synaptophysin was reduced as 76.41% of the CD-Sed group ($P < 0.05$; Fig. 7A and 7B). In the HFD-Sed group, synaptophysin levels were decreased to 79.48% of the CD-Sed group ($P < 0.05$; Fig. 7A and 7B); however, hippocampal synaptophysin levels in the HFD-Ex group were increased to 121.84% of the HFD-Sed group ($P > 0.05$) and not changed (96.84%) when compared with those in the CD-Sed group (Fig. 7A and 7B). In the HFD-CD-Sed group, synaptophysin was restored to 97.64% of the CD-Sed group and increased to 122.85% of the HFD-Sed group ($P > 0.05$; Fig. 7A and 7B). In addition, synaptophysin was at a similar level to the HFD-CD-Sed and HFD-Ex groups in the HFD-CD-Ex group (Fig. 7A and 7B). The synaptophysin level in the HFD-CD-Ex group was increased when compared when the CD-Ex group (129.95%; Fig. 7A, 7B, and Table 1).

Effects of dietary change and/or exercise on NMDAR1, NMDAR2A, and NMDAR2B expression

The mean NMDAR1, NMDAR2A, and NMDAR2B protein expression in the CD-Sed group were used as the standard value. The expression level of three subunits of the NMDAR protein was not significantly changed in the HFD-Sed and HFD-CD-Sed groups when compared with those in the CD-Sed group ($P > 0.05$; Fig. 7A, 7C, 7D, and 7E). The level of these proteins was changed compared to those in the respective sedentary groups after exercise. In CD-Ex group, the protein

expression of NMDAR1, NMDAR2A, and NMDAR2B subunits increased to 149.30%, 163.90%, and 152.14%, respectively, of the expression in the CD-Sed group ($P < 0.01$; Fig. 7A, 7C, 7D, and 7E). The expression of all three NMDAR subunits was increased in the HFD-Ex group to 160.31% ($P < 0.01$), 157.90% ($P < 0.01$), and 132.41% ($P < 0.05$) of the NMDAR1, NMDAR2A, and NMDAR2B, respectively, in the HFD-Sed group (Fig. 7A, 7C, 7D, and 7E). The protein expression of NMDARs in the HFD-Ex group was not statistically different from the CD-Ex group ($P > 0.05$; Fig. 7A, 7C, 7D, and 7E). Additionally, the expression of NMDAR1, NMDAR2A, and NMDAR2B in whole hippocampus was increased in HFD-CD-Ex group (150.47%, $P < 0.01$; 160.67%, $P < 0.01$; 133.07%, $P < 0.05$, respectively) of the HFD-CD-Sed group (Fig. 7A, 7C, 7D, and 7E). Furthermore, the expression of NMDARs in this group was not significantly different from the HFD-Ex and CD-Ex groups (Fig. 7A, 7C, 7D, 7E, and Table 1).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Synaptophysin
NMDAR1
NMDAR2A
NMDAR2B
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CD
HFD
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CD-Ex
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Synaptophysin ROD (% CD-Sed group)
C
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D
NMDAR2A ROD (% CD-Sed group)
E
NMDAR2B ROD (% CD-Sed group)
CD-Sed
HFD-Sed
HFD-CD-Sed
CD-Ex
HFD-Ex
HFD-CD-Ex

Fig. 7. Western blot analysis of synaptophysin, NMDAR1, NMDAR2A, and NMDAR2B in the hippocampus of the CD-Sed/Ex, HFD-Sed/Ex, HFD-CD-Sed/Ex groups (A). The relative optical density (ROD) of the immunoblot bands is demonstrated as percent values of CD-Sed in synaptophysin (B), NMDAR1 (C), NMDAR2A (D), and NMDAR2B (E) in the whole hippocampus of the CD-Sed/Ex, HFD-Sed/Ex, HFD-CD-Sed/Ex groups ($^{a}P < 0.05$, between CD vs. HFD groups, $^{b}P < 0.05$, between sedentary vs. exercise groups, $^{c}P < 0.05$, between HFD vs. HFD-CD groups). All data are shown as the mean ± SE.

Effects of dietary change and/or exercise on 4-HNE expression

The mean 4-HNE protein level in the CD-Sed group was used as the standard value. 4-HNE was decreased in the CD-Ex group to 73.88% of CD-Sed group ($P < 0.01$; Fig. 8A and 8B). 4-HNE protein expression was significantly increased in the HFD-Sed group to 128.43% of the CD-Sed group ($P < 0.01$; Fig. 8A and 8B). In the HFD-Ex group, 4-HNE protein expression was significantly reduced to 77.99% of the HFD-Sed group ($P < 0.01$) and it was unchanged when compared with that in the CD-Ex group ($P < 0.01$; 135.58%; Fig. 8A and 8B). In the HFD-CD-Sed group, 4-HNE was reduced when compared with that the HFD-Sed group (89.61%) and it was higher than that in the CD-Sed group (115.08%; Fig. 8A and 8B). In addition, 4-HNE was 86.73% of the HFD-CD-Sed group in HFD-CD-Ex group ($P < 0.05$) and at a level to the HFD-Ex group (Fig. 8A and 8B). Furthermore, the expression of 4-HNE protein in HFD-CD-Ex group was higher (135.11%) than in the CD-Ex group and at a similar level to the CD-Sed group (99.81%; Fig. 8A, 8B, and Table 1).

Effects of dietary change and/or exercise on SOD1 expression

The mean SOD1 protein level in the CD-Sed group was used as the standard value. In the CD-Ex group, SOD1 was prominently increased to 135.26% of the CD-Sed group ($P < 0.01$; Fig. 8A and 8C). In the HFD-Sed group, SOD1 expression was significantly decreased to 54.59% of that in the CD-Sed group ($P < 0.01$; Fig. 8A and 8C); however, hippocampal expression of SOD1 in the HFD-Ex

group was significantly increased to 205.12% of that in the HFD-Sed group ($P < 0.01$) and decreased to 82.78% of that in the CD-Ex group ($P < 0.01$; 111.97% of the CD-Sed group; Fig. 8A and 8C). In the HFD-CD-Sed group, SOD1 was enhanced to 152.34% of the HFD-Sed group ($P < 0.01$) and 83.22% the CD-Sed group (Fig. 8A and 8C). Moreover, in the HFD-CD-Ex group, SOD1 was increased to 181.82% and 135.13% of the HFD-CD-Sed and HFD-Ex groups ($P < 0.01$), respectively (Fig. 8A and 8C). SOD1 level in the HFD-CD-Ex group was lower than in the CD-Ex group (111.86% vs CD-Ex; 151.30 % of the CD-Sed group ; Fig. 8A, 8C, and Table 1).

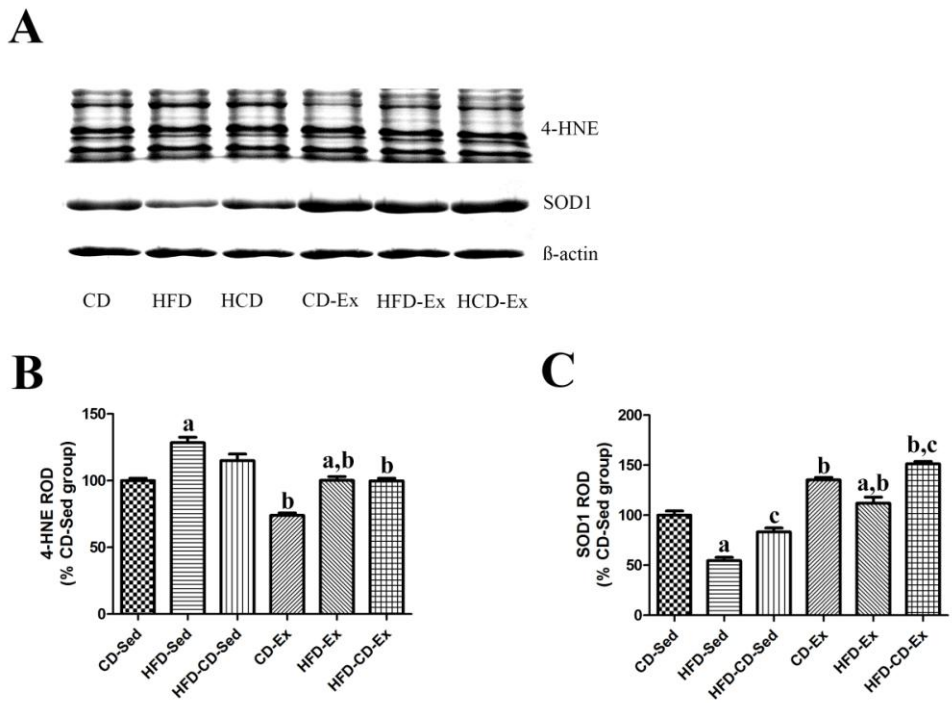


Fig. 8. Western blot analysis of 4-HNE, and SOD1 in the hippocampus of the CD-Sed/Ex, HFD-Sed/Ex, HFD-CD-Sed/Ex groups (A). The relative optical density (ROD) of the immunoblot bands is demonstrated as percent values of CD-Sed in 4-HNE (B) and SOD1 (C) in the whole hippocampus of the CD-Sed/Ex, HFD-Sed/Ex, HFD-CD-Sed/Ex groups ($^{a}P < 0.05$, between CD vs. HFD groups, $^{b}P < 0.05$, between sedentary vs. exercise groups, $^{c}P < 0.05$, between HFD vs. HFD-CD groups). All data are shown as the mean ± SE.

Experiment 1	Target	HFD-induced obesity	Dietary change	Treadmill exercise
Neurogenic markers	Nestin	- (IHC) ± (WB)	++ (IHC) ± (WB)	+ ~ ++ (IHC) ++ (WB)
	Ki67	-- ~ - (IHC)	+ (IHC)	+ (IHC)
	DCX	-- (IHC) -- (WB)	± ~ + (IHC) + ~ ++ (WB)	+ ~ ++ (IHC) ++ (WB)
Neurotrophic factor	BDNF	-- (WB)	+ (WB)	++ (WB)
	pCREB	-- ~ - (WB)	± ~ + (WB)	++ (WB)
Synaptic protein	Synaptophysin	- (WB)	± (WB)	- ~ ± (WB)
	NMDAR1	± (WB)	± (WB)	++ (WB)
	NMDAR2A	± (WB)	± (WB)	++ (WB)
	NMDAR2B	± (WB)	± (WB)	+ ~ ++ (WB)
Oxidative stress	4-HNE	++ (WB)	± (WB)	-- ~ - (WB)
	SOD1	-- (WB)	++ (WB)	++ (WB)

Table 1. Summary of immunohistochemical and immunoblot results in the experiment about dietary change and/or treadmill exercise to HFD-fed obese mice (-- strongly negative; - moderatetly negative; ± weakly negative or positive; + moderately positive; ++ strongly positive).

Experimental design 2

Effects of D-gal-induced aging and/or exercise on body weight, food intake, and non-fasting blood glucose levels in HFD-induced obese mice

The results of experiment 1 showed differences in physiological parameters that were dependent on dietary choice or exercise treatment. Body weight and food intake tended to be higher in the HFD groups when compared with that in the CD groups, and these differences persisted until the end of present experiment (Fig. 9A and 9B). Body weight in the HFD-Ex and HFD-D-gal-Ex groups was reduced after exercise and food intake in these groups was not significantly different from HFD-Sed and HFD-D-gal-Sed groups (Fig. 9A and 9B). Non fasting-blood glucose levels showed a similar pattern to body weight (Fig. 9C). Blood glucose increased in the HFD-fed sedentary groups, while glucose levels in the HFD-fed exercised groups continuously decreased to a level similar to the CD-Sed group (Fig. 9C). However, there were no significant differences in above physiological parameters between saline- and D-gal-treated groups (Fig. 9).

