Maternal Aerobic Exercise during Pregnancy Can Increase Spatial Learning by Affecting Leptin Expression on Offspring’s Early and Late Period in Life Depending on Gender

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Maternal exercise during pregnancy has been suggested to exert beneficial effects on brain functions of the offspring. Leptin is an adipocytokine which is secreted from adipose tissues and has positive effects on learning, memory, and synaptic plasticity. In this study, pregnant rats were moderately exercised and we observed the effects of this aerobic exercise on their prepubertal and adult offspring’s spatial learning, hippocampal neurogenesis, and expression of leptin. All the pups whose mothers exercised during pregnancy learned the platform earlier and spent longer time in the target quadrant. Their thigmotaxis times were shorter than those measured in the control group. It is shown that hippocampal CA1, CA3 neuron numbers increased in both prepubertal and adult pups, in addition that GD neuron numbers increased in adult pups. Leptin receptor expression significantly increased in the prepubertal male, adult male, and adult female pups. In our study, maternal running during pregnancy resulted in significant increase in the expression of leptin receptor but not in prepubertal female pups, enhanced hippocampal cell survival, and improved learning memory capability in prepubertal and adult rat pups, as compared to the control group. In conclusion, maternal exercise during pregnancy may regulate spatial plasticity in the hippocampus of the offspring by increasing the expression of leptin.

1. Introduction

It is known that aerobic exercise is necessary for human health. Regular aerobic exercise decreases body fat rate, increases skeletal muscle strength, improves respiratory capacity, and increases the HDL cholesterol in blood. In addition to the positive effects of the exercise on the body, it also has improving effects on brain functions [1]. It is shown that it increases cerebral angiogenesis and cerebral blood flow [2], capillary growth [3], and dendritic connections [4] and improves cognitive performance in young rats [5, 6]. Brain development in mammals begins intrauterine period and continues till the end of the adolescent period [7]. The brain may easily be affected by internal and external factors within this development process. It is observed that some events and factors encountered during this period have positive or negative effects on the brain development. For example, stressful situations such as maternal deprivation in the early development period may cause neuropsychiatric disorders such as anxiety disorders, schizophrenia, and depression [8, 9]. Besides, enriched environment and physical exercise increase the number of brain cells and improve learning and memory [10].

Hippocampus, which is one of the regions of the brains related to cognitive functions, is the center of spatial learning and memory [11]. Differently from the other regions of the brain, cells are more affected by external factors during their lifetime and situations such as exercise or enriched environment increase the number of neurons in this area [10, 12]. Psychological traumas such as stress also mostly
influence the hippocampus in the brain [8, 12]. While the increase in the number of hippocampal cells enforces learning and memory, its decrease affects them negatively [13, 14].

Regular aerobic exercise during pregnancy is beneficial both for the mother and for the infant. It is observed that as a result of the exercise during pregnancy the muscle power and strength increase, excessive weight gain is prevented, as well as back pain, anxiety, and depression [15]. Other studies demonstrated that the aerobic exercise during pregnancy improves brain functions of the offsprings [16]. Hippocampal neurogenesis may continue lifelong under appropriate conditions, and especially enriched environment and physical exercise are seen to increase neurogenesis especially in gyrus dentatus (GD) in adults [13]. It is also shown in one of our previous studies that the exercise during the adolescent period increased hippocampal neurogenesis and spatial learning [14].

Leptin is a protein, the product of obesity (ob) gene. It is primarily produced in adipose tissue, and it provides nutrition and control of energy balance [17]. It is also thought to be produced and secreted locally in the central nervous system [18]. It is shown that leptin is carried by cerebrospinal fluid [19], enters into the brain by overcoming the blood brain barrier by means of the receptor, and is transferred to most regions of the brain by special carriers [20]. There is leptin receptor expression in many regions of the brain. In humans and rodents, leptin receptor mRNA is expressed in hypothalamus [21], cerebellum, hippocampus, and amygdala [22, 23]. Consequently it is thought that leptin may have an important effect on the brain development. It is indicated that leptin-deficient rats have smaller brain [24] and less myelin [25] and administering leptin to these rats corrects these abnormalities [26]. Leptin is also necessary for the regulation of locomotor activity and for neuronal and glial maturation in brain [27].

