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A thesis of the Master's Degree

Hippocampal Neurogenesis and Regional Cerebral Glucose Metabolism, Functional Connectivity Evaluation Using F-18 FDG PET in Type II Diabetic Rat Model

제2형 당뇨 쥐 모델에서 F-18 FDG PET을 이용한 뇌의 해마 신경발달과 국소 뇌 당대사, 기능성 연결성 평가

August 2017

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Hippocampal Neurogenesis and Regional Cerebral Glucose Metabolism, Functional Connectivity Evaluation Using F-18 FDG PET in Type II Diabetic Rat Model

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Abstract

Diabetes is a representative risk factor of various neurodegenerative and neurovascular diseases. Many epidemiological and clinical studies have shown that diabetic patients more develop cognitive decline. We investigated the effect of diabetes on adult neurogenesis (AN) and functional connectivity using a rat model and fluorine-18-fluorodeoxyglucose (F-18 FDG) positron emission tomography (PET). For type II diabetes (T2DM) model, Zucker diabetic fatty (ZDF) male rats (6 weeks old 5, 12 weeks old 5) were used, and same number and same aged of Zucker lean control (ZLC) male rats were used as control. F-18 FDG brain PET images of each rat were acquired to evaluate regional cerebral glucose metabolism which reflects regional neuronal activity. Image normalization to the MRI template and analysis were performed using SPM8. We extracted 58 count of brain regional volume of interest (VOI). Functional connectivity was evaluated. Doublecortin (DCX) immunohistochemistry (IHC) was done to evaluate the neurogenesis in the dentate gyrus (DG). GLUT3 IHC was performed to evaluate the correlation between the AN and glucose metabolism. 12 weeks old ZDF rats showed the decreased regional glucose metabolism in the bilateral hippocampus compared to age-matched ZLC group ($p<0.05$). In the 6 weeks old group, there was no significant difference of regional glucose metabolism. In functional connectivity analysis, there was no significant difference between 6 weeks old group.

However, in 12 weeks old ZDF showed increased or decreased hippocampus connectivity compared with control ($p<0.005$). The number of neuroprogenitor cells was decrease in the hippocampus of 12 weeks old ZDF compared with same aged ZLC, although there was no significant difference of neuroprogenitor cells in 6 weeks. 12 weeks old ZDF rats showed decreased of the GLUT3-positive cells in the hippocampus compared with same age ZLC rats. That means the decreased glucose metabolism in the hippocampus of the T2DM rats is due to decreased GLUT3 expression and the result of decreased DCX expression in the same area may suggest decreased adult neurogenesis, which may affect variable functioning connectivity with other brain loci.

This result may help to understand the pathophysiologic mechanism of cognitive dysfunction occurring in diabetic patients, and to our knowledge, this is the first functional study with connectivity which is correlated with pathologic alteration using T2DM rat models and F-18 FDG PET. The hippocampal early changes may play an important role in treating and preventing neurodegenerative disease such as Alzheimer's disease (AD) in diabetic patients.

Keyword : Diabetes mellitus, Neurogenesis, GLUT3, FDG-PET, functional connectivity

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Introduction

Adult neurogenesis (AN) is the process of generating functional neurons in the adult brain. AN has been reported not only in the most animal but also in human (Eriksson et al., 1998). AN performs important role in the brain function such as learning and memory (Derakhshan and Toth, 2013). There are several factors regulating AN process. Energy metabolism is one of the important factors that have effect on AN with regard to both the proliferation of neural stem cell and the differentiation of cells. Especially glucose metabolism influences on AN, and it is supported by microarray studies (Geschwind et al., 2001, Ivanova et al., 2002). Therefore, diabetes should effect on adult neurogenesis. The cerebral glucose metabolism is coupled with the neuronal activity (Pellerin and Magistretti, 1994). Glucose transporter (GLUT) expression related with the glucose or FDG uptake coupled with the neuronal activity (McEwen and Reagan, 2004; Vannucci et al., 1997). Among the subtypes of GLUTs, GLUT3 is specifically expressed in the neuron and has high correlation with the brain metabolism (McEwen and Reagan, 2004).

There are many evidences that diabetes induces an impairment of neuronal function in the brain (Hill and Williams, 2004). The most common diabetic brain complications include cognitive decline and depression (Ho et al., 2013a). The incidence of cognitive decline may be as high as 40% in people with diabetes (Dejgaard et al., 1991). Meta-analyses concluded that diabetes

is associated with lower cognitive performance and increased risk for dementia (Gaudieri et al., 2008; Lu et al., 2009). F-18 FDG uptake in PET has a meaning of regional cerebral glucose metabolic rate (rCMRglu), (Alavi et al., 1986) which reflects the regional neuronal activity (Cheah and Amiel, 2012). In this respect, PET can become accepted as an effective tool to estimate the brain metabolic change in diabetic patients.

Furthermore, there has been an increasing number of complex network analysis to identify characteristics of functional brain connectivity, mostly from functional neuroimaging modalities such as functional magnetic resonance imaging (fMRI) and PET (Lang et al., 2012). Neurons in different brain regions interact with other to make harmonization in order to enable various sensorimotor and cognitive tasks to be performed (Horwitz, 2003). The concept of functional connectivity is introduced to explain the functional strengths of such interactions whether direct or indirect, irrespective of the existence of real structural connection (Horwitz, 2003). It provides an opportunity to understand and investigate complex brain processes as well as different brain pathophysiology in a new point of view (Reijneveld et al., 2007). Especially default mode network (DMN), has a meaning of network on resting state conditions, has been a main focus of the researchers (Musen et al., 2012).

In this study, we investigated the effect of type 2 diabetes mellitus (T2DM) on neurogenesis by using F-18 FDG PET and the possible association of the functional changes with the cellular changes of hippocampus in diabetic rats.

Materials and Methods

Experimental animals

Zucker diabetec fatty (ZDF, *fa/fa*) male rats 6 weeks old 5 and 12 weeks old 5 were used. For the aged-matched control, Zucker lean control (ZLC, +/+) male rats 6weeks old 5 and 12 weeks old 5 were used. Genotype herein was determined with the strategy described previous study (Hwang et al., 2010). All procedures were performed in accordance with the National Institutes of Health Guidelines for Animal Research (Guide for the Care and Use of Laboratory Animals), and approved by the Institutional Animal Care and Use Committee at Seoul National University Bundang Hospital. Animals acclimatized to the laboratory for a week before the beginning of experiments. They were housed in a conventional state under adequate temperature (23℃) and humidity (60%) control with a 12-hr light/12-hr dark cycle, and free access to food and water. Purina 5008 rodent diets (7.5% fat) were provided as recommended by Genetic Models (Purina, Korea, Korea). All the experiments were conducted to minimize the number of animals used and the suffering caused by the procedures used in the present study.