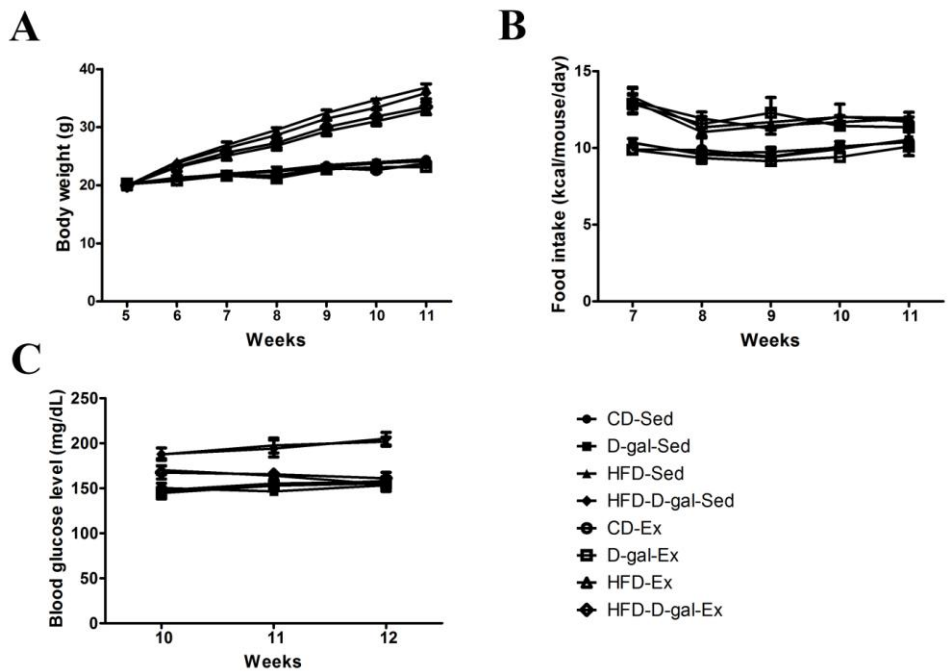
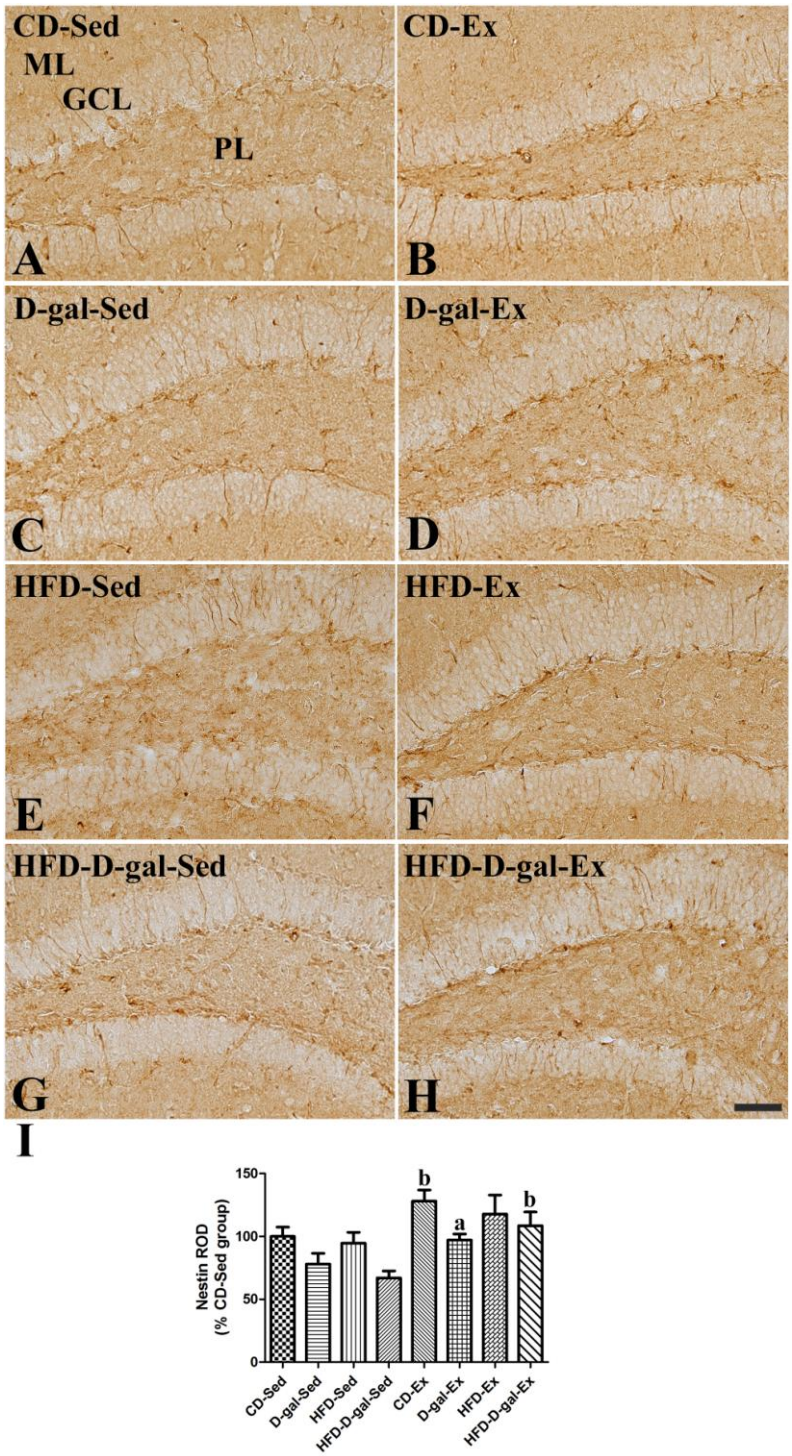


Fig. 9. Changes in body weight (A), food intake (B), non-fasting blood glucose level (C), and blood glucose during glucose tolerance test of the CD-Sed/Ex, D-gal-Sed/Ex, HFD-Sed/Ex, and HFD-D-gal-Sed/Ex groups. Data are presented as the mean ± the standard error of the mean (SEM).

Effects of D-gal-induced aging and/or exercise on neural stem cells in HFD-induced obese mice

In the CD-Sed group, nestin immunoreactive cells and fibers were detected in the subgranular zone and granule cell layer of dentate gyrus (Fig. 10A). In the CD-Ex group, nestin-immunoreactive cells and fibers were higher than that in the CD-Sed group (Fig. 10B). In this group, nestin immunoreactivity increased significantly to 128.04% of the CD-Sed group ($P < 0.05$) (Fig. 10I). In addition, hippocampal nestin protein levels were significantly increased to 248.23% of the CD-Sed group ($P < 0.01$; Fig. 13A and 13B). In the D-gal-Sed group, very few nestin immunoreactive cells and fibers were detected in the dentate gyrus (Fig. 10C). In this group, nestin immunoreactivity and protein level decreased to 74.34% ($P > 0.05$) and 71.57% ($P < 0.05$), respectively, of the CD-Sed group (Fig. 10I). In the D-gal-Ex group, the number of nestin immunoreactive cells and fibers was higher than that in the D-gal-Sed group (Fig. 10D). In this group, nestin immunoreactivity and protein was increased to 125.61% ($P > 0.05$) and 148.02% ($P < 0.05$), respectively, of the D-gal-Sed group, however, their levels were still lower as 72.93% ($P < 0.05$) and 70.32% ($P < 0.01$) of those in the CD-Ex group, respectively (Fig. 10I, 13A, and 13B). In the HFD-Sed group, nestin immunoreactivity and protein levels were not significantly different when compared with those in the CD-Sed group (87.17% and 109.93% of the CD-Sed group, respectively; $P > 0.05$; Fig. 10I, 13A, and 13B). In the HFD-Ex group, early exercise treatment increased the number of nestin immunoreactive cells and fibers to 130.78% ($P > 0.05$) when compared with that in the HFD-Sed group (114.00% of the CD-Sed group; Fig. 10F and 10I). Nestin was increased to 182.85% ($P <$

0.01) of the HFD-Sed group (201.02% of the CD-Sed group; Fig. 13A and 13B). In this group, nestin immunoreactivity and protein expression was 89.04% and 80.98%, respectively, of the CD-Ex group ($P > 0.05$; Fig. 13A and 13B). The number of nestin immunoreactive cells and fibers was lowest in the HFD-D-gal-Sed group (76.90% and 90.17% of the HFD-Sed and D-gal-Sed groups, respectively; $P > 0.05$; Fig. 10G and 10I). Nestin expression was the lowest when compared with that in all other groups (HFD-Sed: 55.05%, $P < 0.01$; D-gal-Sed: 84.55%, $P > 0.05$; Fig. 13A and 13B). Finally, in the HFD-D-gal-Ex group, nestin immunoreactivity and protein expression was increased as 161.95% ($P < 0.05$) and 241.63% ($P < 0.01$), respectively of the HFD-D-gal-Sed group (Fig. 10I, 13A, and 13B). In addition, nestin immunoreactivity in this group was 95.22% ($P > 0.05$) and 116.25% ($P > 0.05$) of the HFD-Ex and D-gal-Ex groups, respectively (84.78% when compared with that in the CD-Ex group; Fig. 10I). Furthermore, nestin expression was 72.75% ($P < 0.01$) and 138.02% ($P > 0.05$) of the HFD-Ex and D-gal-Ex groups, respectively (58.91% of the CD-Ex group; Fig. 13A, 13B, and Table 2).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Nestin ROD
(% CD-Sed group)
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100
50
0
b
a
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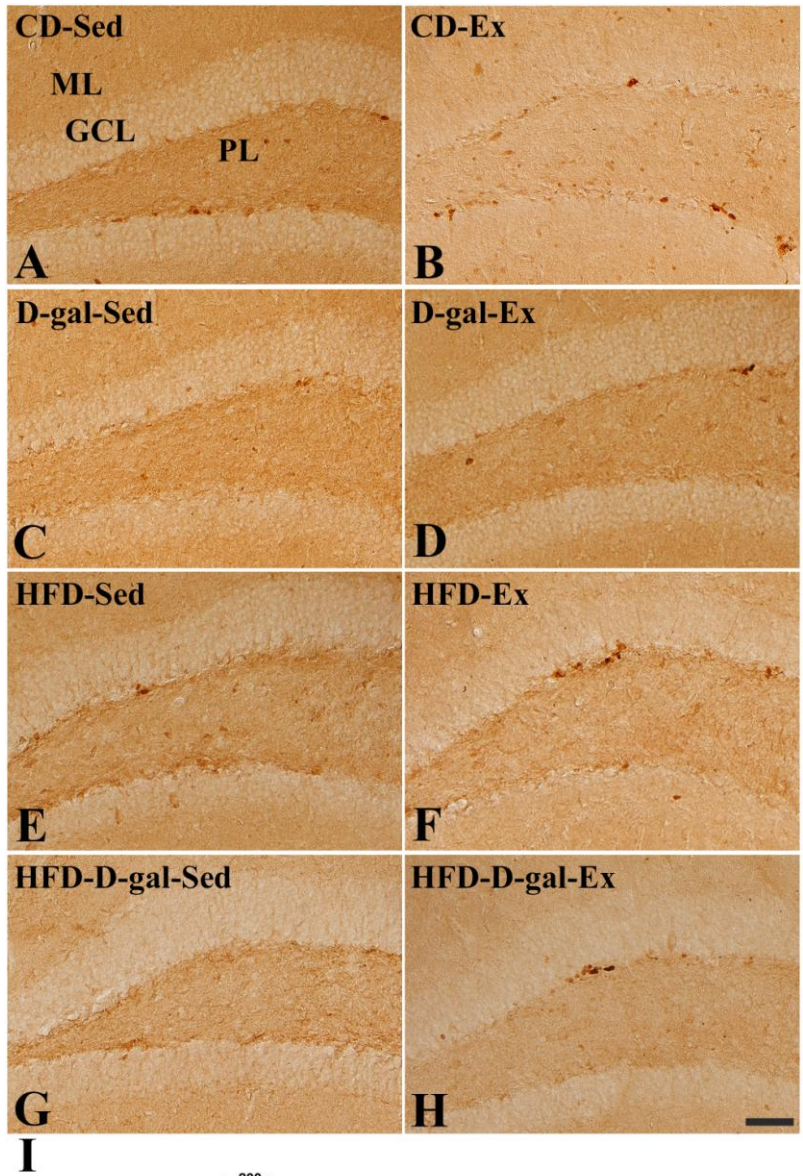
CD-Sed
D-gal-Sed
HFD-Sed
HFD-D-gal-Sed
CD-Ex
D-gal-Ex
HFD-Ex
HFD-D-gal-Ex

Fig. 10. Immunohistochemistry for nestin in the dentate gyrus of the CD-Sed (A) CD-Ex (B), D-gal-Sed (C), D-gal-Ex (D), HFD-Sed (E), HFD-Ex (F), HFD-D-gal-Sed (G), and HFD-D-gal-Ex (H) groups. Nestin-immunoreactive cell bodies are located in the subgranular zone and their fibers are found in the granule cell layer. Note nestin-immunoreactivity is decreased in the D-gal-Sed, HFD-Sed, and HFD-D-gal-Sed groups. However, physical exercise was effective in enhancing the nestin immunoreactivity in exercised groups compared to their respective sedentary groups. GCL, granule cell layer; PoL, polymorphic layer; ML, molecular layer. Bar = 50 μm. (I) Relative optical density (ROD) demonstrated as percentages of CD-Sed in nestin-immunoreactivity per section in each groups. (J) ROD of the immunoblots is demonstrated as percent values ($^{a}P < 0.05$, indicates a significant difference from D-gal treatment. $^{b}P < 0.05$, indicates a significant difference from exercise treatment. $^{c}P < 0.05$, indicates a significant difference from HFD treatment). Data are presented as the mean ± SEM.

