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

Effects of marginal vitamin B6 deficiency on hepatic transcriptome profile in control and obese mice

경도의 비타민 B6 결핍이 대조군과
비만 마우스의 전사체 발현에 미치는 영향

February, 2019

Department of Food and Nutrition
Graduate School
Seoul National University

Hyun-Jee Um

Abstract

Effects of marginal vitamin B6 deficiency on hepatic transcriptome profile in control and obese mice

Hyun-Jee Um
Department of Food and Nutrition
Graduate School
Seoul National University

Although vitamin B6 is present in a wide rage of foods, vitamin B6 deficiency is quite common worldwide. A metabolically active form of vitamin B6, pyridoxal 5′-phosphate (PLP), acts as a coenzyme involved in amino acid, carbohydrate and lipid metabolism. Vitamin B6 also plays an essential role in maintenance of normal immunity. Therefore, vitamin B6 deficiency affects several aspects of metabolism *in vivo*. Research on the metabolism of amino acids and amino acid derivatives in case of marginal vitamin B6 deficiency has been actively conducted. However, the molecular mechanisms of vitamin B6 are not clearly known. In addition, patients with fatty liver, metabolic syndrome, type 2 diabetes or those who are obese are more likely to have suboptimal vitamin B6 levels compared to healthy individuals. Nevertheless, no studies have examined vitamin B6 metabolism in obese animal models induced by a high-fat diet. Therefore, in this

study, we investigated the effect of marginal vitamin B6 deficiency in non-obese or obese mice on transcriptome profiling in the liver. Also, vitamin B6 status in obese mice fed high-fat diets was assessed. Mice of control diet (CD) groups were fed a CD in combination of adequate (7 mg pyridoxine/kg diet; CD7) or deficient (1 mg; CD1) amount of vitamin B6 for 16 weeks. Mice of high-fat diet (HFD) group were fed a HFD in combination of adequate (HFD7) or deficient (HFD1) amount of vitamin B6 for the same period. RNA was extracted from the liver and microarray examinations were performed. Biochemical parameters in serum and the liver were measured. Serum and hepatic lipid levels were significantly changed by high-fat diet, but not by marginal vitamin B6 deficiency. Marginal vitamin B6 deficiency changed serum glucose level and the concentration was significantly lower in HFD1 than in HFD7. According to a transcriptome analysis of four groups, the expressions of 4,000 genes were significantly changed. Changes in the expression levels of differentially expressed genes (DEGs) were affected more by the dietary fat content than by marginal vitamin B6 deficiency. In an enrichment analysis of the 54 upregulated DEGs in comparison of CD7 and CD1 (CD1/CD7), it was found that the Gene Ontology (GO)-based terms of biological processes, such as sterol metabolic process were enriched. Expression levels of *Hmgcr, Cyp51,* and *Msmo1* involved in cholesterol synthesis and *Cyp39a1* involved in bile acid biosynthesis were significantly higher in CD1 than in CD7. In the enrichment analysis of the 54 downregulated DEGs in CD1/CD7, GO-based terms of biological processes, such as immune response were significant. Functional analysis of genes with increased expression in HFD1/HFD7 revealed significant GO-based terms associated with amino acid metabolism. Serum and hepatic PLP

concentrations were lowered by HFD consumption. Microarray results showed that the expression of *Aox* which converts PLP to pyridoxate significantly changed and the expression pattern of *Aox* differed depending on the dietary fat content. In conclusion, results of microarray suggest that obesity may differentially regulate vitamin B6-associated metabolic pathways such as those associated with amino acid, lipid and carbohydrate metabolism in the liver. In addition, these results demonstrate that obesity induced by a high-fat diet may lower vitamin B6 level.