FDG PET Data acquisition and processing

F-18 FDG PET/CT images were acquired on 6 week and followed up on 12 week. All rats were fasted for at least 6 h before F-18 FDG intravenous

injection (Fueger et al., 2006). Each animal was placed under a heating lamp in a cage and warmed for at least 30 min before the F-18 FDG injection (Fueger et al., 2006). F-18 FDG (500 μCi/100 g body weight) was injected through tail vein 1 h prior to PET image acquisition. Brain Image was acquired for 40 min and we allowed 60 min of F-18 FDG uptake (Nano PET/CT, Bioscan, Washington DC, US)

Each brain images were co-registered to the FDG and T2 MRI rat brain template (Schiffer et al., 2006) in PMOD 3.8 (PMOD group, Zurich, Switzerland). Brain image was masked to exclude the extra-cranial area. Each image was spatially normalized to FDG rat brain template in SPM8, SPnM, and global count normalization was done. For the comparison of regional cerebral glucose metabolism, we performed 5,000 times permutation and non-parametric comparison was done. Uncorrected P-value 0.005 was used as statistical threshold.

Functional connectivity and brain network construction

To make brain network, we used 53 nodes that were represented by the 58 VOIs (Dorr et al., 2007). We extracted intensity-normalized FDG uptake in the VOIs of each rat. With FDG uptake, correlation coefficients were obtained. Pearson's correlation coefficients (r) between each pair of the VOIs were calculated in an inter-subject manner and correlation matrix (58 × 58) was obtained from each group (Choi et al., 2014).

To evaluate statistical differences of connectivity between the groups, we performed permutation test on all possible connections between nodes. Correlation matrix of each groups were transformed to Z scores using Fisher transformation. Randomly reassigned labels were permuted 5,000 times for each of 58 VOIs and interregional correlation matrices were calculated, followed by Fisher transformation. We obtained Type I error by the comparison between the observed Z score for each connection and Z score from permuted data. To determine statistically different connections in comparison between vehicle and test groups, a threshold was set as $p<0.005$.

Doublecortin (DCX) expression

After acquisition the PET images, all subjects were euthanized using established method (Perera et al., 2007; Perera et al., 2011a). Briefly, the subjects were anesthetized to a surgical depth with intraperitoneal injection of ketamine (100mg/kg) and xylazine (20mg/kg), after anesthetization, transcardially perfused with saline and formalin. The brains were removed and postfixed in 4% paraformaldehyde for immunohistochemistry staining and analysis. The left hippocampus was dissected into 40 μm sections. Every 40^{th} section (approximately 10-12 sections per antibody) through the rostrocaudal extent of the left hippocampus was immune-stained with standard peroxidase to detect and quantify neurogenesis rated using previous methods (Hwang et al., 2008). New neurons that were still

immature are detected by the expression of the microtubule-associated protein doublecortin (DCX) (goat anti-doublecortin, 1:200; Santa Cruz, CA). The secondary antibody is biotinylated anti-mouse IgG (1:200; Vector Laboratories, Burlingame, CA), and was visualized with avidin-biotin complex solution (Vector Laboratories, Burlingame, CA) and diaminodenzidine (DAB; Sigma-Aldrich, St Louis, MO). The density of DCX-labeled cells per mm^3 of the subgranular zone (SGZ) of the dentate gyrus was estimated for each animal. Two independent raters, masked to the group, counted all unambiguously DCX-labeled cells in the SCZ of the dentate gyrus (defined as a two-cell-body-wide zone on either side of the border of the granule cell layer) using 40x primary magnification. Between the number of DCX-rective cells of ZDF and that of ZLC groups, the unpaired, two-sided t-test comparison–Student's t-test (SPSS 8.0) was performed. The significance level was assumed as $P < 0.05$ unless otherwise indicated.

Glucose Transporter 3 (GLUT3) expression

The same brains section was immunostained with standard peroxidase to detect and quantify neurogenesis rated using previous methods (Perera et al., 2007; Perera et al., 2011a). New neurons that were still immature, detected by the expression of the glucose transporter 3 (GLUT3) (goat anti-GLUT3, 1:200; Santa Cruz, CA). The secondary antibody is biotinylated anti-mouse

IgG (1:200; Vector Laboratories, Burlingame, CA), and was visualized with avidin-biotin complex solution (Vector Laboratories, Bulringame, CA) and diaminodenzidine (DAB; Sigma-Aldrich, St Louis, MO). The density of GLUT3-labelled cells per mm^3 of the Cornu Ammonis (CA)-1, CA-2/3, and dentate gyrus was estimated for each animal. Two independent raters, masked to the group, counted all unambiguously GLUT3-labelled cell in the SCZ of the dentate gyrus (defined as a two-cell-body-wide zone on either side of the border of the granule cell layer) using a previous method (Hwang et al., 2008). Briefly, the staining intensity of all GLUT3 immunoreactive cells was evaluated on the basis of an optical density (OD), which was obtained after the transformation of the mean gray level using the formula; OD=log (256/mean gray level). The OD of background was taken from areas adjacent to the measured area. After the background OD was subtracted, a ratio of the OD of image file was calibrated and then analyzed using NIH Image software.

Blood glucose level, Insulin and IGF-1 level

Prior to the injection of F-18 FDG, blood samples were taken from the tail vein of anesthetized rats, for the measurement of plasma IGF-1, insulin and glucose levels using IGF-1 ELISA assay kit (Quantikine), insulin RIA kit (Merckmillipore), and portable glucose monitoring device (Roche).

Results

Regional Cerebral Glucose Metabolism between ZDF and ZLC

At 6 week, there was no significant regional cerebral glucose metabolism difference between ZDF and ZLC. However, at 12 weeks old, ZDF showed significantly decreased regional glucose metabolism mostly in dentate gyrus area (Figure 1, $p<0.005$). ZLC rats at 12 weeks old still showed no significant metabolic decrease in that area.

Metabolic Connectivity Differences between ZDF and ZLC

At 6weeks old, there was no difference of functional connectivity between ZDF and ZLC rats. However, at 12 weeks old, the ZDF rat showed increase of some functional connectivity and decrease of other functional connectivity connected to hippocampus compared with control rats ; decreased with frontal association cortex, right medial prefrontal cortex, frontal cortex, both motor cortex, right cingulate gyrus, left thalamus, right somatosensory cortex, both anterior hippocampus, right parahyppocampal gyrus, both auditory cortex, and right visual cortex and increased connectivity with olfactory bulb, both cerebellum, left caudate nucleus-putamen, right insular cortex, both anterior/posterior hippocampus, central amygdala, left ventricular accumbens, medulla, and right cerebellar hemisphere (Figure 2, p-value < 0.005).