Effects of D-gal-induced aging and/or exercise on cell proliferation in HFD-induced obese mice

In the CD-Sed group, Ki67-immunoreactive nuclei were located mainly in the subgranular zone of the dentate gyrus (Fig. 11A). In this group, the mean number of Ki67-immunoreactive nuclei was used as the standard value. After exercise treatment, Ki67-immunoreactive nuclei were increased in the CD-Ex group and the average number was highest as 146.84% of that in the CD-Sed group ($P < 0.01$) (Fig. 11B and 11I). Ki67-immunoreactive nuclei were dramatically decreased in the D-gal-Sed group with the average number as 65.82% of that in the CD-Sed group ($P < 0.05$) (Fig. 11C and 11I). In the D-gal-Ex group, Ki67-immunoreactive nuclei were increased and the level was higher than that of D-gal-Sed ($P < 0.01$) and lower than that of CD-Ex ($P < 0.01$) groups (as 149.04% and 67.67% of that in the D-gal-Sed and CD-Ex groups, respectively) (Fig. 11D and 11I). In the HFD-Sed group, the number of Ki67-immunoreactive nuclei was significantly decreased compared to the CD-Sed group, with the average number of Ki67-immunoreactive nuclei being 67.09% of the CD-Sed group ($P < 0.05$) (Fig. 11E and 11I). In the HFD-Ex group, the average number Ki67-immunoreactive nuclei was prominently increased as 166.04% of that in the HFD-Sed group ($P < 0.01$) and its level was lower than that of CD-Ex groups (as 75.86% of CD-Sed group, $P < 0.05$) (Fig. 11F and 11I). In the HFD-D-gal-Sed group, Ki67-immunoreactive nuclei were lowest as 46.84% of that in the CD-Sed group (Fig. 11G and 11I). In this group, average number was decreased compared to the HFD-Sed (as 69.81% of the HFD-Sed group, $P < 0.05$) and D-gal-Sed (as 71.15% of the D-gal-Sed group, $P > 0.05$) (Fig. 11G and 11I). In the HFD-D-gal-Ex group, the average number of Ki67-

immunoreactive nuclei was transparently increased compared to that in the HFD-D-gal-Sed group (163.51% of that of the HFD-D-gal-Sed group, $P < 0.05$) (Fig. 11H and 11I). However, the average number of Ki67-immunoreactive nuclei in this group was lower compared to those in the HFD-Ex (68.75% of HFD-Ex group, $P < 0.01$) and D-gal-Ex (78.06% of D-gal-Ex group, $P > 0.05$) groups (Fig. 11I and Table 2).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150
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50
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b
a,b
b,c
a,b
CD-Sed
D-gal-Sed
HFD-Sed
HFD-D-gal-Sed
CD-Ex
D-gal-Ex
HFD-Ex
HFD-D-gal-Ex

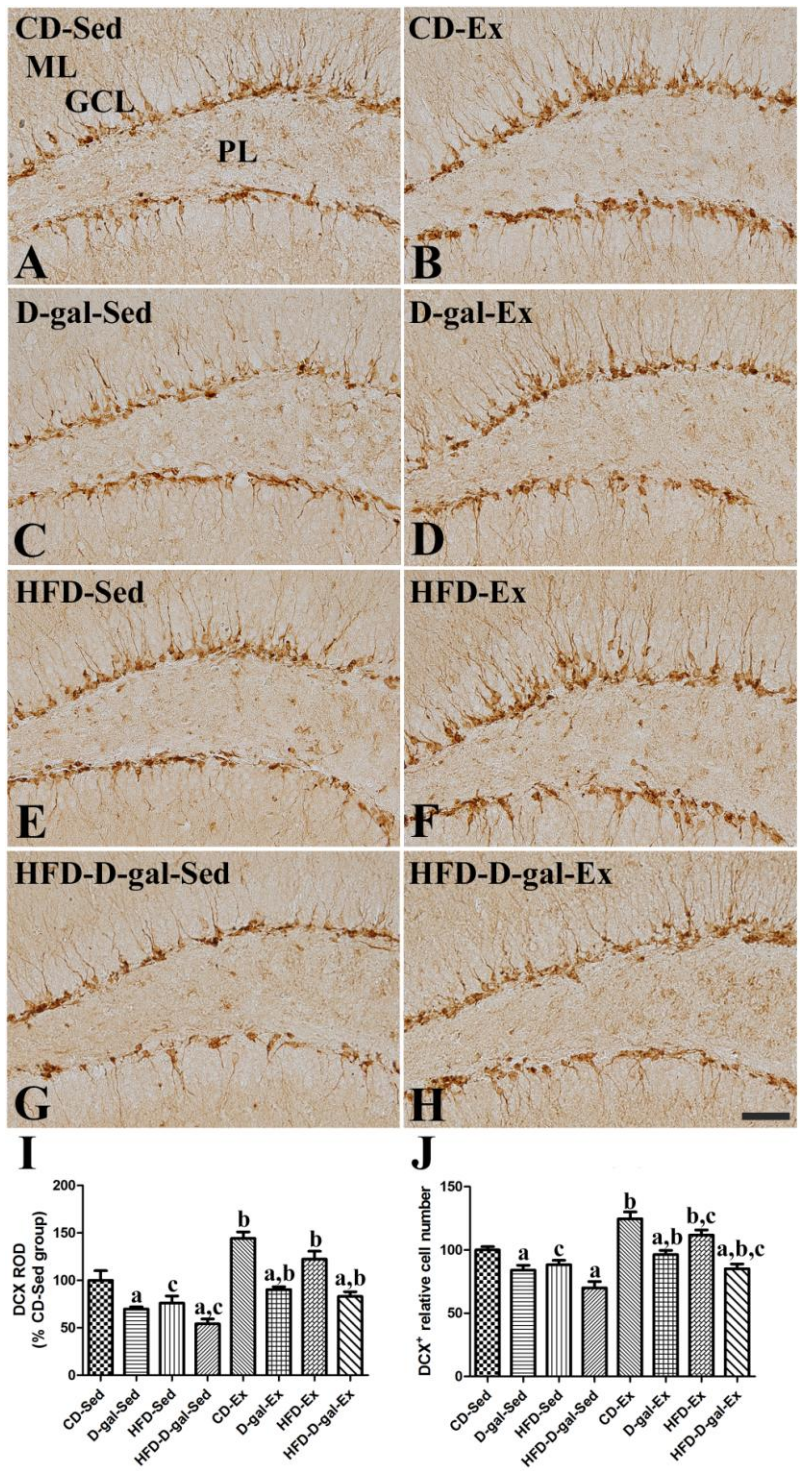
Fig. 11. Immunohistochemistry for Ki67 in the dentate gyrus of the CD-Sed (A) CD-Ex (B), D-gal-Sed (C), D-gal-Ex (D), HFD-Sed (E), HFD-Ex (F), HFD-D-gal-Sed (G), and HFD-D-gal-Ex (H) groups. Ki67-immunoreactive cells are located in the subgranular zone. Note that Ki67-immunoreactive cells are significantly decreased in the D-gal-Sed, HFD-Sed, and HFD-D-gal-Sed groups. However, physical exercise was effective in inducing the cell proliferation in the exercised groups compared to their respective sedentary groups. GCL, granule cell layer; PoL, polymorphic layer; ML, molecular layer. Bar = 50 μm. (G) The mean number of Ki67-immunoreactive cells per section in each groups ($^{a}P < 0.05$, indicates a significant difference from D-gal treatment. $^{b}P < 0.05$, indicates a significant difference from exercise treatment. $^{c}P < 0.05$, indicates a significant difference from HFD treatment). Data are presented as the mean ± SEM.

Effects of D-gal-induced aging and/or exercise on neuroblast differentiation in HFD-induced obese mice

In the CD-Sed group, the cell body of DCX-immunoreactive neuroblasts were observed in the subgranular zone of the dentate gyrus and their dendrites were well-developed with extension to the molecular layer of the dentate gyrus (Fig. 12A). In the CD-Ex group, the number of DCX-immunoreactive neuroblasts was highest among all groups as 124.67% of that in the CD-Sed group ($P < 0.01$) and pattern of dendritic branching was complex compared to that in the CD-Sed group (Fig. 12B and 12J). In this group, the DCX immunoreactivity and protein levels were 144.30% and 130.89% of those in the CD-Sed group, respectively ($P < 0.01$) (Fig. 12I, 13A, and 13C). In D-gal-Sed group, the number of DCX-immunoreactive neuroblasts were reduced as 84.11% of that in the CD-Sed group ($P < 0.05$) and dendritic branching showed uncomplicated shape compared to that in the CD-Sed group (Fig. 12C and 12J). DCX immunoreactivity and its protein level of D-gal-Sed group were reduced to the level of 69.83% ($P < 0.01$) and 54.86% ($P < 0.01$) of the CD-Sed group, respectively (Fig. 12I, 13A, and 13C). In the D-gal-Ex group, the mean number of DCX-immunoreactive neuroblasts was increased as 114.45% of the D-gal-Sed group ($P < 0.05$) (Fig. 12J). In addition, its immunoreactivity and protein level were increased to 129.48% ($P < 0.01$) and 130.20% ($P < 0.05$) of those in the D-gal-Sed group (Fig. 12I, 13A, and 13C). In this group, the number of DCX-immunoreactive neuroblasts and their immunoreactivity/protein level were lower compared to those in CD-Ex group ($P < 0.01$) (Fig. 12I, 12J, 13A, and 13C). In HFD-Sed group, the number of DCX-immunoreactive neuroblasts was decreased as 88.36% of that in the CD-Sed group ($P < 0.05$) (Fig. 12E and 12J).

DCX immunoreactivity and protein level were 76.01% ($P < 0.05$) and 85.11% ($P < 0.01$) of those in the CD-Sed group (Fig. 12I, 13A, and 13C). In the HFD-Ex group, the number of DCX-immunoreactive neuroblasts was significantly increased as 126.51% ($P < 0.01$) of that in the HFD-Sed group (Fig. 12F and 12J). In this group, the number of DCX-immunoreactive neuroblasts was still lower as 89.66% of CD-Ex group ($P < 0.01$) (Fig. 12J). DCX-immunoreactivity and protein level was 161.05% ($P < 0.01$) and 145.06% ($P < 0.01$) of those in the HFD-Sed groups, respectively (Fig. 12I, 13A, and 13C). The immunoreactivity and protein level of DCX in this group was 84.84% ($P > 0.05$) and 94.32% ($P < 0.01$) of those in the CD-Ex group (Fig. 12I). There is no significant difference in the degree of reduction between D-gal-Sed and HFD-Sed groups. However, there is transparent difference between D-gal-Ex and HFD-Ex groups in DCX-related parameters. In the HFD-D-gal-Sed group, the number of DCX-immunoreactive neuroblasts was lowest as 70.06% of the CD-Sed group (Fig. 12G and 12J). In this group, the number of DCX-immunoreactive neuroblasts was decreased as 83.29% and 79.29% of that in the D-gal-Sed ($P > 0.05$) and HFD-Sed ($P < 0.05$) groups, respectively (Fig. 12I, 13A, and 13C). Additionally, DCX immunoreactivity in the HFD-D-gal-Sed group was lowest as 54.30% of the CD-Sed group (77.76%, $P < 0.05$ and 71.44%, $P < 0.05$ of the D-gal-Sed and HFD-Sed groups, respectively) (Fig. 12I). In this group, dendritic branches were shortest and uncomplicated shape compared to those in other groups (Fig. 12G). In the HFD-D-gal-Sed group, the protein level was of DCX-immunoreactive neuroblasts was also lowest as 43.06% of the CD-Sed group (78.49%, $P > 0.05$ and 50.60%, $P < 0.01$ of the D-gal-Sed and HFD-Sed groups, respectively) (Fig. 13A and 13C). Finally, in the HFD-D-gal-Ex group, the average number of DCX-immunoreactive neuroblasts was prominently

increased as 121.63% of that in the HFD-D-gal-Sed group ($P < 0.05$) (Fig. 12H and 12J). Their immunoreactivity and protein levels were also increased to 153.10% ($P < 0.01$) and 154.36% ($P < 0.01$) of those in the HFD-D-gal-Sed group (DCX immunoreactivity; 91.95%, $P > 0.05$ and 67.92%, $P < 0.01$ of the D-gal-Ex and HFD-Ex groups, DCX protein level; 93.06%, $P > 0.05$ and 53.84%, $P < 0.01$ of the D-gal-Ex and HFD-Ex groups, respectively) (Fig. 12I, 13A, 13C, and Table 2).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CD-Ex
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D-gal-Sed
HFD-Sed
HFD-D-gal-Sed
CD-Ex
D-gal-Ex
HFD-Ex
HFD-D-gal-Ex

Fig. 12. Immunohistochemistry for doublecortin (DCX) in the dentate gyrus of the CD-Sed (A) CD-Ex (B), D-gal-Sed (C), D-gal-Ex (D), HFD-Sed (E), HFD-Ex (F), HFD-D-gal-Sed (G), and HFD-D-gal-Ex (H) groups. DCX-immunoreactive neuroblasts are detected in the subgranular zone of the dentate gyrus. Note that DCX-immunoreactive neuroblasts in the D-gal-Sed, HFD-Sed, and HFD-D-gal-Sed decreased compared to the CD-Sed group. DCX-immunoreactive neuroblasts were improved by exercise in the CD-Ex, D-gal-Ex, HFD-Ex, and HFD-D-gal-Ex groups compared to their sedentary groups. GCL, granule cell layer; PoL, polymorphic layer; ML, molecular layer. Bar = 50 μm. (I) ROD demonstrated as percentages of CD-Sed in DCX immunoreactivity per section in all groups. (J) The mean number of DCX-immunoreactive neuroblasts per section in each groups ($^{a}P < 0.05$, indicates a significant difference from D-gal treatment. $^{b}P < 0.05$, indicates a significant difference from exercise treatment. $^{c}P < 0.05$, indicates a significant difference from HFD treatment). Data are presented as the mean ± SEM.

