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A THESIS FOR THE DEGREE OF
MASTER OF SCIENCE IN FOOD AND NUTRITION

Effects of maternal high-fat diet on brain transcriptome of weaned offspring and high-fat diet fed-adult offspring

모체 고지방 식이 섭취가 이유기 자손 마우스와
고지방 식이를 섭취한 성체 자손 마우스의
뇌 전사체에 미치는 영향

August, 2017

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Abstract

Effects of maternal high-fat diet on brain transcriptome of weaned offspring and high-fat diet fed-adult offspring

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Maternal high-fat diet (HFD) consumption is known to affect the development of offspring, and its effect may persist until adulthood. However, most of the relevant studies have been conducted at one specific developmental stage of offspring. Therefore, this study investigated the effects of maternal HFD consumption on offspring brain on postnatal day (PD) 21, right after weaning, and PD105, when adult offspring were fed HFD for 12 weeks post-weaning. Five-week-old female C57BL/6 mice were provided with low-fat diet (LFD, 10% calories from fat) or high-fat diet (HFD, 45% calories from fat) before and during pregnancy, and during lactation period. Two of male offspring per dam were sacrificed on PD21, and the other two male offspring per dam were fed with HFD for 12 weeks until the sacrifice on PD105. Whole brain transcriptomes of offspring were analyzed using microarray. There were 25 differentially expressed genes (DEGs, Benjamini

P-value < 0.05) in the comparison between HFD21 and LFD21 (HFD21/LFD21) and 5 DEGs in HFD105/LFD105. Among 5 DEGs of HFD105/LFD105, 3 genes were involved in brain serotonin system (*Tph2*, *Slc6a4*) and showed significantly higher expressions in HFD105 compared to LFD105. However, levels of serotonin, its metabolite (5-hydroxyindoleacetic acid) and its precursor (tryptophan) in the whole brain were not different between LFD105 and HFD105. Also, mRNA level of *Cartpt*, a gene encoding neuropeptide, cocaine- and amphetamine-regulated transcript peptide, was higher in HFD105 compared to LFD105, which may be due to the activation of its transcription factor, cAMP response element binding protein by AMP-activated protein kinase. Such higher gene expressions of *Tph2*, *Slc6a4* and *Cartpt* in HFD105 compared to LFD105 were revealed to be affected by maternal oxidative stress indicated by serum level of thiobarbituric acid reactive substances. On the other hand, there were unique 377 DEGs in HFD105/HFD21, which were associated with immune response. Especially, upregulated genes were mostly involved in gene ontology terms indicating chemokine-mediated inflammation. However, protein levels of phosphorylated c-Jun N-terminal kinases, hyperphosphorylated tau and amyloid-β, which are the markers of neuropathogenesis, showed no differences by maternal diet in PD105 offspring. In conclusion, maternal HFD consumption induced upregulation of genes involved in brain serotonin system and neuropeptide in adult offspring which were fed HFD for 12 weeks, but not in weaned offspring. Also, as weaned offspring were fed HFD for 12

weeks, maternal HFD induced higher gene expressions of chemokine-mediated inflammation, which were not induced in offspring from LFD-fed dams.

Key words: maternal high-fat diet, mouse offspring, post-weaning obesogenic diet, brain, microarray, serotonin, neuropeptide, inflammation

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List of abbreviations

AMPK: AMP-activated protein kinase

BBB: Blood-brain barrier

CART: Cocaine- and amphetamine-regulated transcript

CNS: Central nervous system

CREB: cAMP response element binding protein

DEG: Differentially expressed gene

ERK: Extracellular signal-regu lated kinase

H2-Ab1: Histocompatibility 2, class II antigen A, beta 1

HFD: High-fat diet

5-HIAA: 5-Hydroxyindoleacetic acid

HSC70: 70-kDa heat shock cognate protein

5-HT: 5-Hydroxytryptamine

LFD: Low-fat diet

LPE: Local-pooled error

PCA: Perchloric acid

PD: Postnatal day

TBARS: Thiobarbituric acid reactive substances

TPH: Tryptophan hydroxylase

I. Introduction

1. Effects of maternal overnutrition on offspring brain

According to 'Developmental Origins of Health and Disease' hypothesis, intrauterine environment affects fetal development and the effects may persist until adulthood (Barker, 2007). Both maternal undernutrition and overnutrition may affect various mechanisms and lead to fetal intrauterine growth retardation (Chavatte-Palmer *et al.*, 2016). Maternal overnutrition has been reported to affect diverse metabolisms and susceptibility to diseases in offspring, such as poor fetal growth, liver dysfunction, behavior/emotional alteration (Williams *et al.*, 2014), learning and memory function perturbation (Penfold *et al.*, 2015), dysregulated neurodevelopment (Bale, 2015) and development of psychiatric disorders (Edlow, 2017). Also, maternal obesity is known to induce food intake increase or energy expenditure reduction in offspring, predisposing them to obesity (Rooney *et al.*, 2011).

Maternal overnutrition was induced by various diets in previous studies. Most recently, studies increasingly provide experimental animals with palatable diets which contain high sugar as well as high fat, or "junk food" diets which are similar to street snacks, as they are considered to reflect eating habits of modern society (Alfaradhi *et al.*, 2011). However, high-fat diet (HFD) which has been classically used for inducing overnutrition is still useful to induce maternal overnutrition and its effects on offspring brain has not been completely clarified yet.

Microarray is advantageous in figuring out the alteration of genome wide transcriptome. Several previous studies performed microarray to figure out the effects of maternal HFD consumption on offspring brain, but those studies investigated the effects on offspring brain only at one developmental stage. Maternal HFD showed sex-specific different effects in fetal forebrain (Edlow *et al.*, 2016), and increased appetite-regulating neuropeptide and inflammation in particular, in hypothalamus of offspring on postnatal day (PD) 21 (Rother *et al.*, 2012). The latter study focused on the hypothalamic inflammation which was associated with glucose intolerance. As simultaneous comparison of different developmental stages was not conducted in the previous studies, the effects of maternal HFD consumption on both PD21 and PD105 offspring were investigated in the current study.

2. Effects of high-fat diet consumption on brain

Like any other organs, brain is known to be affected by HFD consumption. Factors that can be affected by HFD in brain are diverse, including insulin resistance, oxidative stress, inflammation, vascular alterations, aging and so on, which can lead to cognition impairment (Freeman *et al.*, 2014) and perturbation of learning and memory function (Cordner *et al.*, 2015). HFD is also known to affect neurobehavior. HFD intake decreased hippocampal extracellular serotonin and it led to anxiety-like behavior accompanied by other metabolic disorders, which were reversed by withdrawal of HFD

(Zemdegs *et al.*, 2016). Juvenile HFD exposure, which didn't necessarily lead to obesity, induced behavioral and limbic alteration in mice (Vinuesa *et al.*, 2016). Also, it was figured out that diet-induced obesity caused alteration of brain reward circuitry and eventually depressive-like behavior in mice (Sharma *et al.*, 2013).

2.1. Brain serotonin system

Serotonin or 5-hydroxytryptamine (5-HT) is a monoamine neurotransmitter, derived from tryptophan. About 95% of serotonin in the body is synthesized and located in the gut, and some of serotonin is transferred to platelets in blood and carried to elsewhere it is needed (Gershon, 2013). Although serotonin itself cannot enter the central nervous system (CNS) from circulation because of blood-brain barrier (BBB) (Young, 2007), tryptophan can cross BBB via large amino acid transporter and be used in serotonin synthesis (O'Mahony *et al.*, 2015). Tryptophan is hydroxylated to 5-hydroxytryptophan by rate-limiting enzyme, tryptophan hydroxylase (TPH). There are two isoforms of TPH and the second isoform, TPH2 exclusively exists in CNS (Walther *et al.*, 2003). After hydroxylation, 5-hydroxytryptophan is decarboxylated by 5-hydroxytryptophan decarboxylase and becomes serotonin. Excess serotonin is metabolized to 5-hydroxyindoleacetic acid (5-HIAA) by monoamine oxidase, and the ratio of 5-HIAA to 5-HT is considered as a parameter of serotonin turnover.

Serotonin is synthesized in the raphe nuclei of brainstem neurons and this serotonergic raphe neurons project extensively throughout the CNS. Inhibition of serotonin synthesis was reported to induce hyperphagia, whereas central injection of serotonin or increasing synaptic serotonin levels by drug administration induced hypophagia, which indicates serotonin as a suppressant of food intake (Donovan *et al.*, 2013). Serotonin is also associated with mood regulation in that selective serotonin reuptake inhibitor is a representative antidepressant by elevating synaptic serotonin level (Jenkins *et al.*, 2016).

Several studies reported that HFD intake suppressed brain serotonin system. HFD-induced oxidative stress might cause degeneration of serotonergic neurons and decrease brain serotonin (Kurhe *et al.*, 2015). Also it was reported that brain serotonin and its metabolite, 5-HIAA were reduced by prolonged-HFD intake, suggesting the association of neuroinflammation (Krishna *et al.*, 2016). In diet-induced obesity mice which showed susceptibility to HFD consumption, binding densities of serotonin receptor 2A/2C were higher than diet-resistant mice, which were positively correlated with fat mass (Huang *et al.*, 2004). Also, diet-resistant mice showed reduced binding density of serotonin transporter, which indicated that serotonergic system seemed to contribute to the development of diet-induced obesity or diet-resistant phenotypes. It was also demonstrated that expression of serotonin receptor 1B was reduced by HFD feeding, which is involved in modulation of energy homeostasis and activity of orexigenic neurons

(Manousopoulou *et al.*, 2016).

2.2. Neuropeptides

Neuropeptides are small peptides which are involved in various physiological functions including reward, behavior and food intake modulation. Especially, appetite regulation is mediated in hypothalamus by various neuropeptides which transfer appetite-regulating signals. Neuropeptides such as agouti-gene related protein and neuropeptide Y are known as orexigenic, while pro-opiomelanocortin and cocaine- and amphetamine-regulated transcript (CART) peptide are generally known as anorectic neuropeptides (Harrold *et al.*, 2012; Parker *et al.*, 2012). CART peptide is also known as a regulator of stress control, reward, and pain transmission as well as food intake, and its neuroprotective role has emerged recently (Zhang *et al.*, 2012).

HFD feeding has been reported to induce various alterations of neuropeptides, which is not always consistent. In particular, regulation of *Cartpt* by HFD is controversial in previous studies, in which *Cartpt* was upregulated (Lee *et al.*, 2010), downregulated (Tian *et al.*, 2004), or differed by regions (Yu *et al.*, 2008).