Key words: marginal vitamin B6 deficiency, amino acid metabolism, high-fat diet, immunity, sterol metabolism, transcriptome, liver
Student number: 2016-21668

Contents

List of figures

List of tables

List of appendices

List of abbreviations

AOX: aldehyde oxidase
CD: control diet
DEG: differentially expressed gene
GO: Gene Ontology
HFD: high-fat diet
KEGG: Kyoto Encyclopedia of Genes and Genomes
NAFLD: non-alcoholic fatty liver disease
PDXK: pyridoxal kinase
PDXP: pyridoxal phosphatase
PL: pyridoxal
PLP: pyridoxal 5′-phosphate
PM: pyridoxamine
PMP: pyridoxamine 5′-phosphate
PN: pyridoxine
PNP: pyridoxine 5′-phosphate
PNPO: pyridoxine 5′-phosphate oxidase
T2DM: type 2 diabetes mellitus
VLDL: very low-density lipoprotein

Ⅰ. Introduction

Epidemiological studies have shown that a low dietary intake of vitamin B6 or low plasma levels of pyridoxal 5′-phosphate (PLP) are independent risk factors for cancer, diabetes, cardiovascular diseases and neurological disorders (DeRatt et al. 2014). The metabolically active form of vitamin B6 is PLP, which serves as a coenzyme for numerous enzymes, most of which are involved in the metabolism of amino acids. Vitamin B6 is also essential for the metabolism of carbohydrates and lipids and the synthesis of neurotransmitters (Ueland et al. 2017). However, the mechanism underlying the effects of PLP at the molecular levels remains unclear (Marzio et al. 2014).

Plasma PLP concentrations of less than 20 nmol/L indicate deficient levels of vitamin B6, while greater than 30 nmol/L indicates adequate levels. In general, 20-30 nmol/L indicates a marginal status (Leklem 1990, Ye et al. 2010). Since vitamin B6 is contained in a variety of foods, it is unusual to find an inadequate intake in people who eat regular diets. However, deficiency of vitamin B6 is quite common worldwide; approximately 30% of the world's population shows plasma PLP concentrations less than 30 nmol/L (Lin et al. 2006, Ye et al. 2010, Chew et al. 2011, Kim et al. 2014).

Deficiency of vitamin B6 affects amino acid metabolism and various other aspects of metabolism *in vivo*. Hepatic very low-density lipoprotein metabolism becomes abnormal (Kitagawa et al. 2015), and the desaturase activity required for fatty acid synthesis decreases (She et al. 1994). Several previous studies have shown that vitamin

B6 deficiency affects hepatic cholesterol biosynthesis (Shah et al. 1960, Lupien et al. 1969, Hinse et al. 1971, Okada et al. 1977). However, the molecular mechanism by which vitamin B6 affects lipid metabolism is not clear. In addition, vitamin B6 deficiency can weaken immunity, including reducing of the antibody and interleukin production (Meydani et al. 1991, Doke et al. 1997, Inubushi et al. 2000).

In many animal studies of marginal deficiency, amino acids and their derivatives such as plasma cysteine, cystathionine, glycine and serine were altered. Concentrations of neurotransmitters, organic acids and bile acids in plasma were also changed (Lima et al. 2006, Mayengbam et al. 2016a, Mayengbam et al. 2016b). In human studies, the plasma concentrations of glycine, glutathione, cystathionine, asparagine, and glutamate were changed in subjects who were marginally deficient in vitamin B6 (Davis et al. 2005, Davis et al. 2006, Gregory et al. 2013). Both human and animal studies investigated changes in the levels of various metabolites. Although several *in vivo* studies investigated the effects of deficiency of vitamin B6, most of them have been conducted in healthy individuals or animals fed normal amounts of dietary fat. Also, studies of vitamin B6 deficiency have adopted a method of measuring the metabolite concentration to investigate the effects of vitamin B6 deficiency. It has not been determined how vitamin B6 deficiency affects the expression of genes involved in metabolism.

Obesity is known to be a risk factor for numerous chronic diseases. An increased risk of hypertension, type 2 diabetes mellitus (T2DM), non-alcoholic fatty liver disease (NAFLD) and colorectal cancer are all associated with obesity (Fabbrini et al. 2010, Na et al.