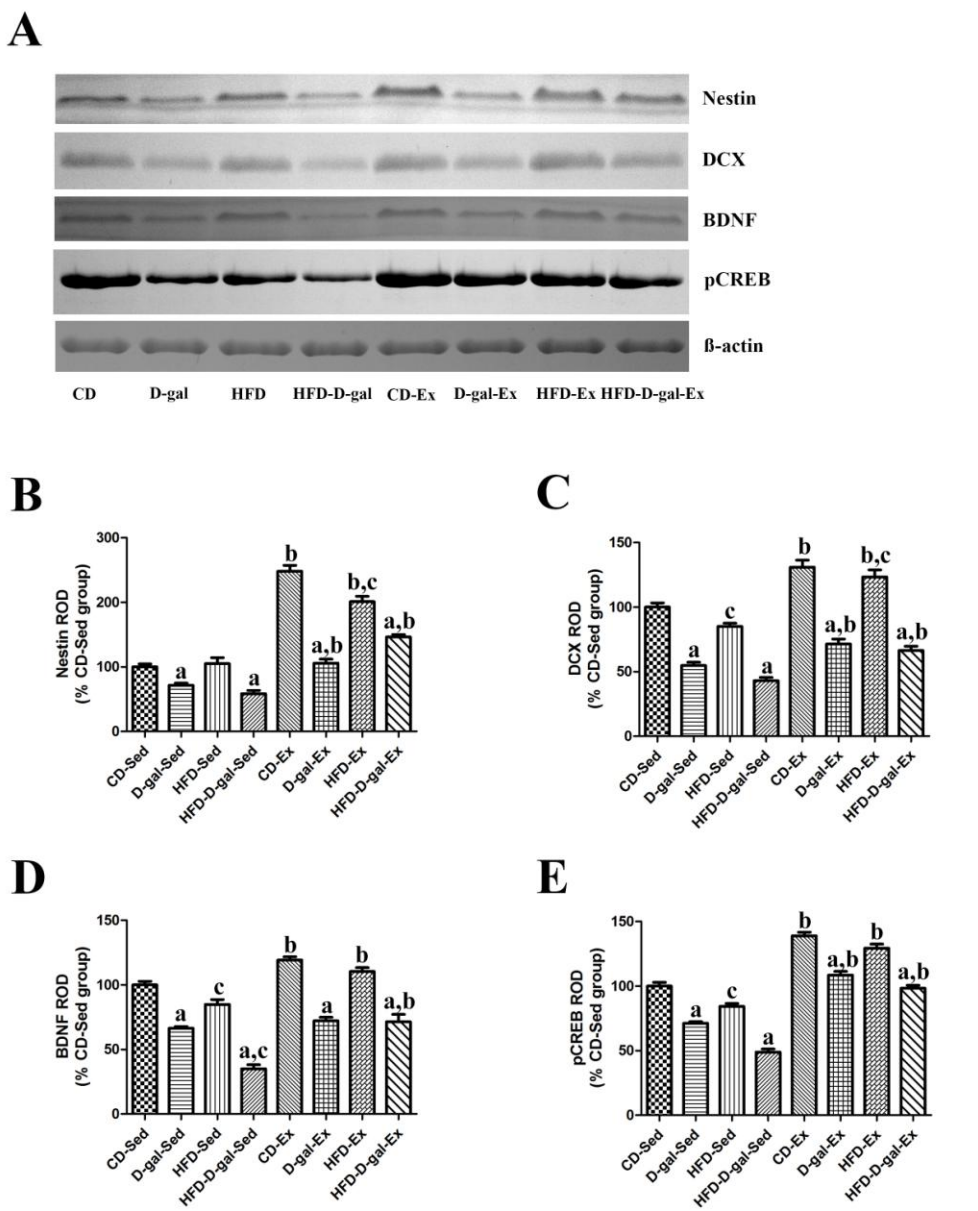
Effects of D-gal-induced aging and/or exercise on BDNF expression in HFD-induced obese mice

Mean BDNF protein level of the CD-Sed group was used as the standard value. In the CD-Ex group, its level was significantly increased as 119.34% of that in the CD-Sed group ($P < 0.01$) (Fig. 13A and 13D). In the D-gal-Sed group, BDNF protein expression was significantly decreased to 66.41% of that in the CD-Sed group ($P < 0.01$). In the D-gal-Ex group, BDNF protein expression was slightly increased to 108.70% of that in the D-gal-Sed group ($P > 0.05$) (Fig. 13A and 13D). However, the level of BDNF in D-gal-Ex was low as 60.48% of that in the CD-Ex ($P < 0.01$) (Fig. 13A and 13D). In HFD-Sed group, BDNF protein expression was reduced as 84.89% of that in the CD-Sed group ($P < 0.05$) (Fig. 13A and 13D). After exercise treatment, the level of BDNF in HFD-Ex group was significantly increased to the level of 130.13% of the HFD-Sed group ($P < 0.01$) and still lower compared to that in the CD-Ex group (92.56% of the CD-Ex group, $P < 0.05$) (Fig. 13A and 13D). The BDNF protein level in the hippocampus of the HFD-D-gal-Sed group was lowest among all groups as 35.02% of the CD-Sed group and it was 52.73% and 41.25% of those in the D-gal-Sed and HFD-Sed groups ($P < 0.01$), respectively. After exercise treatment, BDNF level in HFD-D-gal-Ex group was reversely changed as 203.95% of that in the HFD-D-gal-Sed group ($P < 0.01$) (Fig. 13A and 13D). Additionally, its level was 98.95% ($P > 0.05$) and 64.65% ($P < 0.01$) of that in the HFD-D-gal-Ex and HFD-Ex groups, respectively (Fig. 13A, 13D, and Table 2).

Effects of D-gal-induced aging and/or exercise on pCREB expression in

HFD-induced obese mice

Mean pCREB protein level of the CD-Sed group was used as the standard value. In the CD-Ex group, the pCREB protein expression was prominently increased as 139.31% of that in the CD-Sed group ($P < 0.01$) (Fig. 13A and 13E). In the D-gal-Sed group, pCREB protein expression was significantly decreased to 71.31% of that in the CD-Sed group ($P < 0.01$) (Fig. 13A and 13E). In the D-gal-Ex group, pCREB protein expression was significantly increased to 152.18% of that in the D-gal-Sed group ($P < 0.05$) (Fig. 13A and 13E). However, the level of pCREB in D-gal-Ex was low as 78.03% of that in the CD-Ex ($P < 0.01$) (108.51% of that in the CD-Sed group) (Fig. 13A and 13E). In the HFD-Sed group, the pCREB protein expression was decreased to 84.25% of that in the CD-Sed group ($P < 0.01$) (Fig. 13A and 13E). However, the pCREB protein level in the hippocampus of the HFD-Ex group was increased as 153.65% of HFD-Sed group ($P < 0.01$) (Fig. 13A and 13E). Its level was 93.08% of that in the CD-Ex group ($P > 0.05$) (129.45% of that in the CD-Sed group) (Fig. 13A and 13E). In the HFD-D-gal-Sed group, the expression level of the pCREB protein was reduced to 68.64% and 58.09% of that in the D-gal-Sed and HFD-Sed group, respectively ($P < 0.01$) (Fig. 13A and 13E). Protein level of pCREB was lowest as 48.94% of that in the CD-Sed group (Fig. 13A and 13E). Furthermore, in the HFD-D-gal-Ex group, the protein level of pCREB was significantly increased as 201.14% of that in the HFD-D-gal-Sed group ($P < 0.01$) and it was 90.72% ($P > 0.05$) and 76.05% ($P < 0.01$) of that in the D-gal-Ex and HFD-Ex groups, respectively (Fig. 13A and 13E). The pCREB level of HFD-D-gal-Ex group was low as 70.78% of the CD-Ex group and it was restored to the level of the CD-Sed group (Fig. 13A, 13E, and Table 2).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BDNF
pCREB
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HFD
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CD-Ex
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HFD-D-gal-Ex
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DCX ROD
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D
BDNF ROD
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E
pCREB ROD
(% CD-Sed group)
CD-Sed
D-gal-Sed
HFD-Sed
HFD-D-gal-Sed
CD-Ex
D-gal-Ex
HFD-Ex
HFD-D-gal-Ex

Fig. 13. Western blot analysis of Nestin, DCX, BDNF, and pCREB in the hippocampus of the CD-Sed/Ex, D-gal-Sed/Ex, HFD-Sed/Ex, and HFD-D-gal-Sed/Ex groups (A). The relative optical density (ROD) of the immunoblot bands is demonstrated as percent values of CD-Sed in nestin (B), DCX (C), BDNF (D), and pCREB (E) in the whole hippocampus of the CD-Sed/Ex, D-gal-Sed/Ex, HFD-Sed/Ex, and HFD-D-gal-Sed/Ex groups ($^{a}P < 0.05$, indicates a significant difference from D-gal treatment. $^{b}P < 0.05$, indicates a significant difference from exercise treatment. $^{c}P < 0.05$, indicates a significant difference from HFD treatment). Data are presented as the mean ± SE.

Effects of D-gal-induced aging and/or exercise on synaptophysin expression in HFD-induced obese mice

Mean synaptophysin protein level of the CD-Sed group was used as the standard value. In the CD-Ex group, the level of synaptophysin protein was slightly increased as 112.95% of CD-Sed group ($P > 0.05$) (Fig. 14A and 14B). In the D-gal-Sed, synaptophysin protein expression was significantly decreased to 48.05% of that in the CD-Sed group ($P < 0.01$) (Fig. 14A and 14B). After treadmill exercise treatment, the level of synaptophysin in D-gal-Ex group was 141.52% of that in the D-gal-Sed group ($P > 0.05$) (Fig. 14A and 14B). The synaptophysin level in this group was 60.21% lower than the CD-Ex group ($P < 0.01$) (Fig. 14A and 14B). In the HFD-Sed group, its level was not significantly different from CD-Sed group (as 94.11% of CD-Sed group) (Fig. 14A and 14B). In the HFD-Ex group, synaptophysin protein level was 114.69% of that in the HFD-Sed group and 95.55% of that in the CD-Ex group ($P > 0.05$) (Fig. 14A and 14B). There is no significant difference between CD-Ex and HFD-Ex groups. Additionally, the hippocampal protein level of synaptophysin in the HFD-D-gal-Sed group was lowest at 41.86% of the CD-Sed group and it was 87.11% ($P > 0.05$) and 44.48% ($P < 0.01$) of that in D-gal-Sed and HFD-Sed groups, respectively (Fig. 14A and 14B). After exercise treatment, the level of synaptophysin in HFD-D-gal-Ex was significantly increased to 192.40% of that in the HFD-D-gal-Sed group ($P < 0.01$). Additionally, its level in this group was 118.43% ($P > 0.05$) and 74.63% ($P < 0.05$) of that in D-gal-Ex and HFD-Ex groups, respectively (Fig. 14A and 14B). Finally, it was 71.31% of that in the CD group (Fig. 14A, 14B, and Table 2).