There is a limited number of studies concerning the effects of exercise on leptin, spatial learning, and memory. It is thought that the exercise regulates the glucose metabolism in brain, affects neurogenesis and neuroprotection, and regulates insulin, insulin-like growth factor-1 (IGF-1), and leptin signals in hypothalamus in order to control body weight [28, 29]. Leptin receptor deficient rats (db/db) are obese and have insulin resistance [30]. Deficiency of dendritic branching and lack of hippocampal brain-derived neurotrophic factor (BDNF) are detected in these rats, and regression is observed in these findings when caloric limitation and voluntary exercise are implemented [31]. Besides, the relation between leptin, exercise, and hippocampus-related learning-memory has not been clarified yet.

Various studies revealed that maternal exercise during pregnancy increases hippocampus-related learning and memory in offsprings and improves spatial plasticity; however, it is still not known whether leptin has a role in this process or not. The aim of this study is to investigate the effects of maternal moderate aerobic exercise during pregnancy on hippocampus-related spatial learning, memory, the hippocampal neurogenesis, and hippocampal leptin receptor expression of offsprings in prepubertal and adult periods.

2. Material and Method

Fifty-six Wistar Albino rats were included in the study. The study group consisted of animals whose mothers performed regular aerobic exercise during pregnancy, while the control group consisted of pups whose mothers were sedentary throughout the pregnancy. Each group was further divided into subgroups: male, female, prepubertal (21 days old), and adult (120 days old), resulting in eight groups with 7 animals in each. Pregnancy was controlled by estrous followup. The animals were maintained under standard colony conditions with a 12 h light:12 h dark cycle (lights on 07:00 h) at constant room temperature (23 ± 2°C) and humidity (60%), and food and water ad libitum throughout the experiments. Our study was carried out between 09:00 and 12:00 in a sound-attenuated, air regulated experimental room. All experiments were performed in accordance with the guidelines of the Experimental Animal Laboratory and approved by the Animal Care and Use Committee of the Dokuz Eylul University, School of Medicine.

2.1. Regular Maternal Aerobic Exercise in Pregnancy. Mothers in the exercise group were familiarized to the treadmill, 1 week before the pregnancy, by 10 min/session a day at a speed of 5 m/min for 5 days. These mothers started exercising at 8 m/min for 30 min 5 days a week the day after mating. In the last week of pregnancy, the speed was reduced to 6 m/min and the exercise was done for 30 min 5 days a week. This exercise type is regular mild treadmill exercise [6, 32]. Learning tests were made when offsprings get 21 and 120 days old after birth. At the end of the 5-day learning tests, subjects were sacrificed and their brains were ejected for histological evaluation.

2.2. Learning and Memory Evaluation. Learning and memory were evaluated by using Morris Water Maze (MWM). In tests, a black plexiglass pool having a diameter of 140 cm and a height of 75 cm was filled with warm water (22 ± 1°C). The platform having a 11 cm diameter, used within the pool, was 1.5 cm below the water surface. In all groups, the place of the platform was taught to them by making 5 trials for 4 days. The subject was expected to find the platform within 60 seconds after being placed in the water. It was allowed to stay on the platform for 20 seconds. The start point of the test was changed every day. Their duration of finding the platform, swimming speeds, and the distances covered within the 4-day learning process were calculated and evaluated. In the probe trial made on the 5th day, the platform was removed and the periods spent on the quadrant previously covering the platform and on the counter quadrant were evaluated for 60 seconds. The fact that the rat swam all around the pool wall at a distance of 15 cm in probe trial was evaluated as thigmotaxis which is an index of anxiety.

HVS image video tracking system was used to make the records and analysis of learning tests.

2.3. Histological Evaluation. After the MWM experiments, each group of rats was sacrificed under ether anesthesia by removal of all of the blood in the heart. Brains were removed
and fixed in 10% formalin in phosphate buffer for 24 h. The brains were sectioned coronally into sequential 6 μm slices using a rat brain slicer. The number of neurons in each sample was estimated by taking three coronal sections through the hippocampus that corresponded approximately to plates 21, 23, and 25 in the rat atlas of Paxinos and Watson [33]. All sections were stained with cresyl violet. The images were analyzed using a computer-assisted image analyzer system consisting of a microscope (Olympus BH-2 Tokyo, Japan) equipped with a high-resolution video camera (JVC TK-890E, Japan). The numbers of neurons in hippocampal CA1, CA2, CA3, and gyrus dentatus regions were counted using a 6000 μm 2 counting frame viewed through a 20 X Nikon lens at the monitor. The counting frame was placed randomly five times on the image analyzer system monitor, the numbers of neurons were counted (UTHSCSA Image Tool for windows version 3.0 software), and the average was determined. Hippocampal neuron density then was calculated.

