

# Treadmill exercise in obese maternal rats during pregnancy improves spatial memory through activation of phosphatidylinositol 3-kinase pathway in the hippocampus of rat pups

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Maternal nutrition is necessary for the growth of the fetus, and excessive intake of nutrients interferes with brain development in offspring. In the current study, the effect of treadmill running during pregnancy in obese maternal rats on spatial learning memory and spatial working memory in rat pups was investigated. Phosphorylation of phosphatidylinositol 3-kinase (PI3K), protein kinase B (Akt), and extracellular signal-regulated kinase 1 and 2 (ERK1/2) was also identified in rat pups. Female rats were divided into the normal diet group and the high-fat diet group for 7 weeks, including pregnancy and lactation. Maternal treadmill running was performed for 4 weeks. The born rat pups were classified into a control group, a treadmill exercise group, a high-fat diet group, a high-fat diet and treadmill exercise group according to the sta-

tus of maternal rats. Radial 8-arm maze task for spatial learning memory and Morris water maze task for spatial working memory were done. Western blot analysis was conducted to determine the expressions of PI3K, Akt, ERK1/2. In the current results, maternal treadmill running during pregnancy improved spatial learning memory and spatial working memory in rat pups born to obese maternal rats. This improving effect of memory was due to the enhanced phosphorylation of PI3K, Akt, and ERK1/2 by treadmill running.

**Keywords:** Maternal obesity, Pregnancy, Treadmill exercise, PI3K, Rat pups

## INTRODUCTION

Maternal nutrition is necessary for the growth of the fetus, and excessive intake of nutrients interferes with brain development in offspring. Thus, excessive maternal nutrition leads to obesity, hypertension and metabolic disorders in the pups (Chang et al., 2008; Shankar et al., 2008). The hippocampus integrates all information, including short-term, intermediate, and long-term memory (Snyder et al., 2005). Previously, we reported that treadmill running of obese maternal rats improved short-term memory by increasing brain-derived neurotrophic factor expression in the

hippocampus of rat pups born to obese maternal rats (Ji et al., 2020). This improving effect of treadmill exercise on short-term memory was attributed to increased cell proliferation in the hippocampus of rat pups born to obese maternal rats (Ji et al., 2020).

Brain-derived neurotrophic factor (BDNF) activates various intracellular signals such as phosphatidylinositol 3-kinase (PI3K), protein kinase B (Akt), and extracellular signal-regulated kinase (ERK) (Numakawa et al., 2010). PI3K is associated with cell growth, proliferation, differentiation, and survival. PI3K is a major factor in the signaling pathway of tyrosine kinase (Huang and Reichardt, 2003), and PI3K produces second messengers such as

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phosphoinositide-3,4-diphosphate or phosphoinositide-3,4,5-triphosphate (Banadakoppa et al., 2014). Akt is a serine/threonine-specific protein kinase and regulates many downstream effectors such as nuclear factor- $\kappa$ B and Bcl-2 family proteins (Song et al., 2005). Akt plays critical role in glucose metabolism, apoptosis, cell proliferation, transcription, and cell migration (Numakawa et al., 2010). Activated Akt modulates activation or deactivation the substrate through kinase activity. Mitogen-activated protein kinase (MAPK) is one of the protein kinases and MAPK is specific to serine and threonine. MAPK regulates gene expression, proliferation, differentiation, mitosis, apoptosis, and cell survival (Pearson et al., 2001). ERK includes ERK1, ERK2, ERK5, and ERK7 etc. ERK 1 and 2 (ERK1/2) is called classical MAPK, and is stimulated by proliferation by epithelial growth factor or platelet-derived growth factor, or activated by cell differentiation factors such as nerve growth factor to promote cell proliferation or differentiation.

The purpose of current study was to evaluate the effect of treadmill running during pregnancy in obese maternal rats on spatial learning memory and spatial working memory in rat pups. Phosphorylation of PI3K, Akt, and ERK1/2 was also identified in rat pups to evaluate the mechanism of exercise.