Effects of D-gal-induced aging and/or exercise on NMDAR1, NMDAR2A, and NMDAR2B expression in HFD-induced obese mice

Mean level of NMDAR1, NMDAR2A, and NMDAR2B proteins in the CD-Sed group was used as the standard value. After exercise treatment all three subunits of NMDAR were increased as 170.91% (NMDAR1, $P < 0.01$), 138.76% (NMDAR2A, $P < 0.01$), and 117.72% (NMDAR2B, $P > 0.05$) of those in the CD-Sed groups, respectively (Fig. 14A, 14C, 14D, and 14E). D-gal treatment reduced NMDAR1 and NMDAR2B to the level of 61.31% and 26.48% of those in the CD-Sed groups ($P < 0.01$) (Fig. 14A, 14C, 14D, and 14E). However, the protein level of the NMDAR2A was not changed compared to the CD-Sed group. In D-gal-Ex group, the expression of the NMDAR1, NMDAR2A, and NMDAR2B was increased as 153.74% ($P < 0.05$), 122.11% ($P > 0.05$), and 289.91% ($P < 0.01$) of those in the D-gal-Sed group (Fig. 14A, 14C, 14D, and 14E). In this group, the level of NMDAR1 and NMDAR2B was low as 55.15% and 65.22% of those of the CD-Ex group, respectively ($P < 0.01$) (NMDAR2A; 92.49%, $P > 0.05$ of the CD-Ex group) (Fig. 14A, 14C, 14D, and 14E). HFD-feeding did not significantly affect the expression level of the NMDAR1, NMDAR2A, and NMDAR2B compared to those in the CD-Sed group ($P > 0.05$) (Fig. 14A, 14C, 14D, and 14E). After treadmill exercise, the protein level of NMDAR1, NMDAR2A, and NMDAR2B was 154.07% ($P < 0.01$), 125.80% ($P < 0.05$), and 133.54% ($P < 0.05$) of those in the HFD-Sed group, respectively (Fig. 14A, 14C, 14D, and 14E). Additionally, in the HFD-D-gal-Sed group, their NMDAR1, NMDAR2A, and NMDAR2B levels were determined as 62.75% (NMDAR1; 102.36%, $P > 0.05$ and 53.32%, $P < 0.01$ of the D-gal-Sed and HFD-Sed groups, respectively), 84.76% (NMDAR2A;

80.65%, $P > 0.05$ and 75.00%, $P < 0.05$ of the D-gal-Sed and HFD-Sed groups, respectively), and 32.04% (NMDAR2B; 120.98%, $P > 0.05$ and 36.86%, $P < 0.01$ of the D-gal-Sed and HFD-Sed groups, respectively) of those in the CD-Sed group (Fig. 14A, 14C, 14D, and 14E). Finally, in the HFD-D-gal-Ex group, the expression level of the NMDAR1, NMDAR2A, and NMDAR2B was significantly increased as 259.16%, 173.27%, and 333.38% of those in the HFD-D-gal-Sed group ($P < 0.01$) (Fig. 14A, 14C, 14D, and 14E). Further, the protein level of threes subunits showed pattern of increase compared to those in the D-gal-Ex group (as 172.54%, $P < 0.01$ of NMDAR1, 114.44%, $P > 0.05$ of NMDAR2A, and 139.12%, $P < 0.01$ of NMDAR2B in the D-gal-Ex group, respectively) (Fig. 14A, 14C, 14D, and 14E). However, their levels in the HFD-D-gal-Ex group were not significantly different from those in HFD-Ex group (Table 2).

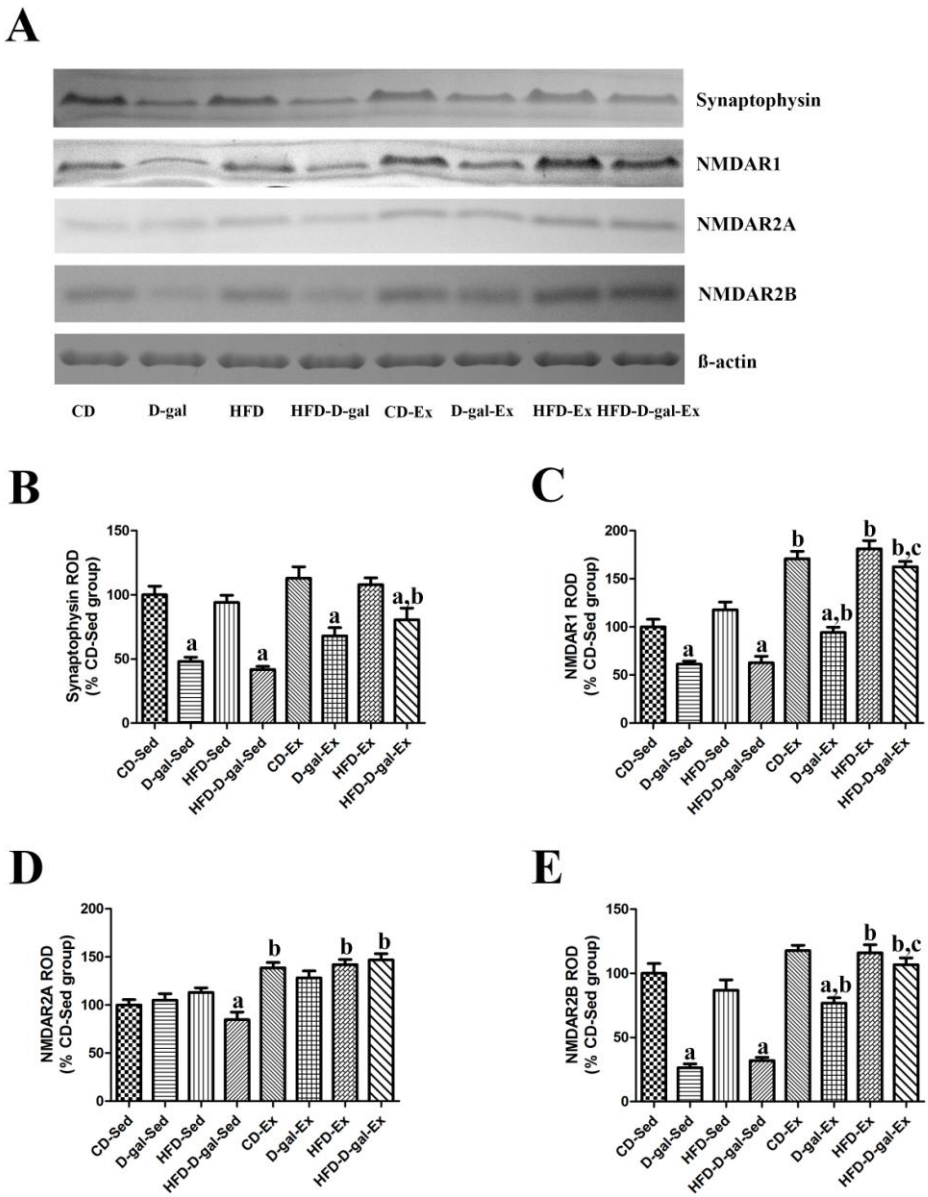
A
Synaptophysin
NMDAR1
NMDAR2A
NMDAR2B
ß-actin
CD
D-gal
HFD
HFD-D-gal
CD-Ex
D-gal-Ex
HFD-Ex
HFD-D-gal-Ex
B
Synaptophysin ROD (% CD-Sed group)
C
NMDAR1 ROD (% CD-Sed group)
D
NMDAR2A ROD (% CD-Sed group)
E
NMDAR2B ROD (% CD-Sed group)
CD-Sed
D-gal-Sed
HFD-Sed
HFD-D-gal-Sed
CD-Ex
D-gal-Ex
HFD-Ex
HFD-D-gal-Ex

Fig. 14. Western blot analysis of synaptophysin, NMDAR1, NMDAR2A, and NMDAR2B in the hippocampus of the CD-Sed/Ex, D-gal-Sed/Ex, HFD-Sed/Ex, and HFD-D-gal-Sed/Ex groups (A). The relative optical density (ROD) of the immunoblot bands is demonstrated as percent values of CD-Sed in synaptophysin (B), NMDAR1 (C), NMDAR2A (D), and NMDAR2B (E) in the whole hippocampus of the CD-Sed/Ex, D-gal-Sed/Ex, HFD-Sed/Ex, and HFD-D-gal-Sed/Ex groups ($^{a}P < 0.05$, indicates a significant difference from D-gal treatment. $^{b}P < 0.05$, indicates a significant difference from exercise treatment. $^{c}P < 0.05$, indicates a significant difference from HFD treatment). Data are presented as the mean ± SE.

Effects of D-gal-induced aging and/or exercise on 4-HNE expression in HFD-induced obese mice

Mean 4-HNE protein level of the CD-Sed group was used as the standard value. In the CD-Ex group, its level was not significantly changed as 88.12% of CD-Sed group ($P > 0.05$) (Fig. 15A and 15B). In the D-gal-Sed group, 4-HNE protein expression was significantly increased to 120.71% of that in the CD-Sed group ($P < 0.01$) (Fig. 15A and 15B). In the D-gal-Ex group, 4-HNE protein expression was significantly reduced to the 88.23% of that in the D-gal-Sed group ($P < 0.05$) and it was high as 120.87% of the CD-Ex group ($P < 0.01$) (Fig. 15A and 15B). Similarly to the protein expression of 4-HNE in the D-gal-Sed group, HFD-Sed group showed prominent increase in 4-HNE protein as 114.46% of that in the CD-Sed group ($P < 0.05$) (Fig. 15A and 15B). However, in HFD-Ex group, the 4-HNE level was decreased to 78.56% of that in the HFD-Sed group ($P < 0.01$) (Fig. 15A and 15B). In this group, the protein level of the 4-HNE was reduced to the similar level to the CD-Ex group. On the other hand, in the HFD-D-gal group, 4-HNE level was increased as 116.23% of the CD-Sed groups (Fig. 15A and 15B). In this group, the protein level of 4-HNE was not significantly different from that in the D-gal-Sed and HFD-Sed group ($P > 0.05$) (Fig. 15A and 15B). Additionally, the level of 4-HNE in the HFD-D-gal-Ex group was significantly decreased as 82.36% of the HFD-D-gal-Sed group ($P < 0.05$) (Fig. 15A and 15B). Further, 4-HNE level in this group was not significantly different from both D-gal-Ex and HFD-Ex groups ($P > 0.05$) (108.63% of the CD-Ex group) (Fig. 15A, 15B, and Table 2).

Effects of D-gal-induced aging and/or exercise on SOD1 expression in

HFD-induced obese mice

Mean SOD1 protein level of the CD-Sed group was used as the standard value. In the CD-Ex group, the SOD1 protein level was increased to 112.76% of that in the CD-Sed group with no significant difference ($P > 0.05$) (Fig. 15A and 15C). In the D-gal-Sed group, SOD1 protein expression was significantly decreased to 75.09% of that in the CD-Sed group ($P < 0.05$) (Fig. 15A and 15C). In the D-gal-Ex group, SOD1 protein expression was prominently increased to 125.26% of that in the D-gal-Sed group ($P < 0.05$) (Fig. 15A and 15C). However, the protein level of SOD1 in D-gal-Ex was low as 83.42% of that in the CD-Ex and was similar to that in the CD-Sed group ($P > 0.05$) (Fig. 15A and 15C). In the HFD-Sed group, the SOD1 protein expression was decreased to 87.93% of that in the CD-Sed group ($P > 0.05$) (Fig. 15A and 15C). However, the SOD1 protein level in the hippocampus of the HFD-Ex group was significantly increased as 126.96% of HFD-Sed group ($P < 0.01$) (Fig. 15A and 15C). The protein level in this group was similar to that in the CD-Ex group ($P > 0.05$) (111.65% of that in the CD-Sed group) (Fig. 15A and 15C). In the HFD-D-gal-Sed group, the SOD1 protein was reduced to 60.30% and 51.49% of that in the D-gal-Sed and HFD-Sed group, respectively ($P < 0.01$) (Fig. 15A and 15C). Protein level of SOD1 was 45.28% of that in the CD-Sed group (Fig. 15C). Additionally, the protein level of SOD1 in the HFD-D-gal-Ex group was prominently increased as 203.97% of that in the HFD-D-gal-Sed group ($P < 0.01$) (Fig. 15A and 15C). Its level was significantly reduced as 89.80% of the HFD-Ex group ($P < 0.05$) and it level was similar to the D-gal-Ex group ($P > 0.05$) (Fig. 15A and 15C). The SOD1 level of HFD-D-gal-Ex group was still lower as 88.91% of the CD-Ex group and it was similar to the level of the CD-Sed group

(Fig. 15A, 15C, and Table 2).

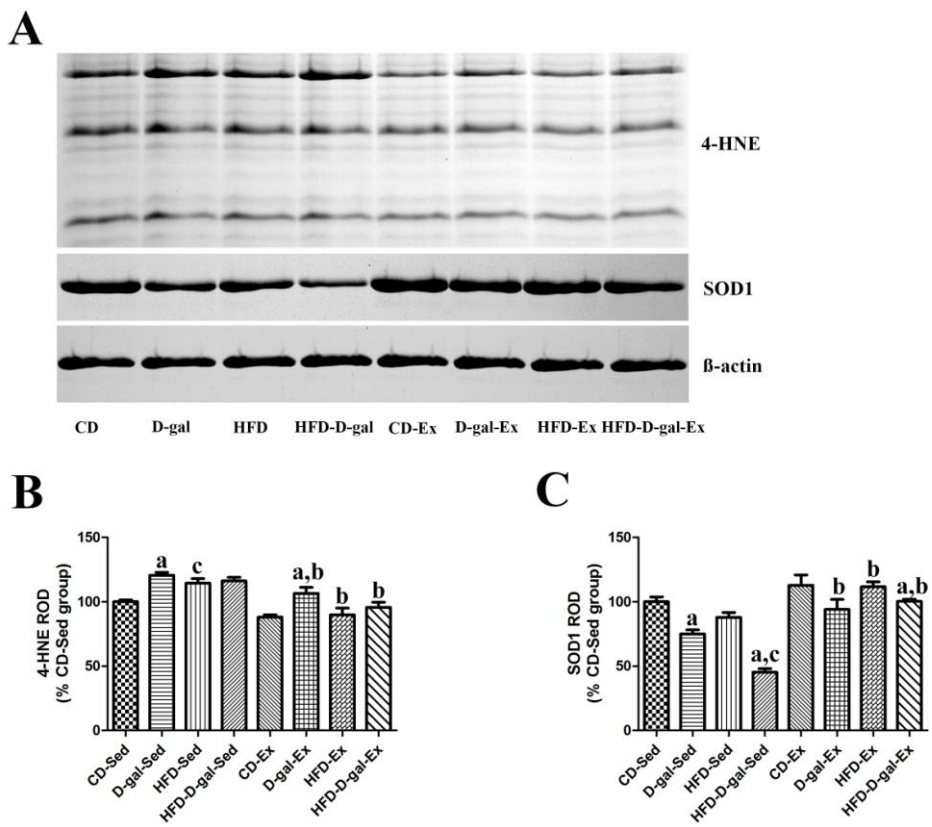

Fig. 15. Western blot analysis of 4-HNE, and SOD1 in the hippocampus of the CD-Sed/Ex, D-gal-Sed/Ex, HFD-Sed/Ex, and HFD-D-gal-Sed/Ex groups (A). The relative optical density (ROD) of the immunoblot bands is demonstrated as percent values of CD-Sed in 4-HNE (B) and SOD1 (C) in the whole hippocampus of the CD-Sed/Ex, D-gal-Sed/Ex, HFD-Sed/Ex, and HFD-D-gal-Sed/Ex groups ($^{a}P < 0.05$, indicates a significant difference from D-gal treatment. $^{b}P < 0.05$, indicates a significant difference from exercise treatment. $^{c}P < 0.05$, indicates a significant difference from HFD treatment). Data are presented as the mean ± SE.