On the other hand, it is widely known that maternal nutrition affects food intake regulation of offspring, especially predisposing offspring to hyperphagia, and eventually to obesity (Ross *et al.*, 2014). Maternal HFD consumption has been reported to induce alteration in expressions of

neuropeptide, but mostly neuropeptide Y, proopiomelanocortin and agouti-gene related protein. (Gupta *et al.*, 2009; Klein *et al.*, 2017; Muhlhausler *et al.*, 2006). However, despite the consistent transcriptional levels of CART peptide, it was reported that CART peptide gene expression showed positive correlation with plasma leptin level in offspring of control-diet fed dams, which disappeared by maternal overnutrition (Muhlhausler *et al.*, 2006). It was demonstrated that maternal overnutrition induced dysregulated development of appetite regulation by leptin resistance.

2.3. Immune responses in the central nervous system

BBB is a brain-specific structure which controls the migration of nutrients and toxic substances from peripheral circulation by its permeability. It also controls the migration of immune cells, which makes the brain immune-privileged organ (Shechter *et al.*, 2013). However, endothelial cells of BBB are sources of pro-inflammatory chemokines, which are involved in lymphocyte and monocyte recruitment (Daneman *et al.*, 2015). Infiltration of leukocyte across BBB is a multi-step process, including rolling, activation, arrest and transmigration (Takeshita *et al.*, 2012). Representative neuroinflammatory disease, multiple sclerosis, is known to be initiated by leukocyte infiltration and chemokines play an important role in recruiting leukocytes (Holman *et al.*, 2011). Also in Alzheimer's disease, microglia, immune cell of CNS, bind to amyloid-β, which activates microglia and

induces inflammatory response. In this response, pro-inflammatory cytokines and chemokines are released by microglia and these chemokines are also suggested to modulate migration of microglia to the inflammatory sites (Heneka *et al.*, 2015). HFD-induced obesity in mice induced recruitment of monocytes which were derived from bone marrow, different from CNS-resident microglia (Buckman *et al.*, 2014). Also, HFD consumption was demonstrated to modulate immunity of T helper 1, 2 and 17 lymphocytes, disrupting BBB permeability (Nerurkar *et al.*, 2011). In the mouse model of multiple sclerosis, immune cell infiltration was exacerbated by HFD consumption (Timmermans *et al.*, 2014).

3. Aim of this study

Aim of this study was to investigate the effects of maternal HFD consumption on brain transcriptome of male offspring. This investigation was performed at two different developmental stages. Offspring mice on PD21 and PD105 were for analysis of recently weaned offspring and adult offspring, respectively. PD105 offspring were also fed post-weaning HFD for 12 weeks so that it could be figured out whether maternal HFD consumption could affect the response of offspring in obesogenic environment.

II. Materials and methods

1. Animals and diets

Female C57BL/6 mice at 4 weeks of age were purchased from Orient Bio Inc. (Korea) and housed four mice per cage. After a week of acclimation, mice were randomly divided into low-fat diet (LFD) group and high-fat diet (HFD) group and provided with LFD (D12450B, Research diets Inc., USA; 70% calories from carbohydrates, 10% calories from fat, 20% calories from protein) and HFD (D12451, Research diets Inc.; 35% calories from carbohydrates, 45% calories from fat, 20% calories from protein) accordingly. After 3 weeks of diet consumption, female mice were allowed to mate with mature male mice of the same strain (2:1) for 5 days. Pregnancy was affirmed by vaginal plug. Pregnant mice were moved to individual cage and after delivery, litter size was adjusted to six. Each diet was maintained before and during pregnancy, and during lactation period. Two of male offspring mice per dam were sacrificed on PD21, right after weaning. The other two male offspring mice per dam were fed HFD same as maternal HFD (D12451, Research diets Inc.) for 12 weeks post-weaning, until the sacrifice on PD105. Therefore, there were 4 groups of offspring mice: PD21 and PD105 offspring from LFD-fed dams (LFD21, LFD105), and PD21 and PD105 offspring from HFD-fed dams (HFD21, HFD105). Overall study design is shown in Figure 1.

All mice were allowed with food and water *ad libitum*. Animals were maintained in temperature (23 ± 2℃) and humidity (55 ± 5%)-controlled

room with 12 h dark-light cycle. All animal experiments were permitted by Institute of Laboratory Animal Resources of Seoul National University (Permission number: SNU-140807-1-3), and performed in accordance with the guidelines of Institutional Animal Care and Use Committee (IACUC) of Seoul National University. After overnight fasting (14 h), mice were sacrificed by cardiac puncture after intraperitoneal injection of 30 mg/kg Zoletil (Virbac, France) and 10 mg/kg xylazine (Rompun; Bayer Korea, Korea). Blood was collected by cardiac puncture and centrifuged at 3,000 rpm, 4℃ for 20 min to obtain serum. Tissues were removed, quickly frozen in liquid nitrogen and stored in -70℃ deep freezer until analysis.

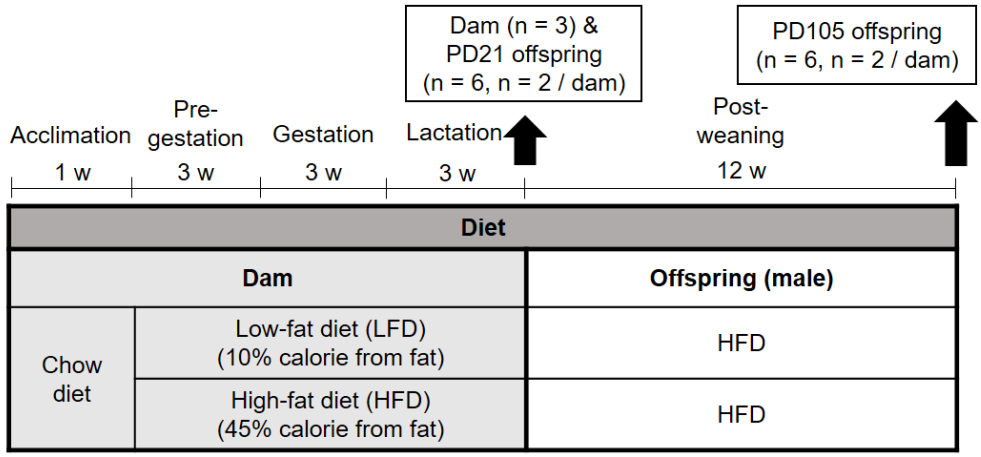


Figure 1. Study design

2. Microarray hybridization

Equal amounts of whole brain samples from two offspring of each dam were pooled for total RNA isolation. Total RNA was isolated using RNAiso Plus (Takara, Japan). RNA purity and integrity were evaluated by ND-1000 Spectrophotometer (NanoDrop, USA) and Agilent 2100 Bioanalyzer (Agilent Technologies, USA). RNA labeling and hybridization were performed by using Agilent One-Color Microarray-Based Gene Expression Analysis protocol (Agilent Technologies, V 6.5, 2010). Briefly, 100 ng of total RNA from each pooled sample was linearly amplified and labeled with Cy3-dCTP. The labeled cRNAs were purified by RNAeasy Mini Kit (Qiagen, USA). The concentration and specific activity of the labeled cRNAs (pmol Cy3/μg cRNA) were measured by ND-1000 spectrophotometer. Six hundred nanograms of each labeled cRNA was fragmented by adding 5 μl 10 × blocking agent and 1 μl of 25 × fragmentation buffer, and then heated at 60℃ for 30 min. Finally, 25 μl 2 × GE hybridization buffer was added to dilute the labeled cRNA. Forty microliters of hybridization solution was dispensed into the gasket slide and assembled to Agilent SurePrint G3 Mouse GE 8×60K, V2 Microarrays (Agilent Technologies). The slides were incubated for 17 h at 65℃ in Agilent hybridization oven then washed at room temperature by using Agilent One-Color Microarray-Based Gene Expression Analysis protocol. The hybridized array was immediately scanned with Agilent Microarray Scanner D (Agilent Technologies). Raw data were extracted using Agilent Feature Extraction

Software (v11.0.1.1).

3. Microarray data analysis

gProcessedSignal value was transformed by logarithm and normalized by quantile method. Statistical significance of the expression data was determined using local-pooled error (LPE) test in which the hypothesis was that no difference exists among 2 groups. Differentially expressed genes (DEGs) were selected according to the criteria of |fold change| ≥ 1.5 and Benjamini-Hochberg adjusted *P*-value < 0.05. Groups were compared in four sets, comparison between HFD21 and LFD21 (HFD21/LFD21), HFD105/LFD105, LFD105/LFD21 and HFD105/HFD21 (Figure 2). In particular, comparison of LFD105/LFD21 and HFD105/HFD21 were for functional analysis of difference sets, which indicate unique genes exclusively altered in each group. Especially, unique genes of HFD105/HFD21 indicate particular alteration by maternal HFD, which was not induced by maternal LFD. Hierarchical cluster analysis was performed using complete linkage and Euclidean distance as a measure of similarity. Functional analysis of DEGs was performed using the functional annotation and clustering tool of the Database for Annotation, Visualization and Integrated Discovery (DAVID) v 6.8, based on gene ontology (GO) term and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway. All data analysis and visualization of DEGs were conducted using R 3.3.1 (www.r-project.org).

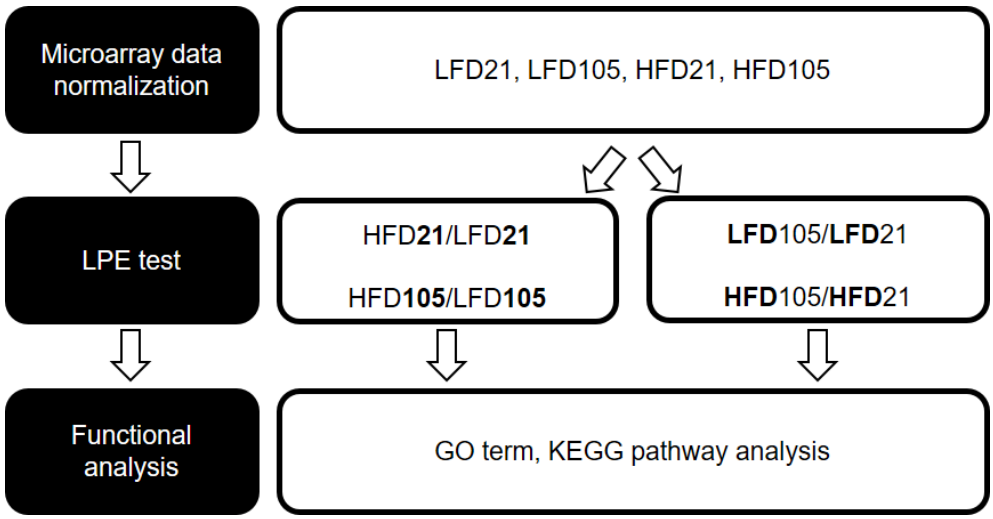


Figure 2. Flow chart of microarray data analysis

4. Quantitative reverse transcriptase polymerase chain reaction analysis

cDNA was synthesized using 2 μg of total RNA with Superscript II Reverse Transcriptase (Invitrogen, USA). mRNA expression levels were analyzed by quantitative RT-PCR using StepOne™ Real Time PCR System (Applied Biosystems, USA) using SYBR® Green PCR Master Mix (Applied Biosystems) according to supplier's protocol. Mouse beta-actin was used as reference gene and relative gene expression levels were analyzed using $2^{-\Delta\Delta Ct}$ method. Primer sequences are described in Table 1.