2.4. Hippocampal Leptin Receptor Expression

2.4.1. RNA Isolation and cDNA Synthesis. Hippocampal tissue samples were each individually homogenized with Qiagen TissueLyser II homogenizer in 1 mL QIAzol lysis reagent, and total RNA was isolated using the RNeasy lipid tissue mini kit (Qiagen, GmbH, Germany) according to the manufacturer’s instructions. DNA contamination was removed by optional on-column DNase-digestion using the DNase-Free DNase Set (Qiagen, GmbH, Germany). The quantity of total RNA was measured by the Nanodrop ND-1000 spectrophotometer. The integrity of total RNA was analyzed in a MOPS-buffered 1.2% agarose gel containing formaldehyde and confirmed by visualization with EtBr staining and UV illumination. The absence of contaminating genomic DNA was also confirmed by setting reverse transcriptase minus (RT-) negative control reaction. The cDNA synthesis was performed with a RevertAid First Strand cDNA synthesis kit (Thermo Fisher Scientific Inc.) using 2 μg of total RNA according to the manufacturer’s instructions.

2.5. Real-Time RT-PCR. Specific rat primer pairs and probes for reference and target genes for real-time polymerase chain reaction (PCR) analysis were designed using the ProbeFinder Software, which is available online (http://www.universalprobelibrary.com/). B-actin was used as the reference gene. The UPL (universal probe library) probe number for the leptin receptor gene was 118, and the primer sequences were as follows: Forward: 5′-TGTACGAAATCTATGTGGTTTTG-3′; Reverse: 5′-TTG-GATAGCAGGTAAAGT (76 bp). The UPL probe number was β-actin gene was 115, and the primer sequences were as follows: Forward: 5′-CTAAACACCTGTGAAAAAG-3′; Reverse: 5′-GCCTCGAGATGCTAGCTACA-3′ (79 bp). All PCR reactions were performed using LightCycler 480.

Probes Master in a 20 μL reaction mixture (2 μL CDNA; 10 μL LightCycler 480 Probes Master, and a 2 μL (10 mM) each specific gene primer pairs and upl probes) in a LightCycler 480 Instrument, 96-well format (Roche Diagnostics, Indianapolis, Ind.). The PCR program was initiated at 95 °C for 10 minutes before 40 cycles, each for 10 seconds at 95 °C, 30 seconds at 60 °C, 1 second at 70 °C, and 30 seconds at 40 °C for cooling, were conducted. Negative control, consisting of real-time RT-PCR reaction mixture using sterile distilled water instead of template RNA, was included in each batch of PCR. In addition, repeatability and reproducibility of the assays were assessed by performing 3 replicates. Each amplification cycle was analyzed with the LightCycler 480 software.

3. Statistical Evaluation

Differences between days in learning tests were evaluated with GLM-repeated measures in SPSS program, and differences between groups were evaluated with Mann-Whitney U test, which is a nonparametric test. Differences between groups in terms of all measurement results were also evaluated with Mann-Whitney U test.

4. Results

It was observed in this study that the duration spent for finding the platform shortened and reached a steady level in all rats during the 4-day learning test process. All of the pre-pubertal and adult pups whose mothers performed regular exercise during pregnancy learned the place of the platform in MWM tests in a shorter time as compared to the control group; however, there were differences between groups in learning process. Among the pups whose mothers exercised during pregnancy, prepubertal females learned the place of the platform on the 2nd day (P < 0.05), prepubertal males learned the place on the 3rd day (P < 0.05), adult females learned the place on the 2nd day (P < 0.05), and adult males learned the place on the 2nd and the 4th days (P < 0.006) of the learning test, which were shorter in comparison with those measured in the control group (Figure 1(a)). It was seen that in probe trial, all pups whose mothers exercised during pregnancy spent more time in the target quadrant and less time in the opposite quadrant (P < 0.05) (Figure 1(b)). It was also determined that, in these groups, thigmotaxis periods were significantly shorter as compared to the control group (P < 0.05) (Figure 1(c)). In histological evaluation, the number of neurons increased CA1 (P < 0.004) and CA3 (P < 0.006) in prepubertal female pups of rats who exercised during pregnancy, CA1 (P < 0.004) and CA3 (P < 0.004) in their male offspring, CA1 (P < 0.003), CA3 (P < 0.05), and GD (P < 0.05) in their adult female offspring, and CA1 (P < 0.004), CA3 (P < 0.05), and GD (P < 0.004) in their adult male offspring (Figures 2(a), 2(b), and 2(c)). Hippocampal leptin receptor expression was found to be significantly higher in prepubertal male (P < 0.05), adult female (P < 0.007), and adult male (P < 0.011) pups whose mothers exercised during pregnancy as compared to the control group. On the other hand, hippocampal leptin expression in prepubertal females was significantly lower compared to the control group (P < 0.002) (Figure 3).
5. Discussion