Experiment 2	Target	D-gal-induced aging	HFD-induced obesity	Treadmill exercise
Neurogenic markers	Nestin	- ~ ± (IHC) -- ~ -(WB)	± (IHC) -- ~ ± (WB)	± ~ + (IHC) + ~ ++ (WB)
	Ki67	-- ~ - (IHC)	- ~ ± (IHC)	+ ~ ++ (IHC)
	DCX	-- ~ - (IHC) -- (WB)	-- ~ - (IHC) -- ~ ± (WB)	+ ~ ++ (IHC) + ~ ++ (WB)
Neurotrophic factor	BDNF	-- (WB)	-- ~ ± (WB)	+ ~ ++ (WB)
	pCREB	-- (WB)	-- ~ ± (WB)	+ ~ ++ (WB)
Synaptic protein	Synaptophysin	-- ~ - (WB)	± (WB)	± ~ ++ (WB)
	NMDAR1	-- ~ ± (WB)	± ~ ++ (WB)	+ ~ ++ (WB)
	NMDAR2A	- ~ ± (WB)	± (WB)	± ~ ++ (WB)
	NMDAR2B	-- ~ ± (WB)	± ~ + (WB)	± ~ ++ (WB)
Oxidative stress	4-HNE	± ~ ++ (WB)	± ~ + (WB)	-- ~ ± (WB)
	SOD1	-- ~ ± (WB)	-- ~ - (WB)	± ~ ++ (WB)

Table. 2. Summary of immunohistochemical and immunoblot results in the experiment about D-gal and/or treadmill exercise to HFD-fed obese mice (-- strongly negative; - moderatetly negative; ± weakly negative or positive; + moderately positive; ++ strongly positive).

Discussion

Effects of dietary change and/or treadmill exercise in HFD-induced obese mice

In the present study, we first investigated the effect of separate or combined dietary change and treadmill exercise on hippocampal neurogenic and synaptic proteins in HFD-fed obese mice.

Initially, we observed changes in body weight, non-fasting blood glucose level, and glucose tolerance in CD-Sed/Ex, HFD-Sed/Ex, and HFD-CD-Sed/Ex groups. Body weight was highest in HFD-Sed group and dietary change and/or exercise were effective in reducing body weight. Non-fasting blood glucose level also showed similar pattern of change after HFD feeding and dietary change and/or exercise were effective in regulating blood glucose levels. The IPGTT results indicate that dietary change and/or exercise positively acting by enhancing glucose tolerance in obese mice. The combined treatment of dietary change and exercise was most efficient in regulating above physiological parameters. However, whether the diet intervention from HFD to CD is effective in enhancing those parameters even after long-term HFD feeding is not known and further studies are required.

Next, we investigated changes in neurogenic markers in the dentate gyrus following dietary change and exercise. As reported in previous studies (Hwang et al. 2008, Nam et al. 2014c, van Praag et al. 1999), we confirmed the negative effect of HFD and positive effect of exercise on neurogenesis. In this study, we revealed that dietary change and/or exercise is effective and the combined therapy was more efficient in restoring the HFD-induced reduction in neurogenesis. Enhancement of

adult hippocampal neurogenesis is connected with consequent functional improvement in learning and memory (Nam et al. 2014a, van Praag et al. 1999). Interestingly, previous studies have reported that both dietary change and exercise are useful for improving HFD-induced memory deficit (Lafenêtre et al. 2010, Maesako et al. 2012). Neurogenesis is also highly correlated with synaptic plasticity for the reason that in their first 4–6 weeks, new neurons are major mediators of plasticity (Ge et al. 2007, Saxe et al. 2006). Specifically, the increase in cell proliferation and neuronal differentiation was similar between the HFD-Ex and HFD-CD-Sed groups. Therefore, we suggest that exercise is as effective as dietary change in increasing neural stem cells, their proliferation, and differentiation when there is no dietary control. As the mechanism for enhancement in the hippocampal neurogenesis, we speculate that dietary change may eliminate the effect of a HFD and could have induced the similar effect of calorie restriction. Calorie restriction is reported to promote cell survival (Lee et al. 2000) and dietary change may act positively on survival of newly generated cells at the time point of dietary challenge in HFD-CD-Sed compared with those in the HFD-Sed group. Additionally, exercise is reported exert its effects by the activation of quiescent neural stem cells, their proliferation, and differentiation into neurons (Lugert et al. 2010, Nam et al. 2014c, Wu et al. 2008). We confirmed that the exercise-induced increase of neural stem cells was significant in the present results. However, additional studies are required to reveal the underlying mechanism and to determine which treatment should be used in the process of dietary change or exercise-induced enhancement of adult hippocampal neurogenesis. The results presented here suggest that a combined therapy of dietary change and exercise is most effective in improving hippocampal neurogenesis.

Several factors, including hyperglycemia, increased levels of glucocorticoids, pro-inflammatory cytokines, down-regulation of BDNF, insulin resistance, and oxidative stress have been suggested as possible candidate mechanisms for the HFD-induced reduction in adult hippocampal neurogenesis. Among them, we primarily focused on the multifunctional neurotrophin, BDNF, because it exerts neuroprotective effects *via* mechanisms of anti-inflammation, anti-oxidative stress, and normalization of glucose metabolism (Joosten et al. 2004, Noble et al. 2011, Numakawa et al. 2010). As a downstream transcriptional factor of BDNF signaling, we investigated changes in the phosphorylation of CREB. HFD-induced obesity reduced BDNF and pCREB expression in the hippocampus and their reduction was restored after dietary change and/or exercise treatment. Others have also reported that diet with high fat content reduces hippocampal BDNF (Molteni et al. 2002a, Park et al. 2010). BDNF functions as mediator of hippocampal neurogenesis and synaptogenesis (Ambrogini et al. 2013, Lee et al. 2000, Marlatt et al. 2012, Mattson 1998). Conversely, reduced BDNF levels are related to hippocampal structural impairments (Gray et al. 2006). The manipulation of CREB results in a decrease in cell proliferation (Nakagawa et al. 2002) and it also involved in the enhancement of hippocampal neurogenesis and synaptogenesis (Tchantchou et al. 2009). Physical exercise stimulates endogenous BDNF-pCREB signaling, which results in cognitive improvement (Marlatt et al. 2012, Nam et al. 2014c, Vaynman et al. 2004). These results corroborate the hypothesis that the BDNF-pCREB pathway and adult hippocampal neurogenesis are closely associated. Moreover, we suggest that the activation of the BDNF-pCREB pathway mediated the dietary change- and/or exercise-induced improvement of adult hippocampal neurogenesis.

Along with neurogenesis and BDNF-pCREB changes in hippocampus, we

further investigated the changes of pre- and postsynaptic membrane proteins to reveal the effect of dietary change and/or exercise on the synaptic plasticity. Pre-synaptic synaptophysin is a synaptic vesicle membrane protein and its increase is related to the enhancement of synapse formation (Bamji et al. 2006). A separate treatment of dietary change and exercise increased its expression in HFD-fed mice; however, combined therapy did not additively act to ameliorate the HFD-induced reduction. This is in line with previous studies that have demonstrated HFD-feeding-induced reductions and exercise-induced enhancements in the expression of presynaptic proteins (Ahlskog et al. 2011, Molteni et al. 2004). We hypothesized that BDNF may be related with changes in the expression of synaptophysin. BDNF is reported to be involved in the mobilization of synaptophysin-inserted synaptic vesicles and synapse formation (Bamji et al. 2006). In addition, synaptophysin can regulate the availability of synaptic vesicles (Kwon et al. 2011). We suggest that dietary change- and/or exercise-induced changes in BDNF and synaptophysin may be correlated.

Post-synaptic NMDAR is a glutamate-binding ionotropic receptor with ubiquitous distribution across several regions of the brain. It allows calcium ion translocation and plays a vital role in new synapse formation, synaptic plasticity, learning, and memory (Liu et al. 2000, Liu et al. 2004, Xu et al. 2010). Among the three subfamilies of the NMDAR subunit (NMDAR1, NMDAR2 and NMDAR3), an essential subunit is NMDAR1, which composes the heterodimer NMDAR assembly predominantly with NMDAR2A and/or NMDAR2B in the adult hippocampus (Liu et al. 2004). In present study, HFD-induced obesity and dietary change did not significantly affect the expression of the three subunits of NMDAR and exercise increased their expression in diet-treatment independent way.

Interestingly, other studies have shown that HFD treatment results in the desensitization of NMDAR and a reduction in NMDA-induced plasticity (Valladolid-Acebes et al. 2012). Brain inflammation caused by HFD-induced obesity is a candidate mechanism for the reduction in the three subunits of NMDAR (Ma et al. 2014a).

It is hard to connect NMDAR expression directly with HFD- or dietary change-induced changes in neurogenesis; however, we can speculate that the exercise-induced increase in neurogenesis may be partially related to NMDAR expression. A previous study using transgenic mice reported that the NMDAR is involved in the regulation of newly generated neuron (Tashiro et al. 2006). An *in vitro* experiment of hippocampal neural progenitor cells showed that NMDAR activation leads to an increase in proliferation and neuronal differentiation (Joo et al. 2007). Another study suggests that NMDAR2B and NMDAR2A are related to cell proliferation and migration, respectively, in the postnatal brain (Fan et al. 2012). Voluntary wheel running-induced neurogenesis is suppressed in NMDAR2A knockout mice (Kitamura et al. 2003). NMDAR2A null mice showed severe impairments in the synaptic and dendritic morphology of mature granule cells (Kannangara et al. 2014). Additionally, NMDAR2B is regarded as irreplaceable for its instructive role in synaptic plasticity of adult-borne neurons (Ge et al. 2007).

The dietary change- or exercise-induced effect on synaptic protein expression suggests that these interventions can improve the expression of these proteins in the hippocampus. Synaptogenesis and concurrent synaptic plasticity are observed in the process of integrating adult-borne neurons into the circuit that mediates spatial information processing and memory formation. Glutamate-BDNF interactions regulate hippocampal synaptogenesis and synaptic plasticity (Mattson 2008).

Antagonist-induced suppression of NMDAR subsequently down-regulates the expression of BDNF in the hippocampus (Wang et al. 2014b). On the other hand, BDNF can potentiate synaptic transmission in hippocampal neurons *via* NMDARs (Song et al. 1998). Cognitive impairments in the aged or demented brain can be delayed or enhanced via exercise-induced enhancements in synaptic plasticity (Ahlskog et al. 2011, Molteni et al. 2004). Environmental enrichment-induced restoration of cognitive impairment is mediated by un-regulating NMDAR1 and BDNF (Sun et al. 2010). Maternal care increases NMDAR with subsequent BDNF elevation (Liu et al. 2000). Taken together, our results suggest that dietary change and/or exercise are protective against HFD-induced synaptic impairment.

The accumulation of 4-HNE can lead to neuronal dysfunction and death (Kruman et al. 1997, Mattson 1998). The high content of lipids, high oxygen consumption, and lack of an antioxidant system make the brain susceptible to oxidative stress-induced lipid peroxidation (Butterfield et al. 2002). Our data matches those in previous reports, showing that HFD-feeding-induced obesity increases the end-product of lipid peroxidation, 4-HNE or malondialdehyde, in the hippocampus (Hwang et al. 2009, Jeon et al. 2012, Park et al. 2010). Although endogenously generated reactive oxygen species play essential role in the process of neurogenesis, oxidative stress overwhelming body's protective ability is an important mechanism of brain dysfunction (Jeon et al. 2012, Park et al. 2010). From the results presented here, we confirm that change in 4-HNE negatively correlated with hippocampal neurogenesis. Though further studies are required to demonstrate whether the present lifestyle intervention-induced improvement in hippocampal neural plasticity is mediated by a stabilization of cell membrane from oxidative stress, we suggest that both dietary change and exercise are effective in

ameliorating lipid peroxidation.