Table 1. Quantitative real-time PCR primer sequences

Gene		Sequence (5′-3′)
Actb	Forward	TGACCCAGATCATGTTTGAGACC
	Reverse	CCATACCCAAGAAGGAAGGC
Cartpt	Forward	CCCGAGCCCTGGACATCTA
	Reverse	GCTTCGATCTGCAACATAGCG
Ccl5	Forward	TGCCCACGTCAAGGAGTATTTC
	Reverse	AACCCACTTCTTCTCTGGGTTG
Cxcl5	Forward	CAGAAGGAGGTCTGTCTGGA
	Reverse	GTGCATTCCGCTTAGCTTTC
H2-Ab1	Forward	GTGGTGCTGATGGTGCTG
	Reverse	CCATGAACTGGTACACGAAATG
Ppbp	Forward	CTGCCCACTTCATAACCTCCA
	Reverse	GTCCATGCCATCAGATTTTCC
Slc6a4	Forward	ACCTGGACACTCCATTCCAC
	Reverse	CCTGGAGTCCCTTTGACTGA
Tph2	Forward	GTGGCTACAGGGAAGACAAC
	Reverse	AAGTCTCTTGGGCTCAGGTA

5. Total protein extraction and immunoblotting

Equal amounts of whole brain samples from two offspring of each dam were pooled for total protein extraction. Brain tissues were homogenized in 10-fold volume of ice-cold protein lysis buffer [50 mmol/L Hepes-KOH (pH 7.5), 150 mmol/L NaCl, 1 mmol/L EDTA (pH 8.0), 2.5 mmol/L EGTA (pH 8.0), 1 mmol/L NaF, 10 mmol/L beta–glycerophosphate, 0.1 mmol/L Na_3VO_4, 1 mmol/L DTT, 0.1% Tween-20, 10% glycerol, protease inhibitor cocktail (Sigma, USA)] using Tissue Lyser system (Qiagen) with 5 mm sterile stainless steel beads. After centrifugation at 10,000 × g, 4℃ for 30 min, supernatant was transferred to new tube and kept in -70℃ deep freezer until analysis. Protein content was measured using protein assay kit (Bio-rad, USA). Equal amounts of protein were loaded into the lanes of polyacrylamide gel, separated by Tris-glycine running buffer system and then transferred to polyvinylidene fluoride membrane using semi-dry electrotransferring unit (Bio-rad) at 15 V for 60 min. Membranes were blocked with 5% nonfat milk or bovine serum albumin in Tris-buffered saline solution containing 0.1% Tween-20 (pH 7.5, TTBS), and then probed with specific antibodies diluted in 5% nonfat milk or bovine serum albumin in TTBS. The specific antibodies used are as follows : anti-cAMP response element binding protein (CREB; #9197, Cell signaling, USA), anti-p-CREB (#9196, Cell signaling), anti-extracellular signal-regulated kinase (ERK; #9102, Cell signaling), anti-p-ERK (#9101, Cell signaling), anti-AMP-activated protein kinase (AMPK;

#2532, Cell signaling), anti-p-AMPK (#2531, Cell signaling), anti-c-Jun N-terminal kinase (JNK; #9252, Cell signaling), anti-p-JNK (#9251, Cell signaling), anti-amyloid-β (sc-28365, Santa Cruz Biotechnology, USA), anti-dephosphorylated tau (Tau-1, MAB3420, Millipore, USA), anti-total tau (Tau-5, AHB0042, Invitrogen), anti-70-kDa heat shock cognate protein (HSC70; sc-7298, Santa Cruz Biotechnology). HSC70 was used as control protein. Afterwards, membranes were incubated with secondary antibody, horseradish peroxidase-conjugated anti-mouse or anti-rabbit IgGs. Immunoreactive protein bands were visualized using Immobilon western chemiluminescent using HRP substrate (Millipore) by exposure to X-ray film (Fuji, Japan). Band intensities were quantified using Quantity One software (Bio-rad).

6. Measurements of brain serotonin, 5-HIAA and tryptophan levels using high-performance liquid chromatography

Brain levels of serotonin, 5-HIAA, a metabolite of serotonin, and tryptophan, a precursor of serotonin, were measured as described previously (Yamada *et al.*, 1983) with some modifications. Protein lysate was precipitated by adding perchloric acid (PCA; Sigma) containing ascorbic acid (Sigma). Final concentrations of PCA and ascorbic acid were 0.1 mol/L and 0.02% respectively. One microgram per liter of N-acetyl tryptophan (Sigma) was added as internal standard. After centrifugation at 15,000 × g, 4℃ for 10 min,

supernatant was transferred to new tube. The residues were resuspended with 0.1 mol/L PCA, and centrifuged again. Supernatants were mixed and filtered using 0.45 μm filter (Millipore). Ten microliters of the filtered supernatant was injected into Inno C-18 column (4.6 mm x 250 mm, 5 μm, Youngjin Biochrom, Korea) and analyzed using high-performance liquid chromatograpy (HPLC) system (Dionex Ultimate 3000, Thermo Fisher Scientific, USA) and fluorescence detector (Agilent 1270 FL detector, Agilent) at excitation wavelength 280 nm and emission wavelength 350 nm. Standards of serotonin (Sigma), 5-HIAA (Sigma) and tryptophan (Sigma) were dissolved in distilled water at concentration of 1 mg/L and then diluted to various concentrations used for HPLC analysis. Mobile phase was composed of 0.01 mol/L ammonium acetate with 30% methanol. Flow rate was 1.2 mL/min and column temperature was 40℃. At these conditions, retention time of serotonin was 3.31 min, 5-HIAA was 4.55 min and tryptophan was 5.11 min.

7. Statistical analysis

All data except microarray data were analyzed using SPSS software (v 23, SPSS Inc., USA). Student's *t*-test was employed to assess statistical significance. Data were expressed as mean ± SEM and differences were considered statistically significant at P-value < 0.05. Correlations between two variables were determined by Pearson's correlation coefficient.

Microarray data were analyzed using R 3.3.1 (www.r-project.org) as mentioned in 3. Microarray data analysis.

III. Results

1. Effects of maternal high-fat diet consumption on body weight and brain weight of offspring

Final body weight and brain weight of PD21 and PD105 male offspring were measured (Figure 3). There was no significant difference by maternal HFD in body weight and brain weight of both PD21 and PD105 offspring. Despite the researches which showed increased body weight by maternal HFD in offspring (Fante *et al.*, 2016; Mendes-da-Silva *et al.*, 2015; White *et al.*, 2009), prenatal obesogenic diet did not always induce body weight increase in offspring (Blackmore *et al.*, 2014; Murabayashi *et al.*, 2013; Niculescu *et al.*, 2009). Also brain weight of offspring from HFD-fed dams remained comparable to that of offspring from LFD-fed dams which was also demonstrated by previous studies (Dudley *et al.*, 2011; Mendes-da-Silva *et al.*, 2015).

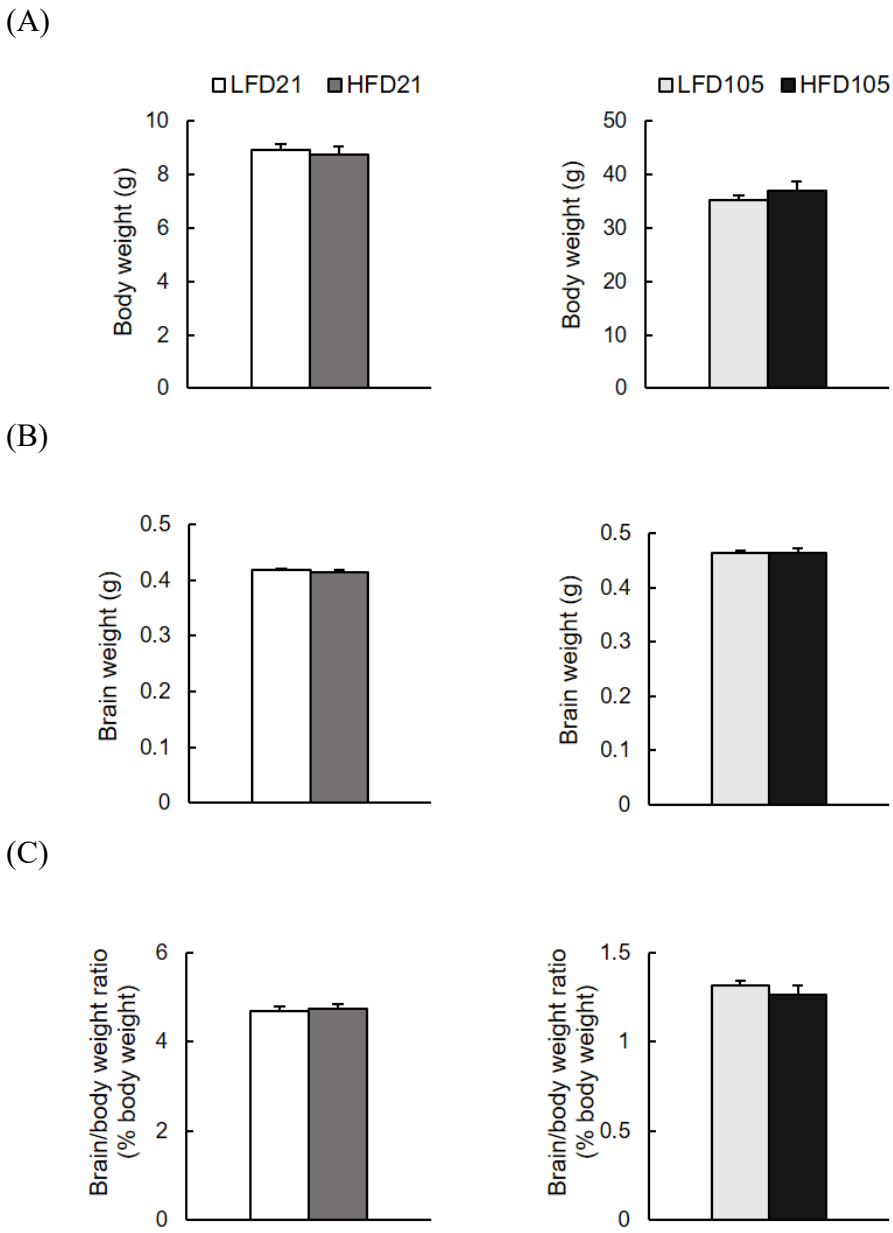


Figure 3. Effects of maternal high-fat diet consumption on body weight and brain weight of offspring

(A) Body weight, (B) brain weight and (C) brain/body weight ratio of PD21 and PD105 offspring from LFD- and HFD-fed dams. Data are presented as mean ± SEM (n = 6).