## MATERIALS AND METHODS

### Animals

Four weeks old Sprague-Dawley male rats ( $n=8$ ) and female rats ( $n=16$ ) were used for this experiment. After mating with male rats for 48 hours, female rats were bred separately. Obesity was caused by feeding female rats a high-fat diet (60% fat). Female rats were divided into two groups: normal diet group ( $n=8$ ) and high-fat diet group ( $n=8$ ). The speed of maternal treadmill running was first 3 m/min for the 5 min, next 5 m/min for 5 min, and lastly 8 m/min for 20 min, once 30 min, 5 times a week, during 4 weeks. The born rat pups were classified into four groups according to the conditions of the maternal rats: control group, treadmill exercise group, high-fat diet group, and high-fat diet and treadmill exercise group ( $n=10$  in each group). The approval number for this experiment was obtained from the Kyung Hee University Institutional Animal Care and Use Committee in Seoul, Korea (KHUASP [SE]-17-099).

### Radial 8-arm maze task

Radial 8-arm maze task was done to detect spatial learning memory as mentioned below (Ko et al., 2019). The radial 8-arm maze

device consisted of a central octagonal plate 30 cm in diameter and 8 radial arms 50 cm long and 10 cm wide, the device placed 1 m above the floor. A small container with a diameter of 3 cm and a depth of 1 cm was filled with water at the tip of the arm. After training 3 times, the work of the radial 8-arm maze was tested. The rat pups were water inhibited for 24 hours prior working with the radial 8-arm maze task, and explored the water for 5 min during the radial 8-arm maze task. The experiment was ended when rat pups found water in the arms or 5 min had passed. The number of correct choices was counted as the number before the first error occurred, and re-entry to the previously visited arm was considered as an error.

### Morris water maze task

Morris water maze task was conducted to detect spatial working memory as mentioned below (Park et al., 2019). To do this, rat pups need to know the spatial location of the hidden platform in a black circular pool with a diameter of 150 cm and a height of 50 cm filled with clear water ( $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$ ). The hidden platform, 15 cm in diameter and 35 cm high, disguised itself as transparent on a black background, 2 cm below the water surface in the center of the northern quadrant. The distal visual cues lie on the walls around the pool, and the position of the cues was not changed through the experiment. All rat pups were trained for 3 consecutive days, 3 times a day prior to the Morris water maze task measurement. Morris water maze task was performed 24 hr after the last training. When finding the platform, rat pups were left on the platform for 30 sec, and if rat pups could not find the platform within 60 sec, the hand guided them to the platform. The rat pups were provided 60 sec for the Morris water maze task, and all data were automatically measured by Smart Video Tracking System (Smart ver. 2.5, Panlab, Barcelona, Spain).

### Tissue preparation

After Morris water maze task, Zoletil 50 (10 mg/kg, Vibac Laboratories, Carros, France) was injected intraperitoneally to anesthesia, and 50 mM phosphate-buffered saline was injected through heart, and then fixed with 4% paraformaldehyde in 100 mM phosphate buffer (pH, 7.4). Brains were excised and the brains were fixed overnight using the same fixative and transferred to a 30% sucrose solution to prevent freezing. A frozen microtome (Leica, Nussloch, Germany) was used to make 40- $\mu\text{m}$ -thick sagittal sections.