Neurogenesis in Dentate Gyrus

For the neurogenesis confirmation, DCX staining was performed in the dentate gyrus and we evaluated DCX-positive cell number. In the DCX staining, 12 weeks old ZDF rats showed decreased of the DCX-positive cells in dentate gyrus compared with ZLC rats of same age on both cryo and paraffin sections (Figure 3,4).

GLUT3 Expression in Hippocampus

For the GLUT3 confirmation, GLUT3 staining was performed in the hippocampal CA-1, CA-2/3 and dentate gyrus and we evaluated GLUT3-positive cell number. In the GLUT3 staining, 12 weeks old ZDF rats showed decreased of the GLUT3-positive cells in CA-2/3 region, dentate gyrus compared with ZLC rats of same age, however, no significant difference of the GLUT-3 positive cells in CA-1 region (Figure 5, 6).

Comparison of 6 hour Fasting Glucose, Insulin, and IGF-1 level

In 6 weeks, there was no significant difference of 6 hr fasting glucose, insulin, and IGF-1 level between ZLC and ZDF rats. However, in 12 weeks, ZDF rats showed significantly decreased insulin level than ZLC rat (Table 1).

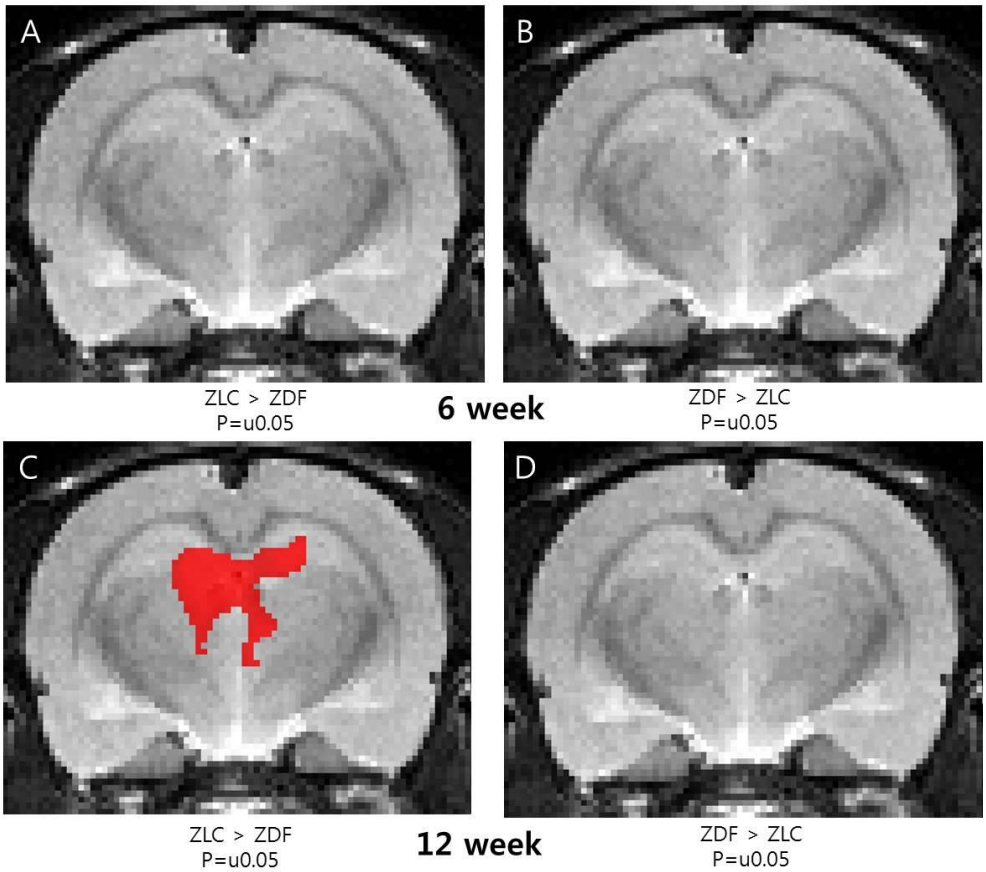

Figure 1. Regional cerebral glucose metabolism comparison between ZDF and ZLC. **A, B.** At 6 weeks of age, no differences in FDG uptake were seen between anterior hippocampus of ZDF rats and ZLC rats. **C, D.** At 12 weeks of age, ZDF rats showed reduced FDG uptake of ZDF rats compared with ZLC rats.