2. Effects of maternal high-fat diet consumption on the gene expression profile of offspring brain

The heat map shows the overall expression pattern of DEGs of the brain transcriptome in PD21 and PD105 offspring (Figure 4). Genes meeting the criteria of |fold change| ≥ 1.5 and Benjamini *P*-value < 0.05 were selected as DEGs. It could be figured out that the effects of post-weaning HFD consumption were greater than the effects of maternal diets.

Numbers of DEGs are shown as Venn diagrams (Figure 5). There were 25 DEGs in HFD21/LFD21 and 5 DEGs in HFD105/LFD105, and there was no common gene among them (Figure 5A). It shows the different effects of maternal HFD on PD21 offspring and PD105 offspring fed HFD.

There were 935 in LFD105/LFD21 and 932 DEGs in HFD105/HFD21, and there were 555 common DEGs (Figure 5B), which indicates 380 and 377 DEGs were exclusively altered in LFD105/LFD21 and HFD105/HFD21, respectively. Common DEGs of LFD105/LFD21 and HFD105/HFD21 showed alteration in the same direction, upregulation or downregulation in both LFD105/LFD21 and HFD105/HFD21. Especially among the unique DEGs of HFD105/HFD21, which were of our interest, there were 227 upregulated genes and 150 downregulated genes.

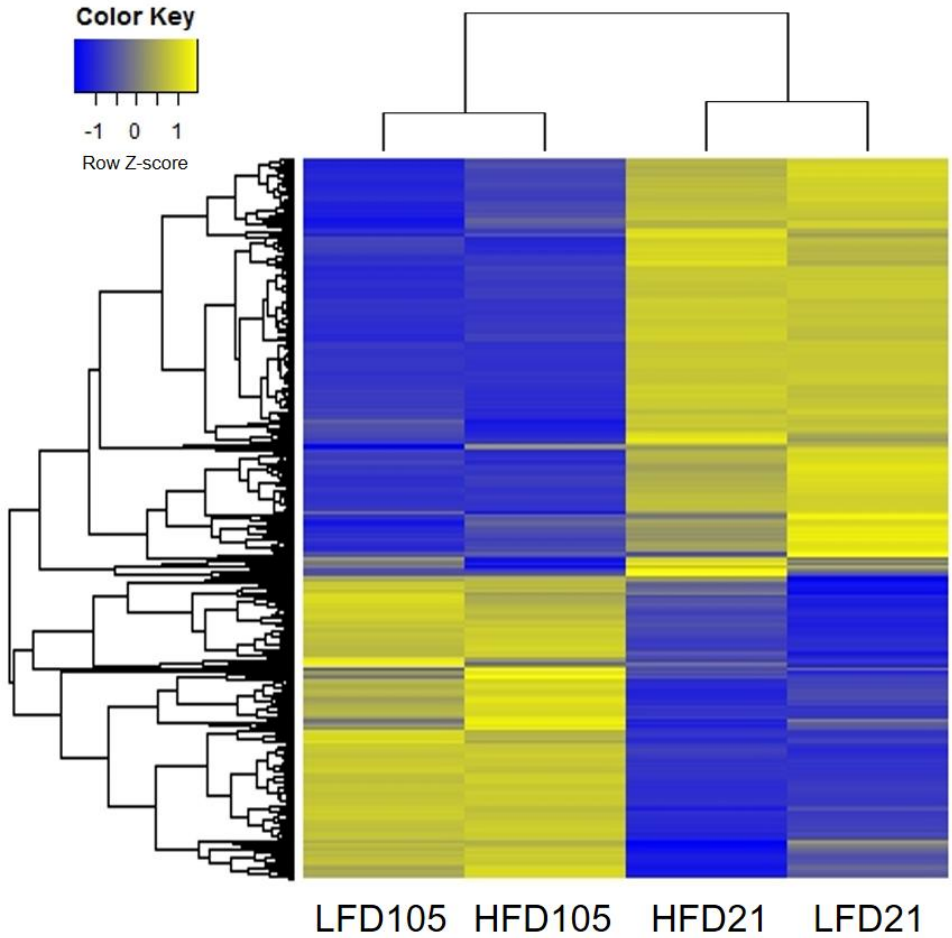
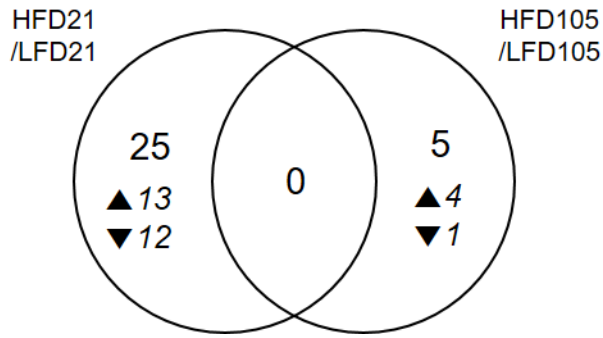


Figure 4. Effects of maternal high-fat diet consumption on gene expression profile of offspring brain

Two-dimensional hierarchical clustering of gene expression profile. Each cell represents the average expression level of differentially expressed genes (DEGs), identified by local-pooled error (LPE test, |fold change| ≥ 1.5, Benjamini *P*-value < 0.05).

(A)



(B)

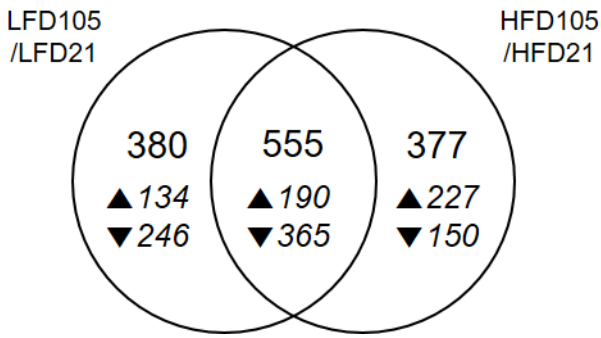


Figure 5. Venn diagrams of the numbers of differentially expressed genes

Numbers of differentially expressed genes (DEGs) in (A) HFD21/LFD21 and HFD105/LFD105, and (B) LFD105/LFD21 and HFD105/HFD21. Numbers of upregulated and downregulated DEGs are also indicated.

3. Comparison of brain transcriptome in HFD21/LFD21 and HFD105/LFD105

DEGs of HFD21/LFD21 and HFD105/LFD105 are presented in Table 2. (Gene names of each gene symbol are indicated in Appendix 1 and 2.) Functional analysis of those DEGs showed no significant GO term and KEGG pathway in HFD21/LFD21. On the other hand, out of 5 DEGs in HFD105/LFD105, there were 3 genes (*Gm5741*, *Tph2*, *Slc6a4*) involved in brain serotonin system, which were also indicated by KEGG pathway ('Serotonergic synapse': Benjamini *P*-value 1.9E-2, Fold enrichment 43.9).

Also among 5 DEGs of HFD105/LFD105, there was *Cartpt* which is a gene encoding neuropeptide CART, known to be involved in various physiological process including appetite regulation (Lau *et al.*, 2014) and associated with leptin signaling (Hill, 2010).

Table 2. Differentially expressed genes of HFD21/LFD21 and HFD105/LFD105

HFD21/LFD21			HFD105/LFD105		
Gene symbol	**Fold change**	**Benjamini *P*-value**	**Gene symbol**	**Fold change**	**Benjamini *P*-value**
Upregulated			**Upregulated**		
1700120B22Rik	6.99	0.014	*Slc6a4*	1.87	<0.001
Trim69	6.58	<0.001	*Tph2*	1.86	<0.001
Gm8298	6.39	0.019	*Gm5741*	1.78	<0.001
Gm36584	3.98	0.047	*Cartpt*	1.58	0.045
LOC105247294	2.33	<0.001			
Camk1d	1.74	0.026			
Pdzd2	1.66	<0.001			
Pisd-ps3	1.64	<0.001			
Alas2	1.63	0.006			
B430316J06Rik	1.62	0.002			
Edil3	1.61	0.042			
Car10	1.53	<0.001			
Cdh18	1.52	0.006			
Downregulated			**Downregulated**		
Cdhr1	-1.53	0.005	*Col11a2*	-1.72	<0.001
Ms4a15	-1.55	0.001			
Xlr3b	-1.55	<0.001			
Nppa	-1.57	0.009			
Ccl17	-1.62	0.045			
Xlr4b	-1.70	<0.001			
1500004A13Rik	-1.73	<0.001			
Mtor	-1.78	0.001			

(Continued on next page)

HFD21/LFD21			HFD105/LFD105		
Gene symbol	**Fold change**	**Benjamini *P*-value**	**Gene symbol**	**Fold change**	**Benjamini *P*-value**
Downregulated			**Downregulated**		
Omp	-2.06	<0.001			
S100a5	-2.68	<0.001			
Gm20098	-6.75	0.011			
Adam26a	-7.65	0.009			

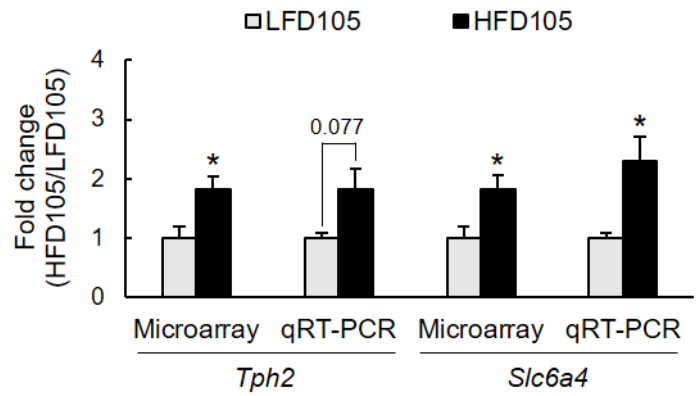
Differentially expressed genes are indicated in descending order of fold change.