Finally, we examined the dietary change- and/or exercise-induced difference in SOD1 expression in the hippocampus of HFD-fed mice. Antioxidant SOD1 is located in the cytoplasm and regarded as the first-line defense to free radical toxicity. In present study, we observed that SOD1 positively correlated with neurogenesis and partially with exercise-induced changes in synaptic proteins. However, SOD1 expression was conversely changed when compared with 4-HNE in the hippocampus. A shortage of SOD1 in mutant mice and a surplus of SOD1 *via* exogenous treatment negatively affect the cell proliferation and neuronal differentiation in the adult hippocampus (Fishman et al. 2009, Yoo et al. 2012). More studies are required to elucidate the direct correlation between SOD1 and neurogenesis. The results presented here suggest that HFD-induced oxidative stress exhausted SOD1 and dietary change and/or exercise induced-SOD1 attenuated the accumulation of 4-HNE in the hippocampus. We suggest that SOD1 may affect hippocampal neurogenesis by maintaining the homeostasis from oxidative stresses.

Taken together, we have confirmed the negative effect of eight weeks of HFD-feeding-induced obesity on neural plasticity in adult hippocampus. Dietary change and/or exercise treatment in the early stage of HFD-induced obesity reversed structural impairments *via* increasing BDNF and reducing oxidative stress. Moreover, the combined therapy was more efficient at restoring the obesity-induced hippocampal impairments.

Effects of D-galactose-induced brain aging and/or treadmill exercise in the hippocampus of HFD-induced obese mice

Our question regarding the effect of aging on the hippocampus in HFD-fed obese mice has not been fully answered; therefore, we additionally established a combined model of brain aging and obesity. To reduce the time required to gain naturally aged mice, we used D-gal-induced brain aging model. Previously, we already revealed that a six-week D-gal treatment induced a brain aging phenotype and its effects are ameliorated by exercise (Nam et al. 2014c). As a follow-up study, we investigated the effects of exercise in the combined model of HFD-induced obesity and D-gal-induced aging in the aspect of hippocampal neurogenic and synaptic protein expression.

We observed changes in body weight and non-fasting blood glucose level in CD-Sed/Ex, D-gal-Sed/Ex, HFD-Sed/Ex, and HFD-D-gal-Sed/Ex mice groups. The body weight was highest in HFD-Sed and HFD-D-gal-Sed groups and exercise was effective at reducing body weight. Non-fasting blood glucose levels showed a similar pattern of change after HFD-feeding and exercise was effective in preventing the increase of blood glucose level. However, there were no significant differences between saline- and D-gal-treated groups. HFD-feeding influenced the physiological parameters and exercise ameliorated the negative effects of HFD. Interestingly, maternal HFD-feeding induced age-dependent metabolic dysfunction (glucose tolerance) in the offspring and exercise prevented this impairment (Laker et al. 2014).

Next, we investigated the change of neurogenic markers that were associated with D-gal-induced aging and/or exercise treatment in the dentate gyrus of HFD-fed obese mice. As reported in previous studies (Hwang et al. 2008, Nam et al. 2014c, van Praag et al. 2005), we confirmed the negative effect of the separate conditions of aging or a HFD on neurogenesis. Furthermore, we revealed that

exercise can reverse the reduction in neurogenic markers in D-gal-induced aging and/or HFD-induced obese mice. The D-gal-induced aging in HFD-fed obese mice aggravated the reduction of neurogenic markers and exercise was efficient in restoring the impairment in the hippocampal neurogenesis. However, we did observe a difference in the efficiency of the exercise treatment between the HFD-Ex and D-gal-Ex groups. Exercise was more effective in the HFD-Ex group, possibly because of HFD-induced hyperglycemia that was prevented by an early exercise intervention. We speculate that the differences are derived from the different mechanisms by which HFD and D-gal treatment reduce the expression of neurogenic markers. According to the previous studies, the accumulation of oxidative stress is commonly observed after D-gal and HFD treatment, but the cause of oxidative stress can be different. While D-gal-induced oxidative stress is elicited by galactitol, HFD-induced oxidative stress is mainly caused by increase of free fatty acid or hyperglycemia. In present study, HFD-induced hyperglycemia was delayed or prevented by early exercise treatment. Moreover, previous studies have reported that physical exercise promotes the consumption of free fatty acid by increasing its modulation (Marques et al. 2010, Sato et al. 2007).

We can speculate from the results presented here that implementation of exercise treatment at the early stages of obesity is effective at restoring adult hippocampal neurogenesis. In addition, the combined model of obesity and aging showed a transparent negative effect on neurogenesis. HFD-feeding in naturally aged mice induces a cognitive impairment *via* increased oxidative stress and decreased antioxidant response (Morrison et al. 2010). Voluntary wheel running in naturally aged mice ameliorates the decline in neurogenesis and spatial learning (van Praag et al. 2005). Exercise is also involved in the activation of quiescent neural stem

cells and increases the number of neural stem cells in aged mice (Blackmore et al. 2009, Lugert et al. 2010.).

BDNF can influence neurogenesis, neuronal protection, synaptogenesis, synaptic transmission, and synaptic plasticity (Ambrogini et al. 2013, Caldeira et al. 2007, Leal et al. 2014, Mattson et al. 2004). In agreement with previous studies, we confirmed that HFD-feeding reduced BDNF expression in the hippocampus (Pistell et al. 2010). It has also been reported that there is an age-dependent reduction in hippocampal BDNF levels (Nam et al. 2014c, Liu et al. 2006). The combined treatment of HFD and D-gal robustly reduced the expression of BDNF. After exercise, BDNF expression was increased in CD-Ex, D-gal-Ex, HFD-Ex and HFD-D-gal-Ex groups when compared with expression in their respective sedentary groups. In accordance with our present results, aerobic exercise-induced BDNF in aged rats or obese mice subsequently improved hippocampal function (Aguiar et al. 2011, Noble et al. 2014). We confirmed that physical exercise is an effective intervention to revert obesity and/or aging-related decline of BDNF levels. Results from mice with BDNF truncation or val66met polymorphic BDNF (valine to methionine substitution at position 66) demonstrate the importance of BDNF on hippocampal neurogenesis (Bath et al. 2012, Waterhouse et al. 2012). In addition, we confirmed that pCREB was changed in a manner similar to BDNF. pCREB is a downstream molecule of BDNF signaling that mediates the transcription of target genes (Yang et al. 2014). CREB mutation experiments have demonstrated that pCREB plays an important role in adult neurogenesis (Nakagawa et al. 2002). Ji et al. (2014) have reported that exercise-induced activation of BDNF-pCREB signaling can attenuate reduce hippocampal neurogenesis by irradiation. In addition, CREB is a mediator of the membrane-depolarization-dependent BDNF induction

and a gain-of-function study has revealed that CREB-induced BDNF is related with a subsequent improvement in memory (Suzuki et al. 2011, Tao et al. 1998). Taken together, these results support our hypothesis that BDNF-pCREB signaling is involved in adult hippocampal neurogenesis and exercise used this signal as one of the mechanism to attenuate HFD- and D-gal-mediated reduction.

Along with the effect of obesity and/or aging on neurogenesis, we investigated their effects on synaptic proteins. First, we examined the change pattern of presynaptic vesicular protein, synaptophysin, and found that it changed similarly to the neurogenic markers. Synaptophysin is highly correlated with synaptic plasticity and its changes are associated with presynaptic plasticity and synaptogenesis (Ujike et al. 2002). The pattern of change in the present results confirmed the negative effect of D-gal or combined treatment of D-gal/HFD. Six weeks of HFD-feeding was not enough to change synaptophysin expression in the hippocampus; however, HFD-feeding from pregnancy to post-weaning impairs the expression of synaptophysin (Page et al. 2014). Consistent to our result, Liu et al. (2006) reported that synaptophysin and 43-kDa growth-associated protein (GAP-43) were significantly reduced in the hippocampus of naturally aged rats. Interestingly, exercise treatment, in accordance with previous research, increased the expression of synaptophysin (Vaynman et al. 2006). Antibody treatment against the BDNF receptor attenuated the exercise-induced increase of synaptophysin (Vaynman et al. 2006). We speculate that obesity- and/or age-induced changes in BDNF and synaptophysin in the hippocampus are highly correlated.

Glutamate is excitatory neurotransmitter and after its release from the presynaptic membrane, it binds to glutamatergic receptors on the post-synaptic membrane. Among these receptors, ionotropic NMDAR is closely related with

neurogenesis, synaptogenesis, and BDNF synthesis (Bernabeu and Sharp 2000, Hardingham 2009, Marosi and Mattson 2014). In addition, synaptic plasticity is closely related with NMDAR-induced signal transduction (Hardingham 2009). There are four types of NMDAR2 subunits and NMDAR2A and NMDAR2B subtypes are predominantly expressed in the adult hippocampus. They form a heterodimer complex with the essential subtype NMDAR1 (Kim et al. 2005). The present results are consistent with those of previous studies showing that D-gal treatment on CD-fed or HFD-fed mice reduces the expression of the three subunits of NMDAR and exercise restores their expression (Nam et al. 2014b). Long-term HFD-feeding- and/or aging-induced reduction in NMDAR expression is regarded as one of the causes of impairment in hippocampus-dependent learning and memory (Brim et al. 2013, Yilmaz et al. 2011, Wang et al. 2014a). In addition to hippocampal function, NMDAR is positively involved in the migration, survival of newly generated neurons, and following new circuit formation (Namba et al. 2011, Tashiro et al. 2006). However, the characterization of NMDAR in terms of cell proliferation is not clearly defined and remains controversial state; therefore, further studies are required. The mechanism of obesity- and/or aging-induced changes in the NMDAR subunits was not examined in our present study; however, Ma et al., (2014a) suggested that brain inflammation is candidate one to elicit the reduction in three subunit of NMDAR. Chronic oxidative stress is also suggested as mechanism of NMDAR reduction (Yilmaz et al. 2011).

Specifically, we observed that the pattern of change was similar between NMDAR1 and NMDAR2B subunits. While D-gal-induced brain aging reduced NMDAR2B in the CD-fed and HFD-fed sedentary groups, NMDAR2A was changed only in the HFD-D-gal-Sed group. Our present results are supported by

previous studies showing that natural aging reduced NMDAR1 and NMDAR2B in the hippocampus; however, the effect of aging on NDMAR2A expression is not clearly defined (Magnusson et al. 2002, Wang et al. 2014a). Though six weeks of HFD-feeding did not cause a significant change in NMDAR2A and NMDAR2B subunits, Yilmaz et al. (2011) have reported that both NMDAR2A and NMDAR2B subunits are negatively affected by long-term HFD-feeding. A previous microarray study supports our present findings by demonstrating exercise as effective way to recover aging-induced reduction of NMDAR (Molteni et al. 2002b). Indeed, exercise or *in vivo* gene transfer induced an increase in hippocampal NMDAR2B enhanced memory in Alzheimer's disease (AD) model or aged mice, respectively (Brim et al. 2013, Revilla et al. 2014). We therefore speculate from our and other results that exercise is protective against obesity and/or aging-induced impairment by positively regulating NMDAR.

We found a positive correlation between synaptic proteins and BDNF expression from results presented here. Others have also reported that BDNF can enhance synaptogenesis by stimulating the production of synaptic proteins (Ambrogini et al. 2013, Leal et al. 2014). Glutamate-mediated activation of NMDAR evoked neuronal activation of BDNF gene and BDNF subsequently increases NMDAR1, NMDAR2A, and NMDAR2B subunits (Marosi and Mattson 2014, Caldeira et al. 2007). Functionally, BDNF-induced synaptic activity is NMDAR dependent and impaired NMDAR resulted in defective response to BDNF in Tau transgenic (Tg) mice (Burnouf et al. 2013). In our present study, exercise was effective in increasing presynaptic synaptophysin and we confirmed positive effects on three subunits of NMDAR. Physical exercise was also beneficial in 3×Tg-AD model mice by reversing decreased hippocampal level of synaptophysin and NMDAR

(Revilla et al. 2014). Collectively, these results suggest that the effect of physical exercise in ameliorating obesity and/or aging-induced reduction of synaptic proteins is highly correlated with BDNF expression.

Furthermore, we investigated the expression of 4-HNE, protein-conjugated lipid peroxidation product, in the whole hippocampus. HFD-induced obesity and/or D-gal-induced aging increased the level of 4-HNE and treadmill exercise reduced its accumulation. As oxidative stress accumulates, the resulting by-products are produced via lipid peroxidation, protein carbonylation, and oxidization of DNA nucleotide (Radak et al. 2011). From the results of present study, we can observe that the expression pattern of 4-HNE was negatively correlated with neurogenic and synaptic marker proteins. Consistently, *in vivo* and *in vitro* experiments support this correlation by showing that ROS negatively affects the process of neurogenesis from hippocampal neural stem cells (Limoli et al. 2006, Rivas-Arancibia et al. 2010). 4-HNE is also regarded as mediator of oxidative stress-induced neuronal apoptosis (Kruman et al. 1997). 4-HNE increases NMDAR-mediated neurotoxicity by accelerating mitochondrial dysfunction (Choi et al. 2013). Lipid peroxidation causes a reduction in NMDAR *via* a change in membrane organization (Yilmaz et al. 2011). Accordingly, we suggest that obesity and/or aging is harmful with respect to lipid peroxidation and exercise can reduce 4-HNE production in the hippocampus.