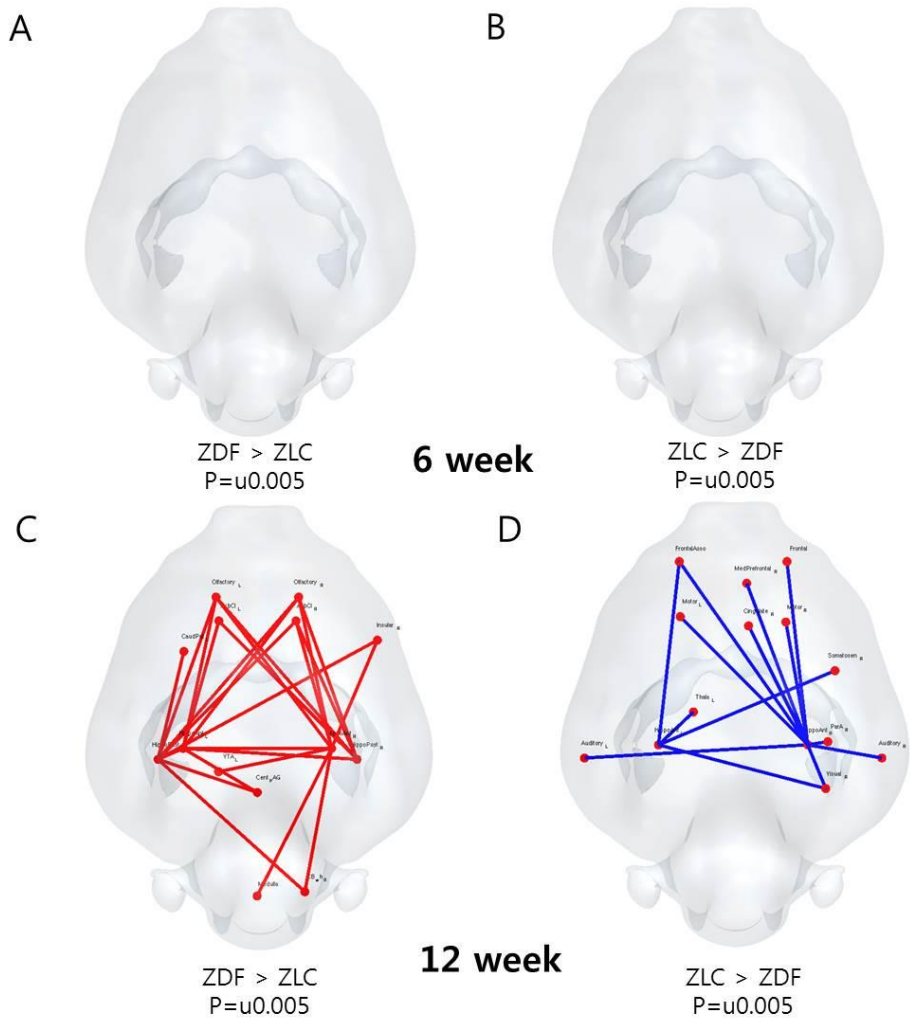

Figure 2. Functional connectivity difference between ZD and ZLC. **A, B.** At 6 weeks of age, no change of functional connectivity with FDG-PET data was detected in both ZDF and ZLC rats. **C,D.** After the comparison of ZDF rats and ZLC rats at 12 weeks of age, different patterns of functional network signal connected to hippocampus were shown as increase of some functional connectivity and decrease of other functional connectivity.

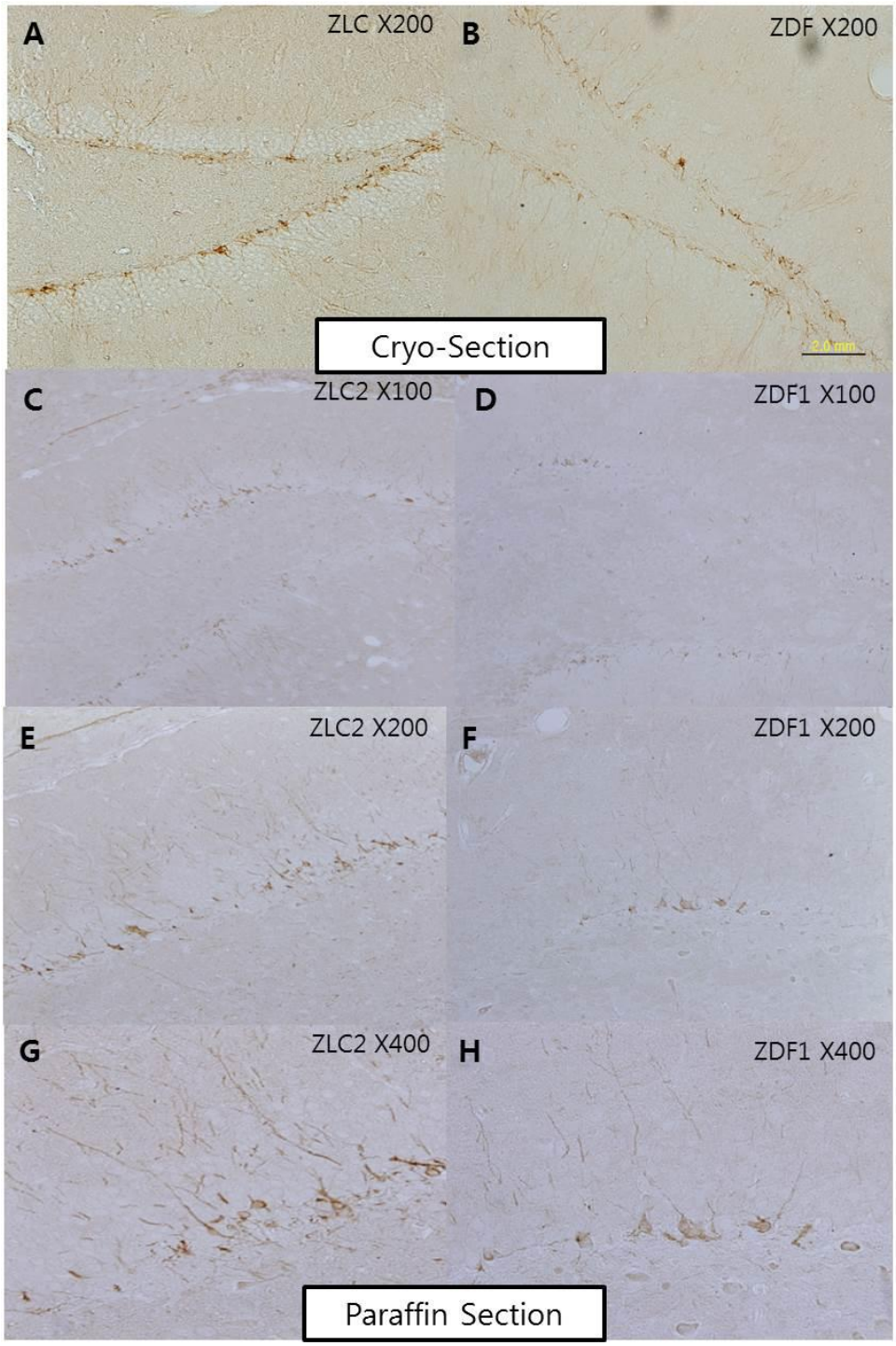

Figure 3. Comparison of neurogenesis between ZDF and ZLC **A, B** The cellular changes in the subgranular zone of the dentate gyrus were shown by cryosection (x200) in ZLC and ZDF rats, respectively. **C~H** showed the

DCX immunoreactive cells by paraffin section (x100, x200, and x400) in ZLC and ZDF rats, respectively. DCX immunoreactive cells are significantly decreased in the ZDF group than in the ZLC group.