3.1. Effects of maternal high-fat diet consumption on brain serotonin system of offspring brain

As *Tph2* and *Slc6a4* were identified to encode serotonin synthesizing enzyme, TPH2 and serotonin transporter respectively, their mRNA levels were validated using qRT-PCR (Figure 6A). The results of qRT-PCR were similar to microarray data, in which gene expressions of *Tph2* and *Slc6a4* were higher in HFD105 compared to LFD105. *Tph2* was reported to be upregulated by maternal HFD (Sullivan *et al.*, 2010) or prenatal restraint stress (Miyagawa *et al.*, 2011). Also protein and mRNA levels of serotonin transporter were upregulated by prenatal auditory stress in offspring mice (Bielas *et al.*, 2014).

As transcriptional level of serotonin synthesizing enzyme was higher in HFD105 compared to LFD105, levels of serotonin, its metabolite (5-HIAA) and its precursor (tryptophan) were measured in whole brain using HPLC (Figure 6B). The results showed no significant difference in the levels of serotonin, 5-HIAA and tryptophan between LFD105 and HFD105.

(A)



(B)

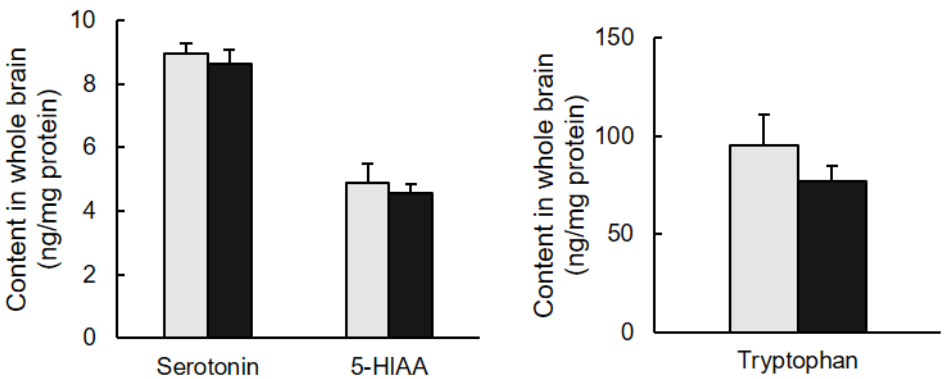


Figure 6. Effects of maternal high-fat diet consumption on serotonin metabolism in the brain of PD105 offspring

(A) Microarray data validation of *Tph2* and *Slc6a4* gene expression levels using qRT-PCR. (B) Brain levels of serotonin, 5-HIAA and tryptophan. Data are presented as mean ± SEM [(A) n = 3, (B) n = 6]. * $P < 0.05$ compared with PD105 offspring of LFD-fed dams (Student's *t*-test).

3.2. Effects of maternal high-fat diet consumption on neuropeptide expression of offspring brain

Microarray data of *Cartpt* in HFD105/LFD105 were validated by qRT-PCR (Figure 7A) and the results were revealed to be similar to microarray data. However, although CART peptide is known as anorectic peptide (Harrold *et al.*, 2012), there was no significant difference in food intake between LFD105 and HFD105 (Figure 7B).

CREB is a transcription factor which can modulate transcriptional level of *Cartpt* (Rogge *et al.*, 2009). Therefore, protein levels of total and phosphorylated CREB were determined by immunoblotting. Despite the consistent expression of total CREB, protein levels of p-CREB were higher in PD105 by maternal HFD (Figure 8A). So it could be demonstrated that transcriptional levels of *Cartpt* were modulated by upregulated phosphorylation of CREB. Higher level of phosphorylated CREB was also reported in another study using male C57BL/6 mice fed HFD, which was related to depressive behavior (Sharma *et al.*, 2013).

Protein levels of ERK and AMPK were measured, as possible kinases of CREB (Alberini, 2009; Huang *et al.*, 2015) (Figure 8B). Although there was no difference in phosphorylation of ERK, p-AMPK level was higher in PD105 by maternal HFD, same as CREB. It indicates that CREB was phosphorylated by AMPK, not ERK in the current study.

(A) (B)

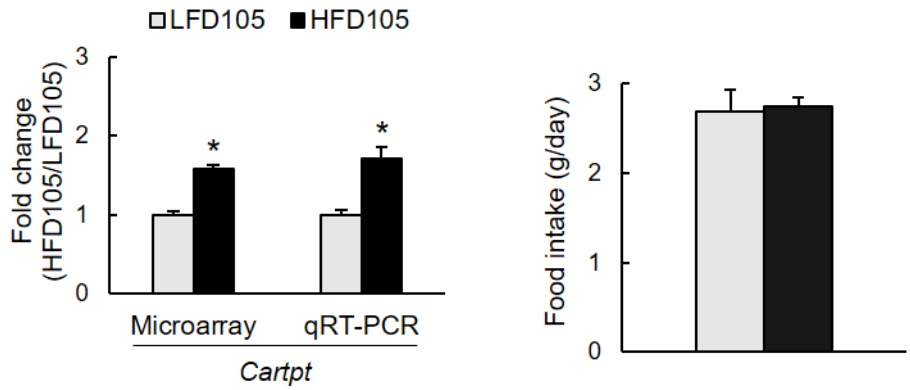
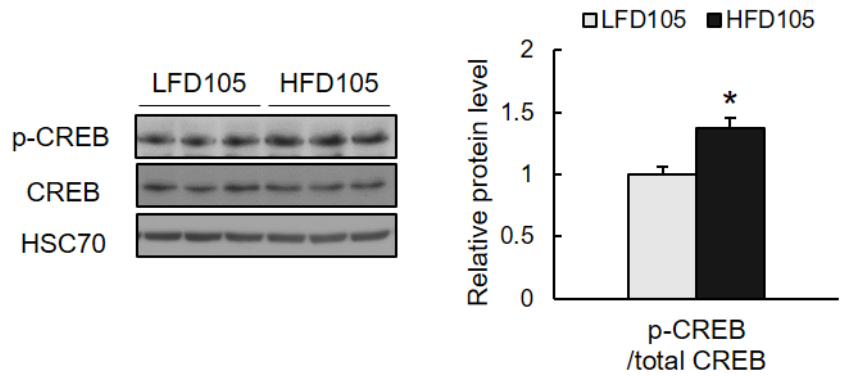


Figure 7. Effects of maternal high-fat diet consumption on expression of *Cartpt* in the brain and food intake of PD105 offspring

(A) Microarray data validation of *Cartpt* gene expression levels using qRT-PCR. (B) Food intake (g/day) of PD105 offspring. Data are presented as mean ± SEM (n = 3). * $P < 0.05$ compared with PD105 offspring of LFD-fed dams (Student's *t*-test).

(A)



(B)

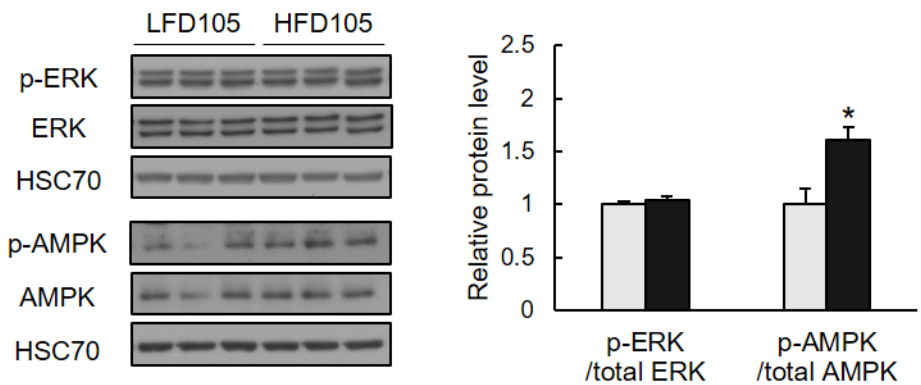


Figure 8. Effects of maternal high-fat diet consumption on regulation of *Cartpt* in the brain of PD105 offspring

Protein levels of (A) CREB and (B) ERK and AMPK determined by immunoblotting. Data are presented as mean ± SEM (n = 3). * $P < 0.05$ compared with PD105 offspring of LFD-fed dams (Student's *t*-test).

3.3. Correlations between parameters of dams and gene expressions of offspring brain

In the previous study, it was reported that maternal liver growth perturbation and oxidative stress by HFD consumption affected cholesterol metabolism in offspring (Kim *et al.*, 2016). Likewise, there were correlations between relevant parameters of dams and gene expressions of *Tph2*, *Slc6a4* and *Cartpt* of PD105 offspring brain (Appendix 3). Especially, gene expressions of *Tph2*, *Slc6a4* and *Cartpt* in the brain of PD105 offspring were positively correlated with serum TBARS level of dams (Figure 9), which indicates the effects of oxidative stress in dams induced by HFD consumption may have affected alteration of gene expressions in the brain of adult offspring when fed HFD. On the other hand, these correlations did not appear in PD21 offspring brain, which indicates that PD105 offspring brains which were challenged with HFD intake were more susceptible to the effects of maternal HFD consumption. Body weight, organ weights and litter size of dams are indicated in Appendix 4.