In this study, the CA1 and CA3 cell counts were significantly higher in prepubertal female and male pups of rats who exercised during pregnancy; CA1, CA3, and GD cell counts were higher in their adult female and male pups as compared to the control group. Furthermore, it was also determined that hippocampal leptin receptor expression increased in prepubertal male and all adult pups of mothers who exercised during pregnancy. Maternal exercise during pregnancy improved the learning process both in prepubertal and in adult offspring as compared to the control group; they become more successful in the probe trial, they spent more time in the target quadrant and less time in the opposite quadrant, and they made less mistakes. Moreover, the thigmotaxis period which is a sign of anxiety was shorter in all pups of rats who exercised during pregnancy. Maternal exercise increased the spatial learning of offspring and hippocampal BDNF expression. It is recently known that leptin, from the cytokine family, also positively affects learning and memory by means of its receptors in hippocampus [40]. It is suggested that synaptic plasticity is regulated by leptin and leptin receptors in rats [41, 42]. It is shown that acute voluntary or forced exercise increases neuronal activity in hippocampus [36]. It is also thought that neurotransmitters such as serotonin, norepinephrine, acetylcholine, and GABA (gamma aminobutyric acid) increase with exercise and regulate regions of hippocampus related to memory [37]. Besides, various studies concluded that exercise increases some neurotrophic factors such as BDNF, IGF-1 and vascular endothelial growth factor (VEGF) [16, 38, 39]. The effects of maternal exercise on the intrauterine hippocampus development are highly complex. Parnpiansil et al. [16] showed that maternal exercise during pregnancy increased the spatial learning of offspring and hippocampal BDNF expression. It is recently known that leptin, from the cytokine family, also positively affects learning and memory by means of its receptors in hippocampus [40]. It is suggested that synaptic plasticity is regulated by leptin and leptin receptors in rats [41, 42]. It is shown that leptin receptors are expressed especially in the CA1 subfield, of the hippocampus [41, 43], facilitate presynaptic neurotransmitter secretion in hippocampal CA1 subfield, and increase the sensitivity of postsynaptic CA1 neurons against neurotransmitters [44]. By increasing NMDA receptors, leptin contributes the conversion of LTP into spatial memory in CA1 region of
Figure 2: Hippocampal neuron density of the groups. (a) Quantitative evaluation of neuronal densities of CA1, CA2, and CA3 regions of hippocampus and gyrus dentatus. (b) Light microscope images of prepubertal pups (Cresyl violet staining). (c) Light microscope images of adult pups (Cresyl violet staining). *P < 0.05 compared to other groups. F: control female pups, M: control male pups, E + F: female pups whose mothers exercised during pregnancy, E + M: male pups whose mothers exercised during pregnancy. GD: gyrus dentatus.
during pregnancy, E + M: male pups whose mothers exercised 
control male pups, E + F: female pups whose mothers exercised 
hippocampus [40, 45]. Spatial memory behaviors related to 
∗
3: Hippocampal leptin receptor expression of the groups. 
Figure 6 The Scientific W orld Journal 
hippocampus [43, 46] and short-term potentiation (STP) 
[44, 47] and facilitates LTP [42]. Leptin is thought to 
hippocampus increases learning and memory performance 
[43] decreased in rodents which have leptin receptor muta-
tion (db/db mice or fa/fa rats). Direct injection of leptin into 
other neurotrophic factors may also play a role in learning 
and memory of females in prepubertal period. How effects 
of leptin on synaptic plasticity are affected by other hormones 
and growth factors has been still not clear. Further studies are 
nEEDED to elucidate the role of leptin and other neurotrophic 
authors contributed equally to this work.

Conflict of Interests

The authors declare they have no competing interests.

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