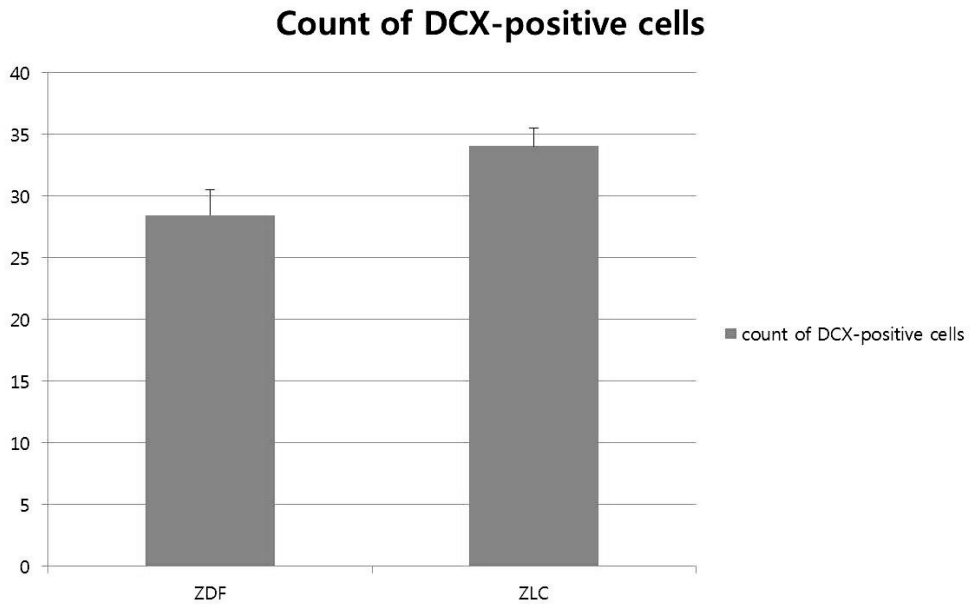

Figure 4. Comparison of count of DCX-positive cells between ZDF and ZLC. This graph showed the count of DCX-positive cells in ZDF rats were significantly decreased compared with that of ZLC rats.

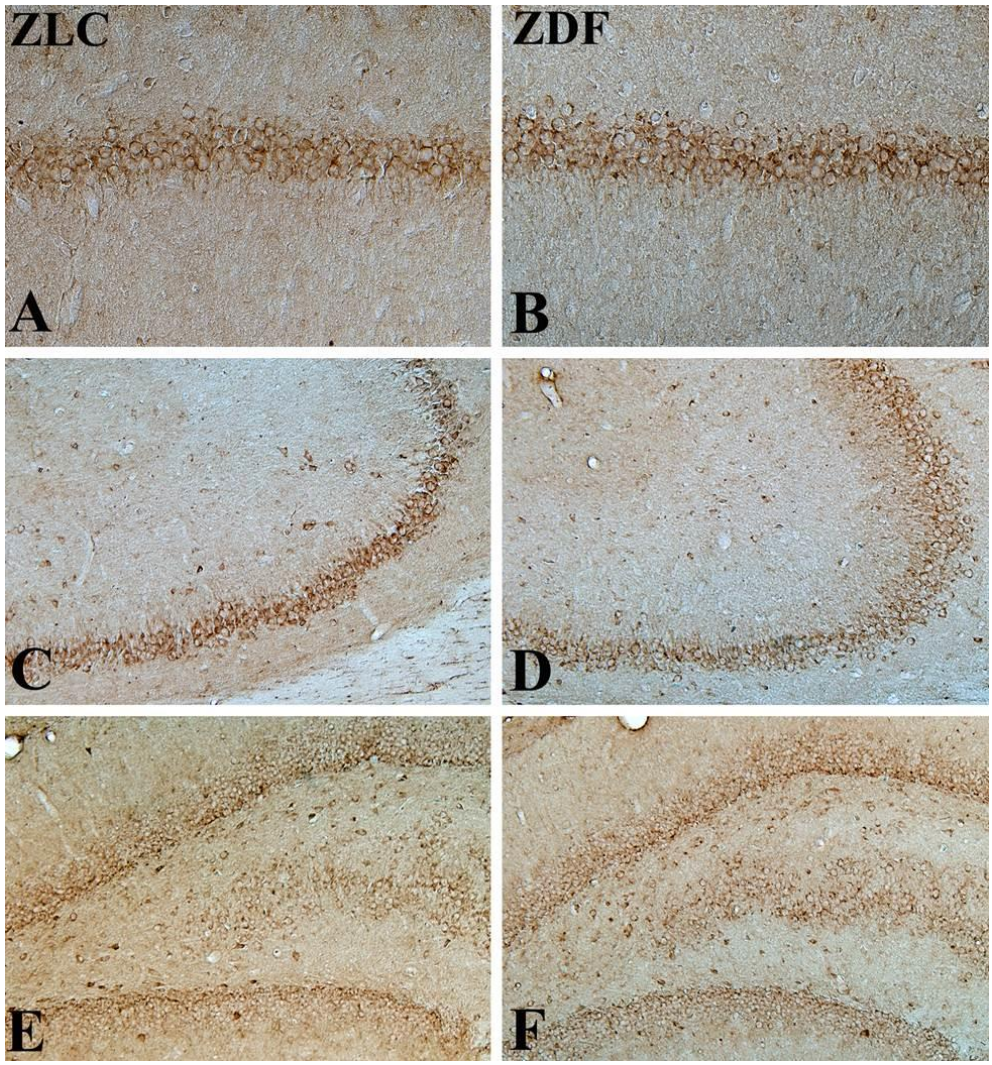

Figure 5. Comparison of GLUT3 immunoreactivity between ZDF and ZLC. **A,B** In the CA-1 region, there was no significant difference of GLUT3 immunoreactivity in the SP of both groups of ZLC and ZDF rats. **C,D** In the CA-2/3 region, there was significant decrease of GLUT3 immunoreactivity in the SP of ZDF groups compared with ZLC groups. **E,F** In the DG region, there was also significant decrease of GLUT3 immunoreactivity in ZDF groups compared with ZLC groups.