2011). Many studies have shown that high-fat diets can lead to obesity in humans (Bray et al. 1998, Schrauwen et al. 2000). High-fat diets also induce obesity in animals. Various animal models fed diet rich in fat have been used in studies on dietary obesity (Hariri et al. 2010). Patients with obesity, fatty liver or T2DM have lower plasma PLP levels than healthy individuals (Aasheim et al. 2008, Odum et al. 2012, Nix et al. 2015, Liu et al. 2016). These results imply that these diseases may alter vitamin B6 metabolism and thus cause vitamin B6 deficiency. The aim of this study was to investigate the effect of marginal vitamin B6 deficiency in control and obese mice using transcriptome profiling of the liver. We also determined the effect of high-fat diet-induced obesity on the metabolism of vitamin B6.

Ⅱ. Literature review

1. Chemical structures and metabolism of vitamin B6

Vitamin B6 is a general name that includes three different pyridine rings replaced at their 4th position. They are denoted pyridoxine (PN), pyridoxal (PL) and pyridoxamine (PM), carrying a hydroxymethyl, aldehyde and aminomethyl group, respectively. Phosphorylated forms at the 5th position are called pyridoxine 5′-phosphate (PNP), pyridoxal 5′-phosphate (PLP) and pyridoxamine 5′-phosphate (PMP) (Coburn 1996).

Since vitamin B6 is not synthesized in human bodies, it must be obtained from foods including vegetables, fruits, dairy products, potatoes and meats. Vitamin B6 from animal products mostly contains PLP and PMP and plant-derived products contain PN and PNP as a prevailing form.

To be absorbed, ingested PNP, PLP and PMP should be dephosphorylated by the tissue-specific intestinal phosphatase. Figure 1 shows the enzymes involved in vitamin B6 metabolism in the liver. The portal vein carries PN, PL and PM to the liver and they are rephosphorylated by pyridoxal kinase. Pyridoxine (pyridoxamine) oxidase catalyzes PNP and PMP to PLP, an active form of vitamin B6 (Albersen et al. 2013, Coburn 2015). PLP released from the liver enters blood stream, strongly binds to albumin (Lumeng et al. 1974, Huang et al. 2012) and circulates throughout the body. Plasma PLP should be converted to PL, a dephosphorylated form, to pass through tissues or cells and to pass the blood-brain barrier. This catalytic

reaction is performed by tissue-specific phosphatases, expressed in germ cells and placenta, and the tissue nonspecific alkaline phosphatase, which is an ecto-enzyme that exists on the outer membrane of cells (Buchet et al. 2013).