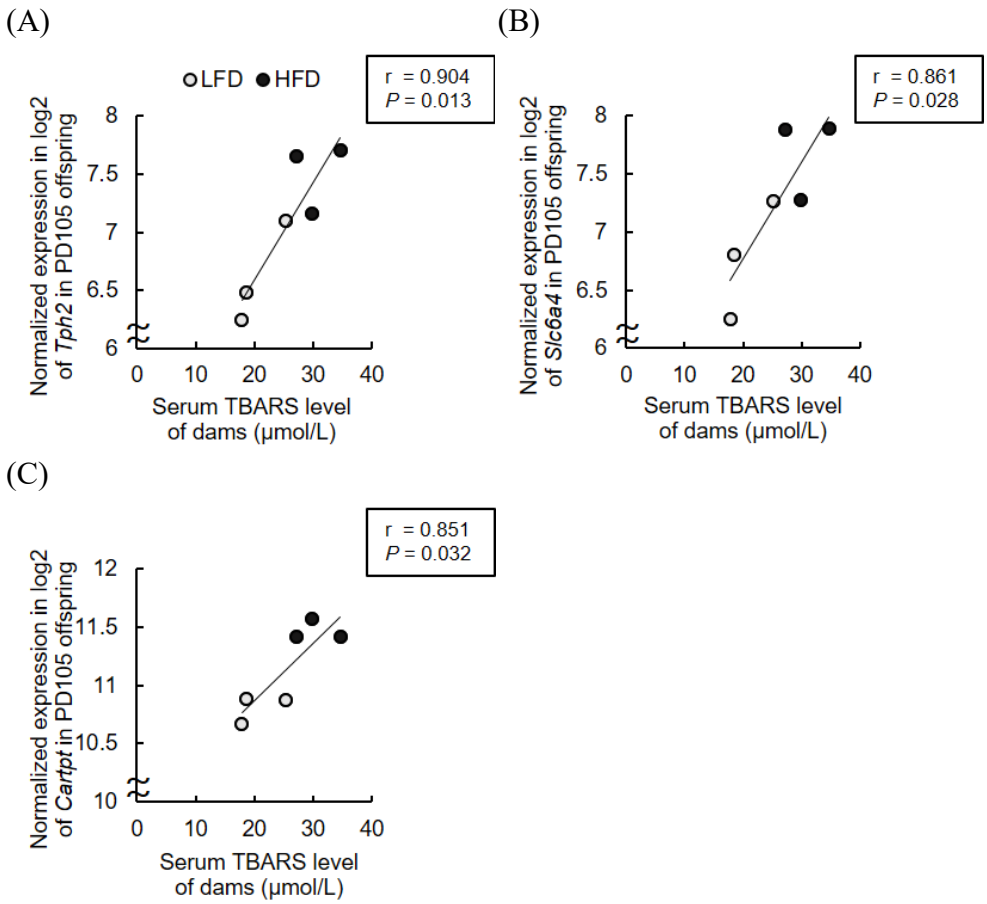


Figure 9. Correlations of serum TBARS level of dams and gene expressions in the brain of PD105 offspring

Pearson's correlation between serum TBARS level of dams and (A) *Tph2*, (B) *Slc6a4* and (C) *Cartpt* gene expressions in the brain of PD105 offspring. Pearson's correlation coefficient, r and *P*-value are indicated for each region (n = 3).

4. Comparison of brain transcriptome in LFD105/LFD21 and HFD105/HFD21

LFD105/LFD21 and HFD105/HFD21 are functionally analyzed based on GO term (Table 3). According to the results, there was upregulation in immune system exclusively in HFD105/HFD21. On the other hand, there was no GO term which was altered significantly, and exclusively in LFD105/LFD21.

To figure out the unique genes which were most affected by maternal HFD, top 20 genes were selected among the upregulated or downregulated genes (Table 4, gene names of each gene symbol are indicated in Appendix 5.), and analyzed based on GO term (Table 5). According to the functional analysis, it was figured out that 3 GO terms, 'Chemokine activity', 'Chemokine-mediated signaling pathway', and 'Immune response' were significant. Therefore 4 genes involved in 3 GO terms, *Ccl5*, *Cxcl5*, *Ppbp* and *H2-Ab1* were validated by qRT-PCR (Figure 10A). Microarray data of validated genes are shown in Appendix 6. Interestingly, gene expressions of *Tph2* and *Slc6a4*, which were higher in HFD105 compared to LFD105, were lower in LFD105 compared to LFD21.

As inflammatory response especially associated with chemokine was higher due to maternal HFD, levels of protein markers were measured to determine whether this led to neuropathogenesis. JNKs are kinases which are activated by diverse inflammatory signals and cellular stresses, and indicated to be involved in pathogenesis of Alzheimer's disease (Zhang *et al.*, 2015). Therefore, phosphorylation of JNK was measured by immunoblotting (Figure

10B), and there was no significant difference among PD105 offspring groups.

Neuroinflammation is observed in the earliest stages of Alzheimer's disease, and it is suggested to be a critical factor of tau pathology (Metcalfe *et al.*, 2010) and amyloid-β pathology (Minter *et al.*, 2016), which are the markers of Alzheimer's disease. Therefore, protein levels of amyloid-β and tau hyperphosphorylation were measured, which were not significantly different between LFD105 and HFD105 (Figure 10C).

Table 3. GO term-based functional analysis of unique differentially expressed genes in HFD105/HFD21

	Term	Benjamini *P*-value	Fold enrichment
GOTERM _MF_DIRECT	GO:0042605~ Peptide antigen binding	<0.001	12.97
GOTERM _BP_DIRECT	GO:0002376~ Immune system process	0.004	3.54
	GO:0002474~ Antigen processing and presentation of peptide antigen via MHC class I	0.013	12.51
	GO:0006955~ Immune response	0.046	3.55

GO terms are indicated in ascending order of Benjamini *P*-value.

Table 4. Top 20 genes among the upregulated and downregulated unique genes in HFD105/HFD21

Gene symbol	Fold change	Benjamini *P*-value	Gene symbol	Fold change	Benjamini *P*-value
Upregulated			**Downregulated**		
Gm11497	6.53	0.000	*Trim69*	-9.26	<0.001
Loc102639603	5.90	0.012	*Ahsp*	-6.86	0.004
5033421B08Rik	5.48	0.012	*Ccdc154*	-6.78	0.014
Rpe65	5.03	0.013	*Bsph2*	-6.55	0.002
Stoml3	4.57	0.007	*1700120B22Rik*	-6.54	0.001
Cxcl5	4.49	0.008	*Rab7b*	-6.50	0.027
Irgm1	4.30	0.030	*Ssxb1*	-6.32	0.018
Pkd2l1	4.09	0.045	*Muc1*	-5.71	0.008
Wfdc17	4.08	0.042	*Gm8298*	-5.42	0.001
Hephl1	3.91	0.032	*Robo2*	-5.36	0.007
Fam81b	3.87	0.049	*Gm13748*	-5.01	0.009
Gm34360	3.68	0.050	*Matn1*	-4.84	0.049
Ccl5	3.31	0.001	*Prnd*	-4.78	0.011
Ppbp	3.02	0.035	*Loc545086*	-4.74	0.040
H2-Ab1	2.98	0.009	*Slco1a1*	-4.71	0.003
6430553K19Rik	2.90	0.003	*Gm36584*	-4.47	0.002
Ceacam18	2.63	0.000	*Olfr1295*	-4.04	0.024
Plbd1	2.62	0.039	*Gm32568*	-3.93	0.038
Gm10378	2.58	0.018	*Gm32633*	-3.49	0.004
Kcnk15	2.52	<0.001	*Gmnn*	-3.18	0.040

Genes are indicated in descending order of |fold change|.

Table 5. GO term-based functional analysis of top 20 upregulated unique genes in HFD105/HFD21

Term		Benjamini *P*-value	Fold enrichment	Genes
GOTERM _MF_DIRECT	GO:0008009~ Chemokine activity	0.021	90.86	*Ppbp, Cxcl5, Ccl5*
GOTERM _BP_DIRECT	GO:0070098~ Chemokine-mediated signaling pathway	0.027	89.66	*Ppbp, Cxcl5, Ccl5*
	GO:0006955~ Immune response	0.049	24.17	*Ppbp, Cxcl5, H2-Ab1, Ccl5*

GO terms are indicated in ascending order of Benjamini *P*-value.

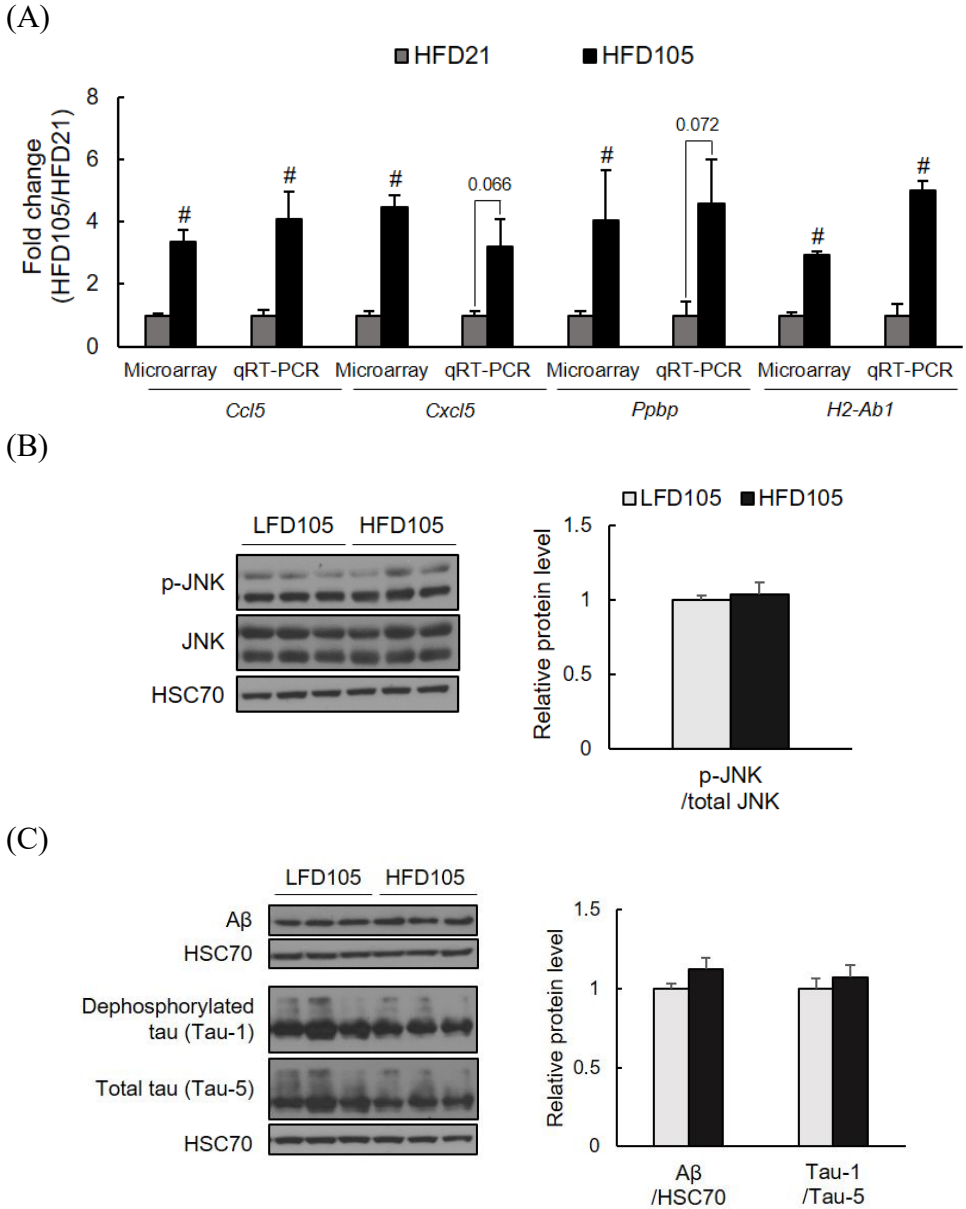


Figure 10. Effects of maternal high-fat diet consumption on inflammatory responses in the brain of offspring

(A) Microarray data validation of *Ccl5*, *Cxcl5*, *Ppbp* and *H2-Ab1* gene expression levels using qRT-PCR. Protein levels of (B) JNK and (C) amyloid-β and tau and determined by immunoblotting. Data are presented as mean ± SEM (n = 3). # $P < 0.05$ compared with PD21 offspring from the same diet-fed dams (Student's *t*-test).