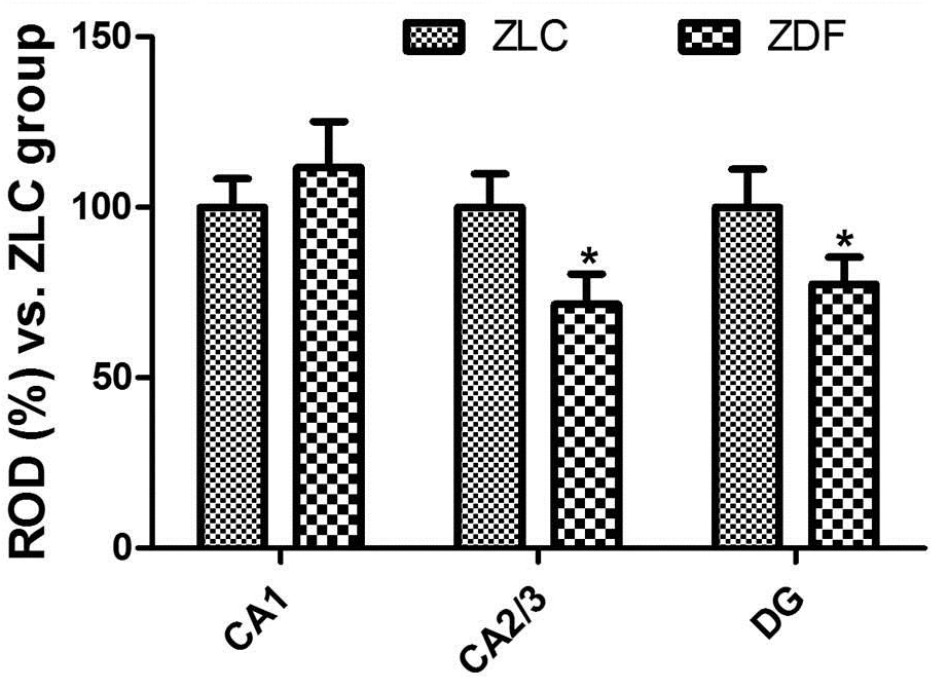

Figure 6. Comparison of GLUT3 immunoreactive cells and relative optical density between ZDF and ZLC. The number of GLUT3 immunoreactive cells and relative optical density of GLUT3 immunoreactivity in the CA1, CA2/3 and DG are expressed as percentage of each grouts ($p<0.05$). All data are represented as the mean±standard errors of mean.

Group	ZLC			ZDF		
	Glucose (mg/dL)	Insulin (ng/mL)	IGF-1 (ng/mL)	Glucose (mg/dL)	Insulin (ng/mL)	IGF-1 (ng/mL)
6 week	89±8.3	12.9±3.5	1362±47	91±9.2	12.5±4.3	1401±54
12 week	92±7.6	13.7±4.1	1428±63	101±11.1	3.3±2.1*	1377±83

Table 1. Comparison of 6 hour fasting glucose, insulin, and IGF-1 level between ZDF and ZLC rats.

Discussion

Neurogenesis is the process that neurons developed from neural stem cells and progenitor cells. Central nervous system (CNS) was thought to be incapable of regeneration after birth. However, it is established that new neurons are continuously generated even in adulthood of most mammals including human (Altman, 1963; Altman and Das, 1967). The hippocampus, the olfactory bulb, subgranular zone (SGZ), and subventricular zone (SVZ) are the region that continuous neurogenesis happens in mammals (Taupin and Gage, 2002). Adult neurogenesis is also reported in human (Eriksson et al., 1998). In a point of brain function, adult neurogenesis (AN) has an important role in several brain functions including olfaction, learning and memory, pattern separation, and mood control (Carlén et al., 2002). AN in hippocampus contributes to the learning and memory process (Deng et al., 2010), and a recent study demonstrated that about 700 neurons are newly developed daily in the dentate gyrus of adult human hippocampus (Spalding et al., 2013). It is known that above 80% of the granule cells in the dentate gyrus are produced postnatally, unlike other neuronal cells in the hippocampus (Bayer, 1980; Bayer and Altman, 1974; Schlessinger et al., 1975). Reduced of AN in the hippocampus with antimitotic drugs or radiation impaired spatial learning and memory. AN is a dynamic biological process that is regulated by several factors. Energy metabolism affects AN through the proliferation of neural stem cells and the differentiation of new

neuronal cells (Rafalski and Brunet, 2011). Among them, the glucose metabolism has essential role in neuronal stem and progenitor cells.

Glucose is the main energy source in the mammalian brain (Mergenthaler et al., 2013). Human brain consumes about 20% of the body's total energy consumption (Raichle, 2006). The glucose consumption has essential role in brain such as maintaining synaptic function and neuronal activities (McEwen and Reagan, 2004; Pellerin and Magistretti, 1994). It is showed that neural stem cells demand higher glycolysis for energy production, proliferation and survival than do neurons in vitro and the glycolytic rate of neural stem cells decreases during differentiation (Candelario et al., 2013). During AN process, mRNAs and proteins for glycolytic metabolism such as GLUTs express to regulate AN (Maurer et al., 2006), and GLUT expression changes on tissue levels as well (Yoo et al., 2016). Among the subtypes of GLUTs, GLUT3 expresses in the neuron and has high correlation with the brain metabolism (McEwen and Reagan, 2004). Several evidences show that changes of GLUT3 expression is correlated with rates of glucose metabolism based on 2-deoxy[^{14}C]glucose uptake in postnatal rats (Nehlig et al., 1988; Vannucci et al., 1989). Compensatory increase of GLUT3 expression in neuron to induce neurogenesis has been observed in brain damage such as ischemia (Huang et al., 2010), traumatic brain injury (Hamlin et al., 2001), and hyperglycemia from diabetes mellitus (Vannucci et al., 1994). These observations could be supported by the study in which GLUT3 expressed cells were found to overlap with DCX expressed

neuroblastic cells in the postnatal brain (Jung et al., 2016a). The down-regulation of GLUT3 accelerates neuronal damage (Fung et al., 2010) and decreases ischemic tolerance (Iwabuchi and Kawahara, 2011). Though these previous studies, we can regard the GLUT3 as an important component in modulating the hippocampal development and AN.

AN is impaired in obese and diabetic animal models (Alvarez et al., 2009; Suh et al., 2005). We used Zucker Diabetic Fatty (ZDF) rat and FDG PET imaging to evaluate the effect of T2DM on AN. ZDF is typical transgenic rat for T2DM model with the deletion of *fa* gene. ZDF rat has missense mutation in leptin receptor gene (Lepr). ZDF rat develops initial hyperinsulinaemia at around 8-10 weeks of age followed by insulin resistance and decreases insulin level at about 10-14 weeks that are not compensated for due to increased apoptosis in the pancreatic beta cells (King, 2012; Leonard et al., 2005). However, it is known that there is no change of IGF-1 level in ZDF rat regardless of insulin and glucose level (Schmidt et al., 2003). Development of T2DM is of spontaneous origin involving genetic factors and the animals develop characteristic features resembling human T2DM. Inbred animal models are genetically homogeneous. Therefore the variability of results is minimal and requires small sample size. However, mortality due to ketosis problem is high, so sophisticated maintenance is required (Srinivasan and Ramarao, 2007). At 6 weeks of age when the diabetic condition is not expressed yet, no difference of rCMRglu was noted between ZDF and ZLC rats. However, at 12 weeks

of age when the T2DM phenotype starts to express, the diabetic condition of ZDF rats showed reduced rCMRglu in hippocampus of ZDF rats compare to ZLC rats. Furthermore, there is no change of IGF-1 level even though the insulin resistance exists in ZDF rat. Therefore, the results of this study are representing only the effect of T2DM such as insulin resistance and hyperglycemia. IGF-1 and insulin have mitotic effect on NSCs. The diabetic condition decreases brain glucose utilization in genetically diabetic mice on the age-related manner proportional to the diabetic condition (Garris et al., 1984). In T2DM patients hippocampal atrophy is consistently reported even in elderly subjects with the prediabetes state (Convit et al., 2004, Gold et al., 2007). Furthermore, Hippocampal atrophy is correlated with cognitive impairment (Gold et al., 2007). The hippocampus is also susceptible to acute metabolic change such as hypoglycemia (Auer et al., 2004). However, considering our result, hippocampus is susceptible to change of glucose level and it is correlated with AD. It is suggestingT2DM is particularly correlated to diabetes-related metabolic change of hippocampus and AN in hippocampus.