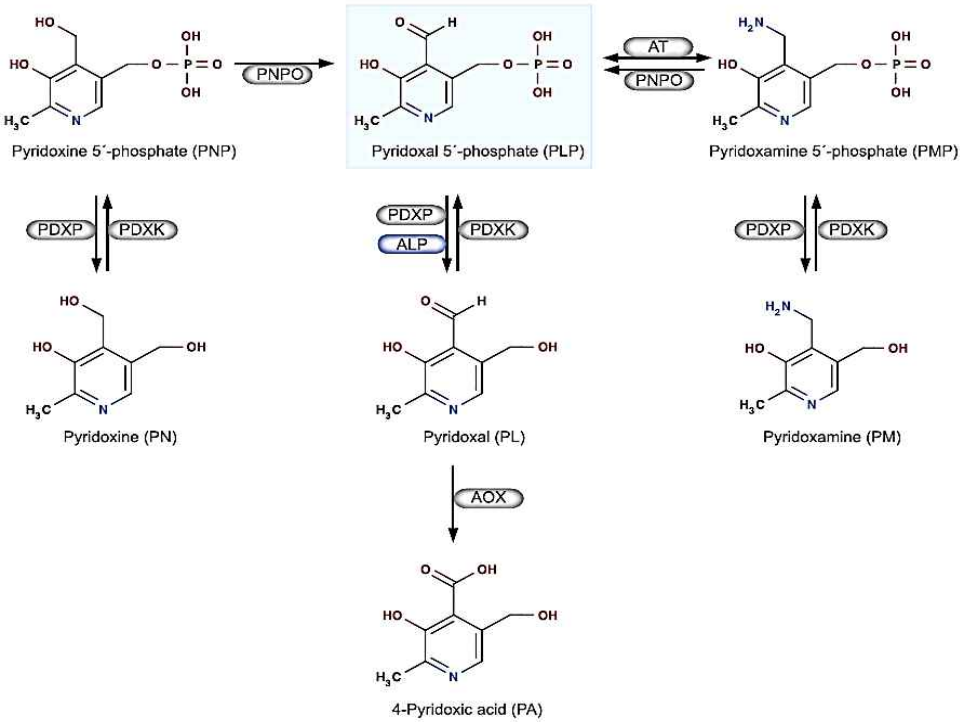

Figure 1. Vitamin B6 structures and metabolism

ALP, alkaline phosphatase; AOX, aldehyde oxidase; AT, aminotransferase; PDXK, pyridoxal kinase; PDXP, pyridoxal phosphatase; PNPO, pyridoxine 5′-phosphate oxidase (Ueland et al. 2017)

2. The function of vitamin B6 and the effect of vitamin B6 deficiency on metabolic changes

PLP, the active form of vitamin B6, acts as a cofactor for more than 140 enzymes (Percudani et al. 2009). PLP-dependent enzymes catalyze numerous reactions involving transamination, α-decarboxylations, aldol cleavages, racemizations, replacement reactions, β- and ɣ-eliminations (Eliot et al. 2004). Most reactions are related to the synthesis and degradation of amino acids, while others are involved in one-carbon metabolism, gluconeogenesis, lipid metabolism, neurotransmitter and heme biosynthesis (Eliot et al. 2004, Percudani et al. 2009). Vitamin B6 also plays an important role in the production of antibodies and cytokines, which is essential for normal immunity (Meydani et al. 1991, Doke et al. 1997, Inubushi et al. 2000).

Because vitamin B6 is involved in many metabolism, the following metabolic changes occur when vitamin B6 is deficient. Vitamin B6 deficiency affects carbohydrate metabolism. Animals that did not feed vitamin B6 had lower blood glucose level and hepatic glycogen content (Ribaya et al. 1977, Guggenheim et al. 1957, Choi et al. 2008). In addition, blood levels of pyruvic acid and lactic acid related to glucose metabolism were significantly decreased (Ribaya et al. 1977). Glucokinase activity and glycolytic activity were decreased by vitamin B6 deficiency (Suzuki et al. 1982). Results of previous studies suggest that vitamin B6 deficiency may reduce glycogen storage in the liver and glucose utilization.

Vitamin B6 deficiency also affects lipid metabolism through various pathways. Severe vitamin B6 deficiency affects one-carbon metabolism and eventually changes hepatic very low-density

lipoprotein (VLDL) metabolism. Vitamin B6 deficiency increases the concentration of homocysteine (Hcy) and S-Adenosyl-homocysteine. S-Adenosyl-homocysteine decreases the activity of phosphatidylethanolamine methyltransferase (She et al. 1995). Reduced phosphatidylethanolamine methyltransferase activity inhibits the synthesis of phosphatidylcholine and low phosphatidylcholine synthesis results in suppression of VLDL assembly (She et al. 1995). Finally, the VLDL secretion is suppressed and excess lipid is accumulated in the liver (Suzuki et al. 1984, Kitagawa et al. 2015). In addition, although vitamin B6 does not act as a cofactor for desasturases, the activity of these enzymes is reduced by vitamin B6 deficiency. The arachidonic acid/linoleic acid ratio, which indicates the degree of desaturation, decreased in the liver due to depressed activity of desaturases (She et al. 1994). Restriction of vitamin B6 to HepG2 cells impairs fatty acid synthesis (Zhao et al. 2013). Several studies have shown that vitamin B6 also affects cholesterol metabolism. The incorporation rate of hepatic acetate to cholesterol increases with vitamin B6 deficiency (Shah et al. 1960, Lupien et al. 1969, Hinse et al. 1971, Okada et al. 1977). However, the results of studies on cholesterol accumulation in vitamin B6 deficiency are inconsistent. Shah et al. reported that serum and hepatic cholesterol levels are not altered by vitamin B6 deficiency (Shah et al. 1960). On the other hand, according to Williams et al.'s study, vitamin B6 deficiency lowers plasma and hepatic cholesterol levels (Williams et al. 1966). Vitamin B6 deficiency does not appear to affect 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase activity, which is the rate-controlling enzyme of cholesterol synthesis, to explain the acetate incorporation rates to hepatic cholesterol (Lupien et al. 1969). There is

no clear mechanism to explain the effect of vitamin B6 deficiency on cholesterol metabolism.