### Western blot analysis

As mentioned below, western blotting for PI3K, Akt, and

ERK1/2 was done (Park et al., 2019). Hippocampal tissues were lysed in a lysis buffer comprising 1 mM phenylmethylsulfonyl fluoride, 50 mM Tris-HCl (pH, 7.5), 150 mM NaCl, 0.1% sodium dodecyl sulfate, 0.5% deoxycholic acid, 1% Nonidet P40, 100-mg/mL leupeptin. Rabbit anti-GAPDH antibody (1:1,000; Cell Signaling Technology, Beverly, MA, USA), mouse anti-total PI3K (t-PI3K) antibody (1:1,000; Santa Cruz Biotechnology, CA, USA), rabbit anti-phosphorylated-PI3K antibody (p-PI3K) (1:1,000; Santa Cruz Biotechnology), rabbit anti-total-Akt (t-Akt) antibody (1:1,000; Santa Cruz Biotechnology), rabbit anti-phosphorylated-Akt (p-Akt) antibody (1:1,000; Santa Cruz Biotechnology), rabbit anti-total ERK1/2 (t-ERK1/2) antibody (1:1,000; Cell Signaling Technology), rabbit anti-phosphorylated-ERK1/2 (p-ERK1/2) antibody (1:1,000; Cell Signaling Technology) were used as the primary antibody. Horseradish peroxidase-conjugated secondary antibodies were treated after washing. Image-Pro Plus computer-assisted image analysis system (Media Cybernetics Inc., Silver Spring, MD, USA) was used for the quantification of the bands.

### Data analysis

Data was analyzed by IBM SPSS Statistics ver. 21.0 (IBM Co., Armonk, NY, USA), and one-way analysis of variance followed Duncan *post hoc* test compared among the groups. The results were expressed as the mean  $\pm$  standard error of the mean with  $P < 0.05$  considered as statistically significant.

## RESULTS

### Spatial learning ability

For the effect of treadmill running during pregnancy of obese maternal rats on the spatial learning ability of rat pups, the radial

8-arm maze task was conducted (Fig. 1). In the high-fat diet group, the time taken to find all the water in 8 mazes was increased compared to the control group ( $P < 0.05$ ). Treadmill running reduced the time taken to find all the water in the high-fat diet group ( $P < 0.05$ ). In the high-fat diet group, the number of correct choices that found water in succession was reduced compared to the control group ( $P < 0.05$ ). Treadmill running increased the number of correct choices to water continuously in the high-fat diet group ( $P < 0.05$ ). In the high-fat diet group, the number of errors entering the maze was increased compared to the control group ( $P < 0.05$ ). Treadmill running reduced the number of errors in the high-fat diet group ( $P < 0.05$ ).

### Spatial working memory

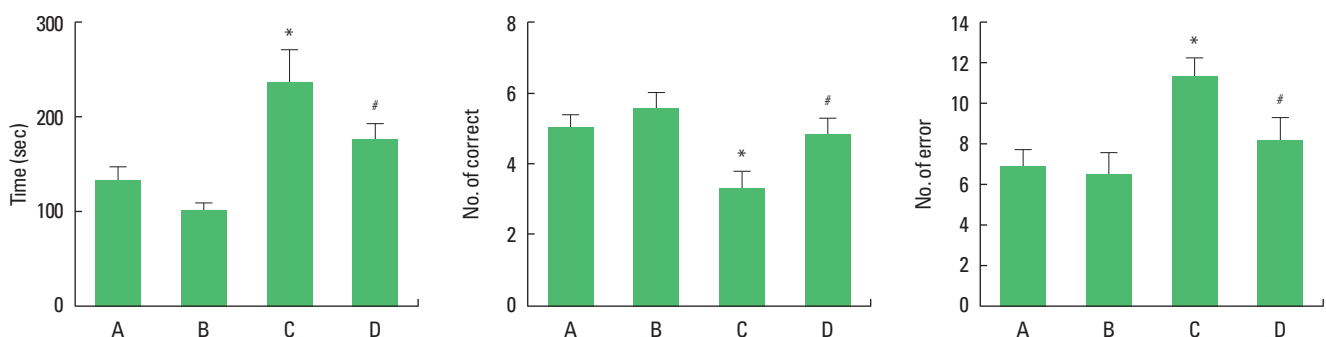
For the effect of treadmill running during pregnancy of obese maternal rats on the spatial working memory of rat pups, the Morris water maze task was conducted (Fig. 2). In the high-fat diet group, the time spent around probe quadrant was decreased compared to the control group ( $P < 0.05$ ). Treadmill running increased the time spent around probe quadrant in the high-fat diet group ( $P < 0.05$ ).