Such impaired brain glucose utilization pattern is similarly observed in AD patients, and its mechanism can be explained as deficient glucose transporters, which function is controlled by insulin level (Piert et al., 1996; Piroli et al., 2007; Simpson et al., 1994). In addition, glucose administration improves the symptoms of AD by mediating insulin secretion (Craft, 2005). These facts show that insulin resistance is closely connected with the

elevated risk of AD. Notice that insulin resistance triggers lower expression and response of glucose transporter responsible for glucose utilization and neuronal activity (Baker et al., 2011). Furthermore, the amyloid protein and insulin are both metabolized by an insulin degradation enzyme in a competitive manner (Haan, 2006). There is also the finding that decreased glucose turnover leads to reduced ATP synthesis, eventually bringing about the generation of β-amyloid characteristic to AD. Acetylcoenzyme A, the key substrate of acetylcholine synthesis, is synthesized in the glycolytic pathway in the brain, which means a single molecule of glucose synthesizes two molecules of acetylcoenzyme A and 38 molecules of ATP. If ATP synthesis is lowered by a decreased glucose turnover, β-amyloid precursor protein (β-APP) cannot be built into the cellular membranes and the β-APP molecule cannot be split off because this process is ATP dependent (Meier-Ruge et al., 1994; Tuček, 1985). In our study, we focused on anterior hippocampus of brain, and found out the decrease of FDG uptake in that area of ZDF rats, an animal model of type II diabetes, which coincides with the results of previous studies in diabetic rats and AD.

There have been investigations to demonstrate the relationship between T2DM and AD by functioning imaging analysis. Insulin resistance is associated with significantly lower regional cerebral glucose metabolism and predicts worse memory performance. Among brain regions, the association between the left temporal lobe and insulin resistance is robust

(Willette et al., 2015). In functional MRI studies, T2DM patients showed reduced functional connectivity compared with control subjects, and insulin resistance of T2DM was inversely correlated with functional connectivity in the right inferior frontal gyrus and precuneus (Musen et al., 2012). Preclinically, the effect on the hippocampal neurogenesis is studied in the mice, rat and primates (Gould et al., 1999; Perera et al., 2011b). High fat diet and diabetes decreased the hippocampal neurogenesis in the animal model (Lindqvist et al., 2006). Furthermore, the decrease of neurogenesis results in the memory dysfunctions (Ho et al., 2013b). Most of the studies were done by histological study. Metabolic connectivity is of interest considering the alterations in glucose metabolism in the brain of neurodegenerative disease. Because of limited availability of FDG PET data, only few studies have demonstrated metabolic connectivity regarding this condition. In this study, we investigated the change of neurogenesis by immunohistochemical staining of neuroprogenitor cells and functional connectivity in diabetes type II animal model using FDG PET/CT.

After the comparison with FDG PET/CT data of ZDF rats and that of ZLC rats at 12 weeks of age, there come out apparently different patterns of functional network signal connected to hippocampus, showing increase of some functional connectivity and decrease of other functional connectivity. In fMRI stud, resting functional connectivity correlated with posterior cingulate cortex was decreased in T2DM patients (Musen et al., 2012).

Frontal and temporal cortices were mainly showed decreased functional connectivity with posterior cingulate cortex (Musen et al., 2012). In our results, the functional connectivity with hippocampus was decreased in 12 weeks old ZDF. Frontal, medial frontal, prefrontal and cingulate cortex were showed decreased functional connectivity with hippocampus. It is similar with the T2DM patients. In early study with magnetoencephalography was performed to detect the changes of functional connectivity in type I diabetic patients, and showed that increased functional connectivity was correlated to better cognitive performance including information-processing speed, attention, and executive functions (van Duinkerken et al., 2009). But, the magnetoencephalography has a limit of low sensitivity, a similar study using fMRI having better sensitivity was performed and revealed several negative findings of increased functional connectivity on sensorimotor network and secondary visual network in type I diabetic patients without microangiopathy (van Duinkerken et al., 2012). This result suggests that relationship between degree of diabetes represented as cognitive impairment and functional connectivity cannot be explained in a simple manner.

There are some possible explanations that the patterns of functional connectivity of hippocampus show an ambivalent feature in accordance with each certain functional network in ZDF rats. First, both excitatory and inhibitory functions of hippocampal neurons are influenced in diabetic rats by altered regulation of cholinergic and GABA receptor binding and gene

expression (Brundel et al., 2010). Which function is more impaired on hippocampal areas connected to each individual domain of brain can make different patterns of functional connectivity. Also, diabetes is associated with pronounced cortical atrophy in the temporal lobe (Aksu et al., 2012; Chen et al., 2012), induces apoptosis of neurons in prefrontal cortex in diabetic rats (Aye et al., 2012), and even accompanies white matter structural changes (Fabel et al., 2003). Those various brain areas forming an interrelationship with hippocampus cannot help showing normal neuronal activities in diabetic patients, so functional connectivity of hippocampus depends on the changes of whole brain activity. In addition, because a functional connectivity value we used was actually composed of sum of multiple values across various brain areas, our results cannot reflect multiple functional networks exactly. Therefore, we should be careful to interpret the meanings of the changes of functional connectivity of hippocampus in our results. The one definite thing was that there is a difference of functional connectivity patterns between in ZDF rats and in ZLC rats.

In type I DM rats, the altered neuronal structure, function and metabolism was accompanied by reduction of dentate gyrus cell proliferation (Jackson-Guilford et al., 2000; Li et al., 2002; Magarinos and McEwen, 2000; Saravia et al., 2001). Using a T2DM rat model, similar cellular change was reported by DCX immunochemistry in the hippocampus (Hwang et al., 2008). We observed the altered function of hippocampus of T2DM rats by PET and the

cellular changes in the same rats, as well. DCX expressing cells were reduced in ZDF rats ith diabetic condition compared to the ZLC rats. DCX protein is essential to neuronal migration, differentiation, and plasticity by regulating and stabilizing microtubule polymerization (Gleeson et al., 1999). In the study using postnatal rat brain, GLUT3 expression was mainly observed in the subgranular zone of the dentate gyrus, and these GLUT3 positive neurons were revealed as neuroblasts based on a double immunofluorescence study for GLUT3 and DCX (Jung et al., 2016b). These results may represent GLUT3 supplies glucose to the neuroblasts in the dentate gyrus, and the observation of the decreased hippocampal metabolism on F-18 FDG PET of T2DM rats is associated with decreased neurogenesis on this region. In addition of neurogenesis after birth, synaptogenesis has been known to rapidly increase in the cerebral cortex up to 25 days in the rat brains. Other molecular mechanisms also give an additional effect on the functional changes such as a decrease of glucose transporters (Cunnane et al., 2011; Ding et al., 2013), endothelial dysfunction, advanced glycation end products, or inflammation by insulin resistance (Craft, 2005).