In correlation with 4-HNE production in the hippocampus, we further examined the effect of the D-gal-induced aging and treadmill exercise on the expression of SOD1 in the hippocampus of HFD-fed mice. SOD1 is the cytoplasmic antioxidant that catalyzes the conversion of superoxide into hydrogen peroxide. Hydrogen peroxide causes reversible oxidative damage while superoxide resulted in

irreversible damage (Lee et al. 2011). In the present study, SOD1 was inversely changed from 4-HNE and positively associated with exercise-induced improvement in hippocampal neurogenic and synaptic proteins. A study employing an Alzheimer's disease mouse model corroborates our findings by showing that voluntary wheel running was effective at reducing oxidative stress *via* the induction of antioxidants (Herring et al. 2010). We suggest that exercise-induced SOD1 attenuated 4-HNE accumulation in the hippocampus of the D-gal and/or HFD-treated mice. Additionally, we can draw that SOD1 may partially affected hippocampal structural plasticity by regulating oxidative stress.

Taken together, D-gal-induced brain aging in the HFD-fed obesity model aggravates the impairment of adult hippocampal neural plasticity. Treadmill exercise-mediated improvement in the neural plasticity seems to be attributable to the activation of BDNF-pCREB signaling and SOD1-mediated reduction of lipid peroxidation.

The limit of present thesis is that we could not differentiate the change of subunit composition of NMDAR in relation with their location in subregions (CA1, CA3, and DG) of hippocampus or extrasynaptic/synaptic membranes; therefore, more thoroughly designed further studies to elucidate these relations are required. NMDAR is activated by its substrate binding and function of NMDAR is regulated by protein kinases (Chen and Roche 2007). An investigation into the phosphorylation of the NMDAR subunits is required to elucidate whether lifestyle interventions can affect this. The early exercise treatment in HFD-fed obese mice was effective at restoring the impaired neural plasticity. However, we could not determine the critical point that lifestyle interventions cannot recover from the

HFD-induced impairment to the control level in present study. A longer period of the HFD-feeding treatment is required to determine the irreversible point of HFD-induced hippocampal neural plasticity. Woo et al. (2013) have reported that after 13 weeks of HFD-feeding, diet change and/or exercise treatment improved BDNF-pCREB signaling. In addition, exercise, but not diet alone, was effective at improving cognitive function. At 18–24 months old, naturally aged rats showed loss of synaptic density and synaptic counts in the dentate gyrus of the hippocampus (McWilliams and Lynch 1984). Dietrich et al. (2008) additionally reported that exercise increases hippocampal synaptogenesis, therefore, further electron microscopic studies are required to determine the effect of HFD-feeding, combined lifestyle interventions, and D-gal-induced aging on the formation of synapse.

In summary, we evaluated the effect of dietary change, aging, and exercise treatment in the HFD-induced obesity model. Combined treatment of dietary change and exercise was efficient in improving weight, glucose control. However, D-gal-induced aging did not affect these physiological parameters. Both dietary change and exercise are positive mediators of hippocampal plasticity. With combined intervention, the improvement in neural plasticity was transparent in HFD-fed obese mice. In addition, D-gal treatment aggravated the impairment of neural plasticity in the HFD-fed obese mice and forced treadmill exercise showed anti-aging and anti-obesity effects in the hippocampus of HFD-fed mice.

Conclusion

The aim of this thesis was to investigate the effect of lifestyle intervention on adult hippocampal plasticity of the HFD-fed obese mice. In particular, we focused on the proliferation and differentiation of neural stem cells in the dentate gyrus of hippocampus. We changes in synaptic protein expression, such as synaptophysin and NMDAR.

In this thesis, we identified that long-term exposure to HFD reduced cell proliferation and neuronal differentiation in adult hippocampus. Dietary change or exercise treatment improved the expression of marker proteins in neural plasticity in the hippocampus of the HFD-fed mice. The earlier start of diet control or exercise intervention was efficient in reversing HFD-induced impairment in neural plasticity in the hippocampus. The BDNF-pCREB signaling was down-regulated in the hippocampus by HFD-feeding. The 4-HNE was reversely changed compared to the antioxidant SOD1. Interestingly, combined dietary control and exercise intervention showed synergistic effects when compared with the monotherapy. The enhancement of the hippocampal neural plasticity was highly correlated with the up-regulation of the BDNF-pCREB signaling and SOD1-induced reduction of 4-HNE accumulation.

Furthermore, we established a combined model of aging and obesity by D-gal and HFD treatment. Importantly, the reduction of adult hippocampal neural plasticity was aggravated by D-gal treatment in the HFD-fed obese mice. Physical exercise was effective in ameliorating D-gal and/or HFD-induced reduction in neural plasticity. We identified that BDNF-pCREB signaling was severely downregulated in the combined aging and obesity model, and treadmill exercise

used this pathway to attenuate the aging and obesity-induced impairments in the hippocampus. The oxidative stress-induced accumulation of 4-HNE was also aggravated by D-gal and/or HFD treatment with a reverse correlation with antioxidant SOD1. In addition, the exercise intervention in the combined model of aging and obesity showed anti-aging and anti-obesity effects *via* SOD1-mediated reduction of 4-HNE.

These results indicate that combined dietary change and exercise intervention is effective in attenuating obesity-induced change in hippocampal neural plasticity. Additionally, the coexistence of aging and obesity aggravated the impairment in hippocampal neuroplasticity. The enhancement of BDNF-pCREB signaling and reduction of 4-HNE accumulation *via* the induction of SOD1 are thought to be common mechanisms between dietary change and exercise interventions. Therefore, lifestyle therapies are helpful protecting hippocampal neural plasticity *via* amelioration of obesity or aging-related changes.

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국문초록

뇌는 환경의 변화에 대한 반응을 조절하는 중추이면서, 자극에 대한 반응을 통해 뇌 내부의 구조적인 변화가 끊임없이 일어남이 여러 연구를 통해 밝혀져 왔다. 뇌 구성 신경세포들은 외부의 자극에 대해 변화하고 재조직화되는 가소성(plasticity)을 가지고 있는데, 최근 기존 신경세포의 가지돌기의 분지 및 시냅스 형성 이외에도 해마의 치아이랑(dentate gyrus) 및 외측뇌실의 뇌실밑구역(subventricular zone)에서는 새로운 신경세포가 형성되는 것으로 알려져 있다. 그중에서 해마의 신경가소성(hippocampal neuronal plasticity)은 지속적인 학습 및 그에 따른 기억형성에 중요한 역할을 하는 것으로 알려져 있으며, 알츠하이머 치매를 비롯한 퇴행성뇌질환에서 나타나는 경도인지기능의 저하(mild cognitive impairment)는 신경가소성 저하와 밀접한 연관이 있다.

신경세포신생(neurogenesis)과 새로 형성된 신경세포의 가지돌기 발달 및 시냅스형성(synaptogenesis)은 환경적인 변화에 민감하며, 이번 연구에서는 서구적인 생활 습관의 확산에 따른 비만과 대사증후군을 모방한 동물모델을 이용하여 식습관변화(dietary change) 및 운동(exercise)이 해마의 신경가소성에 어떠한 영향을 미치는지 알아보고자 하였다. 그리고, 비만과 대사증후군이 뇌의 노화(aging)를 촉진하는 위험 인자로 작용하는 것이 연구로 밝혀지고 있으며, 역으로 노화 또한 비만과 대사증후군의 위험을 높히는 요소 중 하나로 최근 보고되고 있다. 비만 노화에 따른 해마의 기능적 저하는 신경세포신생 저하와 연관이 깊다. 이와 함께 새로 형성된 세포가 시냅스형성을 통해 기존의 신경회로에 연결 또는 새로운 신경회로를 형성하는 것으로 볼 때, 시냅스 이전 부분에 존재하는 synaptophysin이 pre-synaptic plasticity 또는 synaptogenesis의

마커로 사용되며, 시냅스 이후 부분에 있는 *N*-methyl-D-aspartate receptor (NMDAR) 또한 시냅스형성과 신생신경세포의 성숙과 연관이 깊다는 사실은 중요하다. 따라서 이번 연구에서는 고지방사료를 통해 비만을 유도한 동물을 기본 모델로 하여, 식습관변화 및 운동이 해마의 신경가소성에 어떠한 영향을 미치는 지를 규명하고자 하였다. 그리고, 뇌의 노화 phenotype을 유발하는 D-galactose (D-gal) 투여 모델을 통해, 비만과 노화를 동시에 유도하였을 때, 해마의 신경가소성이 어떻게 변화하고, 항비만, 항노화 효과가 있는 운동이 해마의 신경가소성 관련 지표들에 어떠한 영향을 주는 지를 조사하였다.

첫번째 실험에서는 고지방사료(high fat diet, 60% calorie from fat)를 8주 동안 급이한 비만 모델에서 신경세포신생 관련 마커 단백질이 감소하였으며, 고지방사료를 4주 급이하고 일반사료(normal cow diet, 16.71% calorie from fat)로 전환시킨 그룹에서는 신경세포신생 관련 마커 단백질들의 발현이 회복, 증가되는 것을 확인할 수 있었다. 그리고, 트레드밀 운동을 실시한 모든 그룹에서 세포분열의 마커인 Ki67 및 신경모세포(neuroblast)의 마커인 doublecortin의 증가가 확인되었다. 신경세포신생의 기전에 대한 연구로 해마의 brain-derived neurotrophic factor (BDNF) 및 phophorylated cAMP-response element binding protein (pCREB)에 대한 조사를 진행하여, 사료전환 및 운동 모두 BDNF-pCREB 신호경로를 이용하며, 사료전환과 운동을 동시에 실시한 그룹에서 개선의 효과가 가장 뚜렷함을 확인할 수 있었다. 이와 더불어 시냅스 단백질에 대한 조사를 진행한 결과, 비만만 유도하였을 때에는 시냅스 단백질의 발현 저하가 뚜렷하지 않았으나, synaptophysin 및 *N*-methyl-D-aspartate receptors (NMDARs) 단백질이 사료전환 및 운동을 실시한 고지방사료급이 동물군에서는 증가되는 것을 확인하였다. 그리고, 비만 및 노화에서 뇌의

구조 기능적 손상과 연관 깊은 것으로 알려진 산화스트레스 (oxidative stress)가 이번 실험에서는 어떠한 영향을 받는지 알아보기 위한 연구를 진행하였다. 그 결과 사료전환 및 운동 모두 지방의 과산화(lipid peroxidation) 생성물인 4-hydroxynonenal (4-HNE)을 감소시키고, 산화스트레스에 대한 방어기작으로 작용하는 항산화효소 superoxide dismutase 1 (SOD1)을 증가시켰으며, 두 가지를 복합적으로 처리하였을 때, 효과가 더 뚜렷함이 확인되었다.

두번째 실험에서는 D-gal 및 고지방사료 투여를 통해 노화 및 노화 비만복합 모델에서의 신경의 구조적 가소성에 대한 변화를 확인하고자 하였으며, 그 결과 비만모델에서 노화의 유도는 노화가 유도되지 않은 비만 모델에 비해 신경세포신생 및 시냅스형성과 연관된 단백질의 발현이 확연하게 감소되는 것이 확인되었다. 그리고, 이들 모델에서 운동을 실시하였을 때, 노화/비만에 따른 구조적 손상의 정도가 완화, 개선되는 것이 확인되었으며, 메커니즘으로 생각되는 BDNF-pCREB 신호경로가 노화/비만에 의해 저하되었다가 운동에 의해 활성화가 되는 것을 확인할 수 있었다. 추가적으로 노화/비만에 의해 증가된 산화스트레스 생성물인 4-HNE가 운동으로 감소가 되고, 이와 더불어 항산화효소 SOD1가 증가되는 것을 확인할 수 있었다.

이상의 결과들을 종합하여 볼 때, 고지방사료의 급이는 해마의 구조적가소성을 악화시키며, 이번 실험에서 사용된 사료전환은 비만에 따른 악영향을 개선시키는 효과가 있음을 알 수 있었다. 그리고, 사료전환과 운동요법을 복합적으로 적용하였을 때, 시너지 효과가 있음이 확인되었다. 고지방사료를 급이하면서 노화를 유발하는 D-gal의 투여는 해마의 신경가소성 지표들을 더욱 악화시켰으며, 이들 모델에 운동을 실시하였을 때, 신경가소성 관련 지표들이 어느 정도 회복되는 것을

확인할 수 있었다. 이번 연구를 통해 식습관 개선 또는 운동은 비만에 의한 해마의 구조적 변화를 예방하며, 운동에 의한 항노화효과를 고려해 볼 때, 신경가소성을 촉진할 수 있는 exercise-mimetic과 같은 물질의 개발 및 연구가 지속적으로 필요하다고 생각된다.

Keywords: 고지방사료, 트레드밀 운동, 사료전환, D-갈락토오스, 해마, 신경가소성

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