### PI3K expression

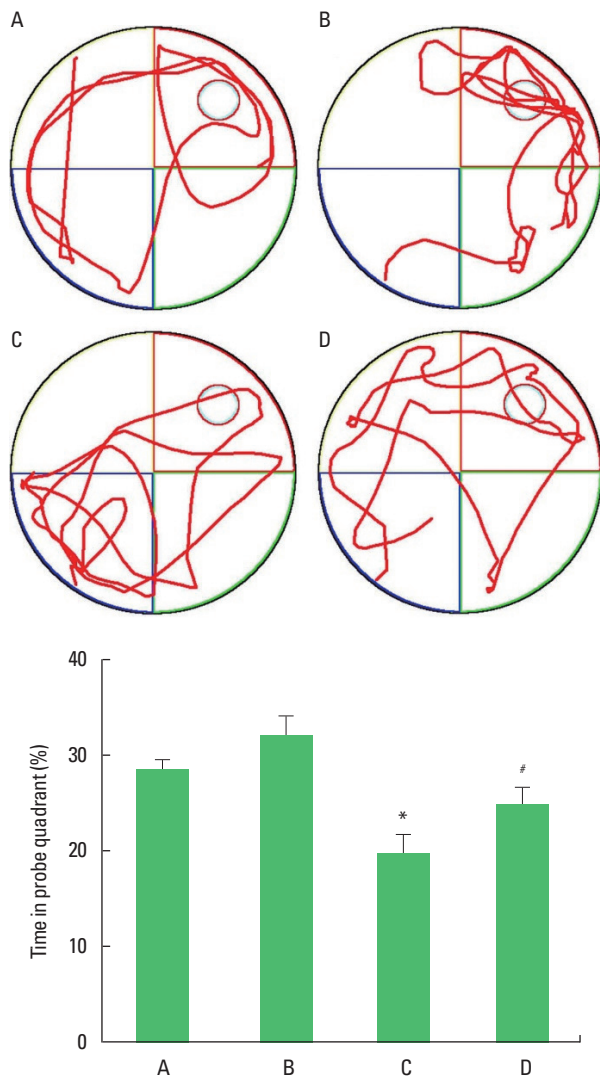
For the effect of treadmill running during pregnancy of obese maternal rats on the PI3K expression in the hippocampus of rat pups, PI3K expression was determined (Fig. 3). The ratio of p-PI3K/t-PI3K was decreased in the high-fat diet group compared to the control group ( $P < 0.05$ ). Treadmill running increased the ratio of p-PI3K/t-PI3K in the high-fat diet group ( $P < 0.05$ ).

### Akt expression

For the effect of treadmill running during pregnancy of obese



**Fig. 1.** Spatial learning ability of rat pups. Left panel: time spent for seeking water. Middle panel: number of correct choices. Right panel: number of errors. A, control group; B, treadmill exercise group; C, high-fat diet group; D, high-fat diet and treadmill exercise group. \* $P < 0.05$  compared to the control group. # $P < 0.05$  compared to the high-fat diet group. Data means the mean  $\pm$  standard error of the mean.

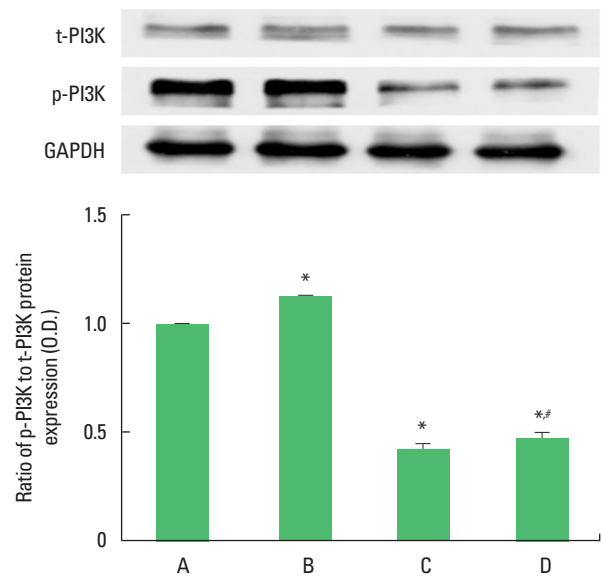


**Fig. 2.** Spatial working memory of rat pups. Upper panel: Morris water maze task. Lower panel: time spent around probe quadrant in each group. A, control group; B, treadmill exercise group; C, high-fat diet group; D, high-fat diet and treadmill exercise group. \* $P < 0.05$  compared to the control group. # $P < 0.05$  compared to the high-fat diet group. Data means the mean  $\pm$  standard error of the mean.