Vitamin B6 deficiency has shown to change the concentration of amino acid metabolites. In human and animal studies, changes in concentration such as amino acids, metabolites of one-carbon metabolism and glutathione have been measured (Lima et al. 2006, Mayengbam et al. 2015, Mayengbam et al. 2016a, Mayengbam et al. 2016b, Davis et al. 2005, Davis et al. 2006, Gregory et al. 2013). In animals, plasma cysteine, cystathionine, and glycine were significantly altered by vitamin B6 deficiency (Lima et al. 2006, Mayengbam et al. 2015, Mayengbam et al. 2016b). Healthy people were also provided with an insufficient vitamin B6 diet to observe changes in the concentration of metabolites in their plasma. Plasma glycine, glutathione, cystathionine, asparagine, glutamate, and glutamine were significantly altered in subjects who were deficient in vitamin B6 (Davis et al. 2005, Davis et al. 2006, Gregory et al. 2013).

It seems that marginal and severe vitamin B6 deficiency affects amino acid metabolism in common. As described above, in severe vitamin B6 deficiency, impaired VLDL metabolism leads to excessive lipid accumulation in the liver. Also, changes in cholesterol metabolism and glucose metabolism were observed in severely deficient studies. However, the effects of marginal vitamin B6 deficiency on hepatic lipid or carbohydrate metabolism have not been studied.

Finally, a deficiency of vitamin B6 can cause an abnormal immune response. Vitamin B6 deficiency can lead to weakened immunity, including regression of the antibody and IL-2 production (Meydani et al. 1991, Doke et al. 1997, Inubushi et al. 2000). Qian et al. found that

the vitamin B6 deficiency diminishes the capacity of lymphocyte proliferation. In particular, cluster of differentiation (CD) 3 proliferation and T cell differentiation are impaired, causing a decrease in immunity (Qian et al. 2017). The expression of interleukin-1β and interleukin-2 was suppressed in CD4-lymphocytes by vitamin B6 deficiency due to decreased activity of serine hydroxymethyl transferase, a PLP-dependent enzyme, resulting in the impairment of one-carbon metabolism and the reducing of mRNA biosynthesis (Trakatellis et al. 1992).

3. Nutritional status of vitamin B6

Since PLP accounts for 70-90% of total vitamin B6, plasma PLP concentration reflects the nutritional status of vitamin B6 (Lumeng et al. 1980). Plasma PLP concentration less than 20 nmol/L reflects deficient status of vitamin B6. PLP concentration greater than 30 nmol/L reflects adequate status and generally 20-30 nmol/L indicates marginal or insufficient status (Leklem 1990, Ye et al. 2010). Vitamin B6 deficiency is very common, even though it is contained in various foods. Especially women of childbearing age and the elderly are very susceptible to vitamin B6 deficiency (Ronnenberg et al. 2000, Kjeldby et al. 2013). Kim et al. examined the vitamin B6 status of 254 healthy Korean adults. This study showed that only 6.3% of subjects ingested total vitamin B6 less than Estimated Average Requirements. However, 19.7% of subjects had marginal vitamin B6 deficiency (Plasma PLP 20-30 nmol/L), while 16% of subjects showed severe vitamin B6 deficiency (Plasma PLP < 20 mnol/L) (Kim et al. 2014). Morris et al. examined the vitamin B6 nutritional status of the US population (Morris et al. 2008). In the US, 14-24% of men and

20-25% of women show vitamin B6 deficiency (Plasma PLP < 20 nmol/L). In the population of Puerto Rico (Ye et al. 2010) and Malaysia (Chew et al. 2011), about 30% of the subjects do not have sufficient vitamin B6 status.