IV. Discussion

To identify the effects of maternal fat consumption on offspring brain, we compared the brain transcriptome of offspring right after weaning and adult offspring from LFD or HFD-fed dams, using microarray analysis. Adult offspring mice were fed post-weaning HFD to figure out how maternal HFD consumption affects the response to additional post-weaning HFD in offspring. Among 5 DEGs in HFD105/LFD105, there were 3 genes involved in brain serotonin system and 1 gene encoding neuropeptide. Also, offspring from HFD-fed dams showed upregulation of chemokine-mediated inflammatory gene expressions as weaned offspring were fed HFD for 12 weeks post-weaning, while offspring from LFD-fed dams did not.

The current study showed no differences in body weight and brain weight of offspring from HFD-fed dams compared to those from LFD-fed dams. Prenatal obesogenic diet did not always induce body weight increase in offspring according to the previous studies (Blackmore *et al.*, 2014; Murabayashi *et al.*, 2013; Niculescu *et al.*, 2009). Such unchanged body weight between offspring from LFD-fed dams and HFD-fed dams indicate that altered gene expressions in the present study were not due to body weight or degree of adiposity in offspring.

Despite the higher gene expression of *Tph2*, there were no significant difference in the levels of serotonin, 5-HIAA and tryptophan. Regulatory mechanism of TPH2 is not widely known, yet. However, it is known as an

unstable enzyme, which was suggested to be stabilized or activated by phosphorylation (Badawy, 2013; Torrente *et al.*, 2012). Therefore, even though transcriptional level of *Tph2* was upregulated, it might not necessarily lead to increase in activated enzyme. Alternatively, upregulated gene expression of *Tph2* might be homeostatic response to prevent reduction in serotonin level. In nonhuman primate model, offspring showed lower serotonin level in cerebrospinal fluid due to maternal HFD, despite the higher mRNA expression of *Tph2* (Sullivan *et al.*, 2010). It was explained as a compensatory response of *Tph2*. While there was no change in serotonin or its metabolite levels, there could be alteration in serotonin neurotransmission, as serotonin transporter (*Slc6a4*) plays an important role in maintaining homeostasis of synaptic serotonin, reuptaking synaptic serotonin for recycling (Kim *et al.*, 2005). Therefore, extracellular serotonin level is suggested to be investigated in further studies. Also, as levels of serotonin and 5-HIAA, and alteration of those levels depend on brain regions, measurement of serotonin level in specific brain regions such as hypothalamus and hippocampus would provide more evidence of brain serotonin system alteration by maternal HFD consumption.

Also, it was observed that gene expressions of *Tph2* and *Slc6a4* were lower in LFD105 compared to LFD21, which were not shown in HFD21 and HFD105. As brain develops, mRNA level of *Slc6a4* or reuptake of serotonin decreases (O'Mahony *et al.*, 2015). Comparable gene expressions of *Slc6a4* in HFD21 and HFD105 could indicate that development of central

serotonergic system was affected by maternal fat consumption.

CART peptide is known to be involved in various physiological processes including food intake regulation (Zhang *et al.*, 2012). *Cartpt* gene expression was higher in PD105 offspring due to maternal HFD in the current study. However, there was no significant difference in food intake between LFD105 and HFD105. HFD feeding induced diverse changes in CART peptide expression in the previous studies (Lee *et al.*, 2010; Tian *et al.*, 2004; Yu *et al.*, 2008). In other words, altered expression of orexigenic or anorectic neuropeptides did not always exert its effect on food intake or obesity induction in the previous studies. Especially, it was reported that upregulation of *Cartpt* by HFD feeding could be considered as an attempt to maintain energy homeostasis against overnutrition, despite the failure of body weight control against HFD (Lee *et al.*, 2010). Therefore, upregulated expression of *Cartpt* in the current study might also be homeostatic response against HFD intake, which was induced by maternal HFD consumption, in particular.

Such upregulation in *Cartpt* expression was suggested to be due to increased phosphorylation of its transcription factor, CREB. Higher level of phosphorylated CREB was also described in another study, which contributed to depressive behavior (Sharma *et al.*, 2013). According to our results, it could be suggested that maternal HFD intensified the activation of CREB by AMPK. Expression of AMPK was reported to be higher in ischemia or hypoxia and activation of AMPK was demonstrated to be critical in phosphorylation of CREB in mouse hippocampal neuron (Huang *et al.*, 2015). Also

phosphorylation of AMPK was reported to be higher by maternal HFD in mouse hypothalamus (Melo *et al.*, 2014).

Gene expressions of *Tph2*, *Slc6a4* and *Cartpt*, which were higher in HFD105 compared to LFD105, showed significant correlations with several parameters of dams. Body weight and adipose tissue weight, which indicate increased adiposity due to HFD consumption in dams, showed positive correlations with gene expressions of *Tph2*, *Slc6a4* and *Cartpt* in PD105 offspring. On the other hand, hepatic DNA content, which was lower in HFD-fed dams compared to LFD-fed dams, indicates liver growth retardation of dams during gestation and lactation period, and showed negative correlation with the gene expressions of PD105 offspring brain. In addition, TBARS levels of liver and serum in dams were positively correlated with gene expressions of PD105 offspring brain. Although underlying mechanisms of such correlations are yet to be clarified, maternal oxidative stress which might have been associated with increased adiposity and disturbed liver growth is suggested to be a mediator of the effects of maternal HFD consumption on the gene expressions of *Tph2*, *Slc6a4* and *Cartpt* in PD105 offspring brain.

Comparisons between PD21 and PD105 offspring from dams fed the same diet, LFD or HFD, indicated the different effects of maternal diet on the response to HFD consumption of offspring. DEGs involved only in HFD105/HFD21 were related to immune response. However, although top 20 upregulated unique genes in HFD105/HFD21 were involved in chemokine-associated inflammatory response, these did not lead to neuropathogenesis.

Chemokines play important roles in recruitment of leukocytes to CNS in autoimmune and neuroinflammatory disease such as multiple sclerosis and Alzheimer's disease (Heppner *et al.*, 2015; Holman *et al.*, 2011; Kempuraj *et al.*, 2016). However, neuroinflammation is not always accompanied by immune cell recruitment (Elahy *et al.*, 2015). Also, although it did not emerge as neuropathological alteration in offspring brain on PD105, it may lead to neuropathogenesis in later life, as aging is accompanied by increase in inflammation (Barrientos *et al.*, 2015; Chung *et al.*, 2011; Tucsek *et al.*, 2014).

To our best knowledge, it is the first study which investigated the brain transcriptome to figure out the effects of maternal HFD consumption on weaned and adult offspring simultaneously. Although there are various regions in brain with different functions and numerous population of cell types, overall transcriptional profiling of whole brain could figure out predominant changes throughout all the regions of brain, providing clues for further investigation on specific regions. Taken together, the present study demonstrates that maternal fat consumption is associated with higher gene expressions involved in serotonin metabolism and neuropeptide in whole brains and these effects were evident in obesogenic diet-fed adult offspring mice rather than weaned offspring. Also, as offspring were fed HFD for 12 weeks, gene expressions of chemokine-mediated inflammation were higher when born to HFD-fed dams.

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Appendices

Appendix 1. Gene names of differentially expressed genes in HFD21/LFD21

Gene symbol	Gene name
Upregulated	
1700120B22Rik	RIKEN cDNA 1700120B22 gene
Trim69	Tripartite motif-containing 69
Gm8298	Predicted gene 8298
Gm36584	Predicted gene, 36584
LOC105247294	Uncharacterized LOC105247294
Camk1d	Calcium/calmodulin-dependent protein kinase ID
Pdzd2	PDZ domain containing 2
Pisd-ps3	Phosphatidylserine decarboxylase, pseudogene 3
Alas2	Aminolevulinic acid synthase 2, erythroid
B430316J06Rik	RIKEN cDNA B430316J06 gene
Edil3	EGF-like repeats and discoidin I-like domains 3
Car10	Carbonic anhydrase 10
Cdh18	Cadherin 18
Downregulated	
Cdhr1	Cadherin-related family member 1
Ms4a15	Membrane-spanning 4-domains, subfamily A, member 15
Xlr3b	X-linked lymphocyte-regulated 3B

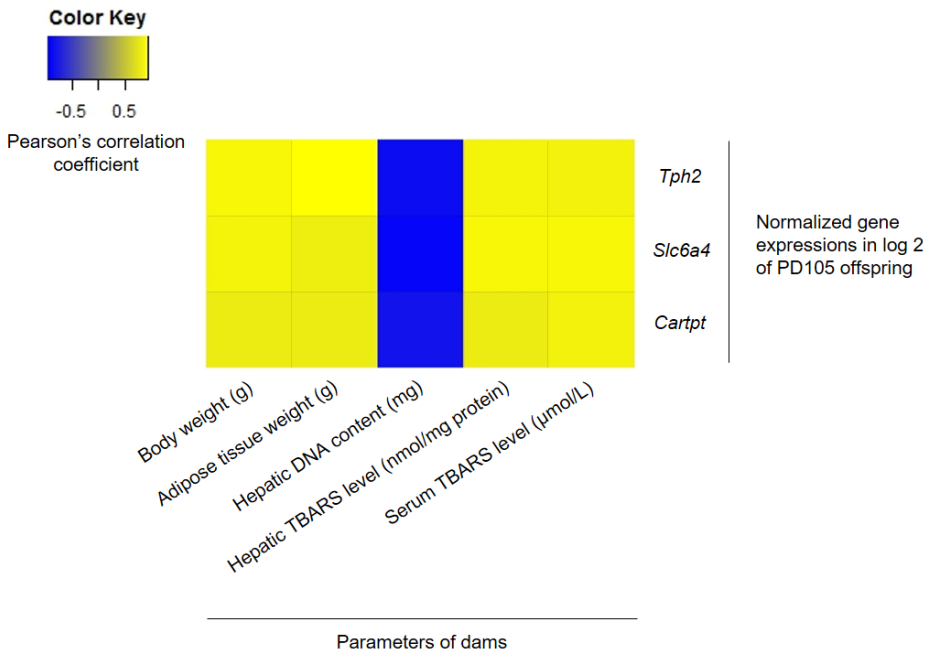
(Continued on next page)