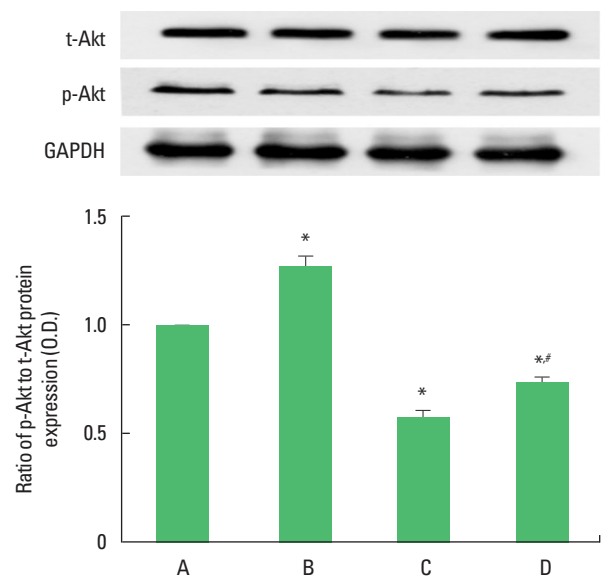
maternal rats on the Akt expression in the hippocampus of rat pups, Akt expression was determined (Fig. 4). The ratio of p-Akt/t-Akt was decreased in the high-fat diet group compared to the control group ( $P < 0.05$ ). Treadmill running increased the ratio of p-Akt/t-Akt in the high-fat diet group ( $P < 0.05$ ).

### ERK1/2 expression

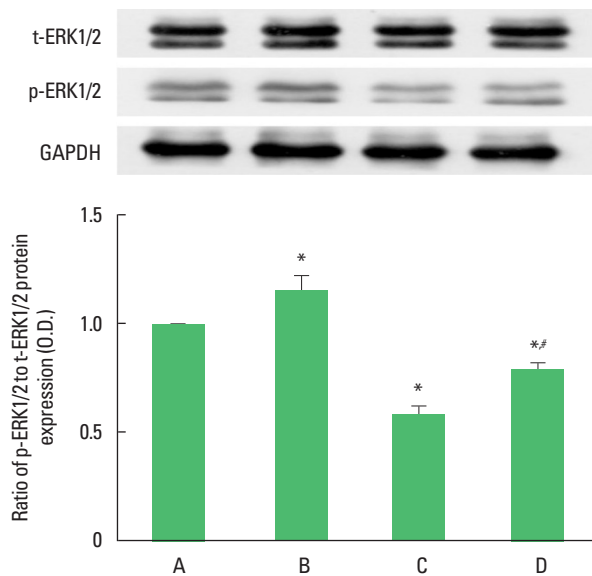
For the effect of treadmill running during pregnancy of obese maternal rats on the ERK1/2 expression in the hippocampus of rat pups, ERK1/2 expression was determined (Fig. 5). The ratio



**Fig. 3.** Phosphatidylinositol 3-kinase (PI3K) expression in the hippocampus of rat pups. Upper panel: western blot analysis of total-PI3K (t-PI3K) and phosphorylated (p-PI3K) in the hippocampus. Lower panel: p-PI3K/t-PI3K ratio in each group. A, control group; B, treadmill exercise group; C, high-fat diet group; D, high-fat diet and treadmill exercise group. \* $P < 0.05$  compared to the control group. # $P < 0.05$  compared to the high-fat diet group. Data means the mean  $\pm$  standard error of the mean.