4. Vitamin B6 status in patients with obesity-related diseases

Obesity is considered to be a major risk factor for chronic diseases. The increased risk of hypertension, T2DM, NAFLD and colorectal cancer are associated with obesity (Fabbrini et al. 2010, Na et al. 2011). In epidemiological studies conducted in the US and China, a positive relationship has been found between the average amount of fat in the diet and incidence of obesity (Tucker et al. 1992, Popkin et al. 1993). Patients of obesity, NAFLD, metabolic syndrome and T2DM have lower plasma PLP levels than healthy individuals (Aasheim et al. 2008, Odum et al. 2012, Nix et al. 2015, Liu et al. 2016). The morbidly obese women and men showed significantly lower concentration of plasma PLP than healthy controls. The average of plasma PLP concentration in women was 46 nmol/L in the control group and 29 nmol/L in the obese group. In men, the control group showed 58 nmol/L and the obese group showed 39 nmol/L (Aasheim et al. 2008). Although subjects with metabolic syndrome were not deficient in vitamin B6, serum vitamin B6 levels were 61.57 nmol/L, which was significantly lower than those in the healthy subjects (77.35 nmol/L) (Odum et al. 2012). The median of plasma PLP concentrations were significantly lower in T2DM patients than in healthy subjects (control, 39.5 nmol/L; diabetes without

microalbuminuria, 26.8 nmol/L; diabetes with microalbuminuria, 22.7 nmol/L) (Nix et al. 2015). NAFLD patients (23.9 nmol/L) had significantly lower serum PLP levels than control subjects (55.8 nmol/L) (Liu et al. 2016). These results implicate that these diseases may alter vitamin B6 metabolism and may cause vitamin B6 deficiency. However, the studies are not actively conducted at the genetic level on how the metabolism of vitamin B6 changes in these patients.

Ⅲ. Materials and methods

1. Animals and diets

Four-week old male C57BL/6 mice were purchased from Raonbio (Korea) and housed four mice per cage. After 12 days of acclimation, mice were randomly divided into four groups (n = 8-9) and fed a control diet (CD, 12% of total calorie from fat) with 7 mg PN/kg diet (CD7), CD with 1 mg PN/kg diet (CD1), high-fat diet (HFD, 45% of total calorie from fat) with 7 mg PN/kg diet (HFD7), or HFD with 1 mg PN/kg diet (HFD1) (Figure 2). The composition of the diets is shown in Table 1. The recommended level of PN in the AIN-93 diet is 7 mg/kg diet (Reeves et al. 1993), and 1 mg PN/kg diet of PN is the minimum level required for preventing growth depression caused by vitamin B6 deficiency (Coburn 1994). The experimental diets were provided ad libitum for 16 weeks. The animals were maintained in a temperature (22 ± 3℃)- and humidity (50 ± 10%)-controlled room with a 12 h dark-light cycle. The experiment was approved by Institute of Laboratory Animals Resources of Seoul National University (SNU-161111-1-2), and performed in accordance with the guideline of Institutional Animal Care and Use Committee of Seoul National University. At the end of the experiments, the animals were fasted for 12 h and sacrificed after intraperitoneal injection of 30 mg/kg Zoletil (Virbac, France) and 10 mg/kg Rompun (Bayer Korea, Korea). Blood samples were collected by cardiac puncture. To obtain serum, blood was left in room temperature for 1 h and centrifuged at 3,000 rpm, 4℃ for 20 min. Liver and epididymal fat were collected.