Because advanced AD is a clinically irreversible condition, it is important to assure whether early signs of AD can be detected in diabetic patients so that the intervention for treatments can be accessed to prevent further progression. Microscopic cellular changes precede macroscopic structural deficits (Serrano-Pozo et al., 2011). Also, the structural deficits precede the

cognitive decline in AD. (Jack et al., 2010; Musen et al., 2012) Exercise has an effect of triggering secretion of brain-derived neurotrophic factor (BDNF), insulin-like growth factor-1 (IGF-1), vascular endothelial growth factor (VEGF) and other neurotransmitter (Trejo et al., 2001; Vaynman et al., 2004; Yi et al., 2009), playing an important role in a learning process and a memory function on hippocampus. (Ma, 2008) The treadmill exercise in ZDF rats reduced blood glucose levels and increased DCX proteins and DCX immunoreactive cells in hippocampus. (Stranahan et al., 2008) Also, impaired hippocampal plasticity and function in diabetic rats exhibiting high levels of corticosterone were reversed by normalizing the levels of corticosterone. Thus the early condition of diabetic patients is good to intervene to protect AD. The critical key for the early intervention is to find out the microscopic cellular changes in early stages. No ideal tools of identifying the microscopic cellular changes of hippocampus except biopsy, which is not able to applied to humans alive, has been existed. However our study has shown the association between the metabolic changes and the cellular changes of hippocampus, and it may provide a potential of functional studies using PET as an effective tool for making a decision of early intervention, as this research field evolves.

There are some caveats in this study. First, our results need to be replicated with a larger sample. Second, it turned out that increases/decreases of the metabolism in brain regions were not simply associated with the changes in the functional connectivity in those regions. Lastly, it is important that our

findings in animal models cannot be directly generalized to human patients with type 2 DM. For example, compared with continuously worsening human T2DM, ZDF rats developed severe hyperglycemia at an age of 13-14 weeks followed by stable state by increased glycogen stores in the skeletal muscle and liver (Szocs et al., 2008).

In summary, here we compared ZDF rats with ZLC rats and found out reduced FDG uptake and changes of functional connectivity in hippocampus in diabetic conditions. The disruption of functional changes of hippocampus is associated with the decline of neuroprogenitor cells in hippocampus. Although determining the exact pathophysiologic mechanism matching the functional changes to the cellular changes remains an unsolved problem now, a functional study with PET may reflect hippocampal cellular changes in early stages and play an important role in treating and preventing AD in diabetic patients.

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ABSTRACT IN KOREAN

당뇨는 다양한 신경퇴행성, 신경혈관성 질환의 대표적인 위험 인자이다. 많은 역학적, 임상적 연구들은 당뇨 환자들에서 인지 기능 저하가 발생할 가능성이 더 높다고 밝혀 왔다. 우리는 F-18 FDG PET과 쥐 모델을 이용하여 당뇨가 성인의 신경발달 (adult neurogenesis) 과 뇌 기능적 연결성 (functioning connectivity) 에 어떤 영향을 주는지 알아보았다. 제 2형 당뇨 모델인 Zucker Diabetic Fatty (ZDF) 수컷 쥐 (6주령 5마리, 12주령 5마리)를 사용하였고, 같은 수와 주령의 Zucker lean control (ZLC) 수컷 쥐를 대조군으로 사용하였다. 각각의 쥐 F-18 FDG PET 뇌영상을 얻어 국소적인 신경 활성화를 반영하는 국소 뇌 포도당 대사를 평가하였고, MRI 템플릿을 이용하여 영상을 정규화하고 SPM8을 이용하여 분석하였다. 58개의 뇌 국소 관심용적 (volume of interest)의 계수를 추출하여 기능적 연결성을 분석하였다. Doublecortin (DCX) 면역화학염색 (immunohistochemistry, IHC) 을 치아이랑 (dentate gyrus, DG)에 시행하여 신경발달을 평가하였다. GLUT3 면역화학염색을 시행하여 성인의 신경발달과 당 대사와의 관계를 알아보았다. 12주령의 ZDF 쥐들은 같은 주령의 ZLC 쥐 그룹과 비교하여 양측 해마에서 국소 당대사가 감소되어 있었다 ($p<0.05$). 6주령 그룹에서는 국소 당대사의 차이가 보이지 않았다. 기능적 연결성 분석에서는 6주령은 두 그룹간 차이가 없었으나, 12주령에서는 ZDF 쥐들이 대조군과 비교하여 해마에서 증가되거나 감소된 연결성의 변화를 보여주었다. 12주령의 ZDF 쥐에서 신경전구세포(neuroprogenitor cells)의 수는 ZLC 쥐와 비교하여 감소되어 있었으나 6주령에서는 차이가 없었다. 12주령의 ZDF 쥐들은 같은 주령의 ZLC 쥐와 비교하여 해마에서의 GLUT3 양성 세포의 수가 감소되어 있었고, 제2형 당뇨의 해마에서 당대사 감소는 감소된 GLUT3와 관련이 있고, 같은 부위의 DCX 양성 세포 수의 감소는 감소된 성인 뇌 신경발달을 의미하며, 이는 다른 뇌 부위와의 다양한 기능적 연결성의 변화를 반영하는 것이라 보여진다.

이 연구 결과는 당뇨 환자들에서 보이는 인지기능 저하의 병태생리적인 메커니즘을 이해하는데 도움이 될 것으로 보이며, 제2형 당뇨 쥐 모델에서 F-18 FDG PET을 이용하여 연결성을 포함한 기능적 변화와 병리적 변화를 연관 지은 최초의 연구이다. 당뇨 환자에서 해마의 조기 변화를 알아봄으로써 알츠하이머형 치매와 같은 퇴행성 뇌질환을 치료하고 예방하는데 도움이 될 것이다.

주요어 : 제 2형 당뇨, 신경 발달, 포도당 운반체 3, FDG-PET, 기능적 연결성

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