**Fig. 4.** Protein kinase B (Akt) expression in the hippocampus of rat pups. Upper panel: western blot analysis of total-Akt (t-Akt) and phosphorylated (p-Akt) in the hippocampus. Lower panel: p-Akt/t-Akt ratio in each group. A, control group; B, treadmill exercise group; C, high-fat diet group; D, high-fat diet and treadmill exercise group. \* $P < 0.05$  compared to the control group. # $P < 0.05$  compared to the high-fat diet group. Data means the mean  $\pm$  standard error of the mean.



**Fig. 5.** Extracellular signal-regulated kinase 1 and 2 (ERK1/2) expression in the hippocampus of rat pups. Upper panel: western blot analysis of total-ERK1/2 (t-ERK1/2) and phosphorylated (p-ERK1/2) in the hippocampus. Lower panel: p-ERK1/2/t-ERK1/2 ratio in each group. A, control group; B, treadmill exercise group; C, high-fat diet group; D, high-fat diet and treadmill exercise group. \* $P < 0.05$  compared to the control group. # $P < 0.05$  compared to the high-fat diet group. Data means the mean  $\pm$  standard error of the mean.

of p-ERK1/2/t-ERK1/2 was decreased in the high-fat diet group compared to the control group ( $P < 0.05$ ). Treadmill running increased the ratio p-ERK1/2/t-ERK1/2 in the high-fat diet group ( $P < 0.05$ ).

## DISCUSSION

Obesity causes systemic inflammatory conditions and is also associated with cognitive impairment (Bilbo and Tsang, 2010). Maternal swimming during pregnancy increased short-term memory through enhancing cell proliferation in the hippocampus of rat pups (Lee et al., 2006). It is well known that exercise of the maternal rats increased memory in offspring (Akhavan et al., 2008; Kim et al., 2007). In the current results, spatial learning memory and spatial working memory were decreased in rat pups born to maternal rats with a high-fat diet compared to control maternal rats. Treadmill running of the maternal rats significantly improved spatial learning memory and spatial working memory in rat pups born to maternal rats with a high-fat diet.

Protein phosphorylation by the tyrosine kinase B receptor (TrkB) induces the activation of PI3K and Akt, regulating the survival of neurons and inducing the activity of ERK, thereby activating the

transcription factor cyclic AMP-response element binding (Kaplan and Miller, 2000). PI3K and ERK activation is mediated by protein binding to phosphorylated TrkB receptor (Numakawa et al., 2010). The MAPK signaling system is a factor that regulates embryogenesis, cell differentiation, proliferation, and death, and ERK1/2 is an important kinase that determines cell fate in the MAPK signaling system. ERK is essential for cell survival by activating PI3K and Akt signaling pathways and promoting cell differentiation and growth (Santos et al., 2010). ERK is essential for cell survival by activating PI3K and Akt signaling pathways to promote cell differentiation and growth (Santos et al., 2010). In the current results, phosphorylation of PI3K, Akt, and ERK1/2 in rat pups born to maternal rats with a high-fat diet was decreased compared to the control group. This means maternal rats with high-fat diet caused dephosphorylation of PI3K, Akt, and ERK1/2 in rat pups.

In a previous study, maternal treadmill running during pregnancy improved short-term memory in rat pups born to obese maternal rats (Ji et al., 2020). The improving effect of short-term memory was achieved by enhancing neurogenesis through increased expression of BDNF and TrkB by treadmill running (Ji et al., 2020). In the current results, phosphorylation of PI3K, Akt, and ERK1/2, which are involved in neuronal birth and survival in the hippocampus, was significantly enhanced in rat pups by treadmill running of maternal rats with high-fat diet group. This indicates treadmill running in maternal rats with high-fat diet enhanced phosphorylation of PI3K, Akt, and ERK1/2 in rat pups.

In the current study, maternal treadmill exercise during pregnancy improved spatial learning memory and spatial working memory in rat pups born to obese maternal rats. This increasing effect was due to the enhanced phosphorylation of PI3K, Akt, and ERK1/2 in rat pups by treadmill running.

## CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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