Gene symbol	Gene name
Downregulated	
Nppa	Natriuretic peptide type A
Ccl17	Chemokine (C-C motif) ligand 17
Xlr4b	X-linked lymphocyte-regulated 4B
1500004A13Rik	RIKEN cDNA 1500004A13 gene
Mtor	Mechanistic target of rapamycin (serine/threonine kinase)
Omp	Olfactory marker protein
S100a5	S100 calcium binding protein A5
Gm20098	Predicted gene, 20098
Adam26a	A disintegrin and metallopeptidase domain 26A (testase 3)

Appendix 2. Gene names of differentially expressed genes in HFD105/LFD105

Gene symbol	Gene name
Upregulated	
Slc6a4	Solute carrier family 6 (neurotransmitter transporter, serotonin), member 4
Tph2	Tryptophan hydroxylase 2
Gm5741	Predicted gene 5741
Cartpt	CART prepropeptide
Downregulated	
Col11a2	Collagen, type XI, alpha 2

Appendix 3. Correlations between parameters of dams and gene expressions of differentially expressed genes in HFD105/LFD105



Appendix 4. Body weight, organ weights and litter size of dams

	LFD (n = 4)	HFD (n = 4)
Final body weight (g)	22.96 ± 0.41	26.81 ± 0.50 *
Organ weight (g)		
Liver	1.73 ± 0.07	1.32 ± 0.05 *
Adipose tissue	0.16 ± 0.03	0.43 ± 0.02 *
Litter size	7.25 ± 0.48	8.25 ± 0.48
Ratio of male offspring (%)	60.57 ± 10.33	52.23 ± 7.40

Data are presented as mean ± SEM (n = 4). * $P < 0.05$ compared with LFD-fed dams (Student's *t*-test).

Data were previously reported (Kim *et al.*, 2016).

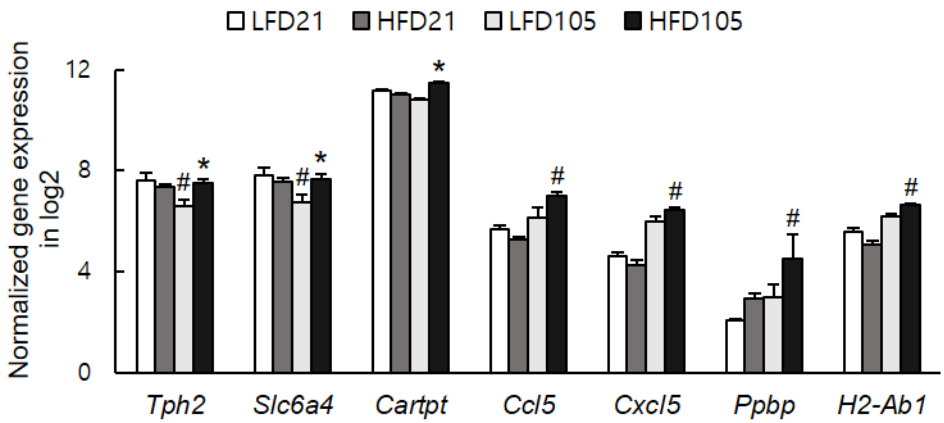
Appendix 5. Gene names of top 20 genes among the upregulated and downregulated unique genes in HFD105/HFD21

Gene symbol	Gene name
Upregulated	
Gm11497	Predicted gene 11497
Loc102639603	Uncharacterized LOC102639603
5033421B08Rik	RIKEN cDNA 5033421B08 gene
Rpe65	Retinal pigment epithelium 65
Stoml3	Stomatin (Epb7.2)-like 3
Cxcl5	Chemokine (C-X-C motif) ligand 5
Irgm1	Immunity-related GTPase family M member 1
Pkd2l1	Polycystic kidney disease 2-like 1
Wfdc17	WAP four-disulfide core domain 17
Hephl1	Hephaestin-like 1
Fam81b	Family with sequence similarity 81, member B
Gm34360	Predicted gene 34360
Ccl5	Chemokine (C-C motif) ligand 5
Ppbp	Pro-platelet basic protein
H2-Ab1	Histocompatibility 2, class II antigen A, beta 1
6430553K19Rik	RIKEN cDNA 6430553K19 gene
Ceacam18	Carcinoembryonic antigen-related cell adhesion molecule 18
Plbd1	Phospholipase B domain containing 1
Gm10378	Predicted gene 10378
Kcnk15	Potassium channel, subfamily K, member 15

(Continued on next page)

Gene symbol	Gene name
Downregulated	
Trim69	Tripartite motif-containing 69
Ahsp	Alpha hemoglobin stabilizing protein
Trim69	Tripartite motif-containing 69
Ahsp	Alpha hemoglobin stabilizing protein
Ccdc154	Coiled-coil domain containing 154
Bsph2	Binder of sperm protein homolog 2
1700120B22Rik	RIKEN cDNA 1700120B22 gene
Rab7b	RAB7B, member RAS oncogene family
Ssxb1	Synovial sarcoma, X member B, breakpoint 1
Muc1	Mucin 1, transmembrane
Gm8298	Predicted gene 8298
Robo2	Roundabout guidance receptor 2
Gm13748	Predicted gene 13748
Matn1	Matrilin 1, cartilage matrix protein
Prnd	Prion protein dublet
Loc545086	Uncharacterized LOC545086
Slco1a1	Solute carrier organic anion transporter family, member 1a1
Gm36584	Predicted gene 36584
Olfr1295	Olfactory receptor 1295
Gm32568	Predicted gene 32568
Gm32633	Predicted gene 32633
Gmnn	Geminin

Appendix 6. Microarray data of validated genes in offspring brain



Microarray data of *Tph2*, *Slc6a4*, *Cartpt*, *Ccl5*, *Cxcl5*, *Ppbp* and *H2-Ab1*. Data are presented as mean ± SEM (n = 3). * $P < 0.05$ compared with PD105 offspring of LFD-fed dams, # $P < 0.05$ compared with PD21 offspring of same diet-fed dams (Student's *t*-test).

국문 초록

모체 고지방 식이 섭취가 이유기 자손 마우스와 고지방 식이를 섭취한 성체 자손 마우스의 뇌 전사체에 미치는 영향

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모체의 고지방 식이 섭취 또는 비만은 자손의 발달에 영향을 미치며, 그 영향은 성인기까지 이어지는 것으로 알려져 있다. 하지만, 모체의 과잉 영양이 자손의 뇌에 미치는 영향에 대한 선행 연구에서는 대부분 특정한 시기의 자손에서만 그 영향을 보고하였다. 따라서 본 연구에서는 모체의 고지방 식이 섭취가 자손의 뇌에 미치는 영향을 갓 이유한 생후 21일 자손과 이유 후 고지방 식이를 섭취한 생후 105일의 성체 자손의 뇌 전사체 분석을 통해 확인하고자 하였다. 5주령 암컷 C57BL/6 마우스에 임신 전부터 수유기까지 저지방 식이 (LFD; 총 에너지 중 10% 지방) 또는 고지방 식이 (HFD; 총 에너지 중 45% 지방)를 제공하였다. 수컷 자손 중 두 마리는 생후 21일에 희생하였고, 다른 두 마리에는 12주간 고지방 식이를 제공한 후 생후 105일에 희생하였으며, 자손의 전뇌를 마이크로어레이를 통해 분석하였다. 그 결과, 저지방 식이를 섭취한 어미의 생후 21일 자손과 비교하였을 때 고지방 식이를 섭취한 어미의 생후 21일 자손에서 발현이 달라진 유전자는 25개로 나타났다. 한편

저지방 식이를 섭취한 어미의 생후 105일 자손과 비교하여, 고지방 식이를 섭취한 어미의 생후 105일 자손에서 발현이 달라진 유전자는 5개로 나타났으며, 이 중 3개의 유전자는 뇌 세로토닌 대사, 1개의 유전자는 신경 펩타이드 발현에 관여하였다. 세로토닌 합성 효소 유전자인 *Tph2*와 세로토닌 운송체 유전자인 *Slc6a4*의 발현이 고지방 식이를 섭취한 어미의 생후 105일 자손에서 저지방 식이를 섭취한 어미의 자손에 비해 높게 나타났지만 전뇌에서의 세로토닌, 세로토닌 대사물질 (5-Hydroxyindoleacetic acid), 그리고 전구체인 트립토판의 양은 두 군 사이에 차이가 나타나지 않았다. 또한 5개의 유전자 중 하나인 *Cartpt*는 신경 펩타이드인 cocaine- and amphetamine-regulated transcript 펩타이드의 유전자로, 고지방 식이를 섭취한 어미의 생후 105일 자손에서 저지방 식이를 섭취한 어미의 자손에 비해 높게 나타났으며, 이는 AMP-activated protein kinase에 의한 cAMP response element binding protein의 활성화 증가로 인한 것임을 확인하였다. 이러한 *Tph2*, *Slc6a4*, *Cartpt* 유전자 발현 증가는 특히 모체의 고지방 식이 섭취로 인한 산화스트레스 증가의 영향을 받은 것으로 보인다. 한편, 고지방 식이를 섭취한 어미의 자손 비교에서만 특이적으로 발현이 달라진 유전자들은 주로 키모카인에 의해 매개되는 염증 반응에 관여하는 것을 확인하였다. 하지만 이는 c-Jun N-terminal kinases 인산화, 타우 과인산화 및 베타 아밀로이드 등의 신경병리학적 변화로 이어지지는 않았다. 결론적으로, 모체의 고지방 식이 섭취는 자손에서 뇌 세로토닌 대사, 신경 펩타이드 관련 유전자 수준의 변화를 일으키며, 이는 이유기 자손보

다 이유 후 고지방 식이를 섭취한 성체 자손에서 나타났다. 또한 이유기 자손이 이유 후 12주간 고지방 식이를 섭취하였을 때, 고지방 식이를 섭취한 어미의 자손에서만 특이적으로 키모카인 매개 염증 관련 유전자의 발현이 높게 나타났다.

주요어: 모체 고지방 식이, 자손 마우스, 이유 후 고지방 식이, 뇌, 마이크로어레이, 세로토닌, 신경 펩타이드, 염증

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