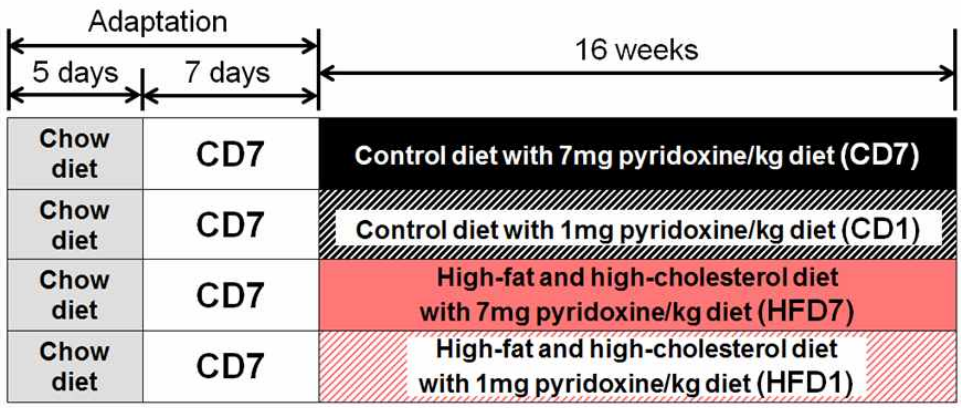

Figure 2. Diagram of experimental timeline

Table 1. Composition of experimental diets

Composition (g/kg diet)	Diet			
	CD7	CD1	HFD7	HFD1
Casein	200.0	200.0	200.0	200.0
L-cysteine	3	3	3	3
Corn starch	417.5	417.5	117.5	117.5
Maltodextrin	132	132	132	132
Sucrose	100	100	200	200
Lard	-	-	187.5	187.5
Soybean oil	50	50	50	50
Cholesterol	-	-	12.5	12.5
Cellulose	50	50	50	50
Mineral mix	35	35	35	35
Vitamin mix [1]	10	-	10	-
Pyridoxine-free vitamin mix [2]	-	10	-	10
Pyridoxine hydrochloride	-	0.001	-	0.001
Choline bitrate	2.5	2.5	2.5	2.5
Total	999.5	999.5	1000.0	1000.0

CD7, a control diet containing recommended amount of vitamin B6 (pyridoxine hydrochloride (PN) 7 mg/kg diet); CD1, a control diet containing low amount of vitamin B6 (PN 1 mg/kg diet); HFD7, a high-fat diet with recommended vitamin B6 (PN 7 mg/kg diet); HFD1, a high-fat diet with low vitamin B6 (PN 1 mg/kg diet).

[1]AIN-93-VX vitamin mix

[2]AIN-93-VX vitamin mix (without pyridoxine-HCl)

2. Measurement of PLP content in serum and the liver

The method of Gregory (Gregory 1980) was slightly modified to analyze the serum and liver PLP using Ultimate 3000 HPLC system (Dionex Corporation, USA). Twenty-five mg of wet liver tissue was homogenized with 500 μl of 0.1 M KH_2PO_4 to obtain 5% homogenate. Two hundred μl of perchloric acid was added to 200 μl of homogenate or serum. Mixture was centrifuged at 1,500g for 20 min at 4℃ and the supernatant (A) was transferred to a new tube. To the precipitate, 200 μl of 1 M perchloric acid was added, mixed vigorously, and centrifuged to obtain supernatant (B). Supernatant A and B were mixed, neutralized with 6 M KOH to pH 6-7. The precipitate ($KClO_4$) in neutralized solution was allowed to settle in the dark at 4℃ for 30 min. After the precipitate settled, 200 μL of the supernatant was transferred to a new tube and mixed with 50 μL of 0.2 M semicarbazide hydrochloride, heated at 95℃ for 5 min, and cooled in ice to terminate the reaction. Fifty μL of 0.2 M phosphoric acid was added, vortexed, and the sample was filtrated with a 0.45 μm syringe filter. Method of Ubbink et al (Ubbink et al. 1985) was used to analyze under optimal chromatographic conditions. A solution of 0.05 M KH_2PO_4 (adjusted to pH 2.9 with concentrated phosphoric acid) containing 7% acetonitrile was used as mobile phase. The flow rate was 1.1 mL/min and the injection volume was 20 μl. Using Ultimate 3000 Fluorescence Detector (Dionex Corporation) at an excitation wavelength of 368 nm and an emission wavelength of 478 nm. The column used was Luna® C18(2) column (250 x 4.6 mm I.D., 5 μm, Phenomenex, USA).

3. Biochemical analysis of serum

Serum was analyzed using commercial colorimetric assay kits (Asan Pharmaceutical Co., Korea) for glucose by glucose oxidase and peroxidase method, for triglyceride (TG) by glycerokinase-glycerolphosphateoxidase-peroxidase method, for total cholesterol (TC) by cholesterol oxidase-peroxidase method, and for Glutamate-pyruvate transaminase (GPT) by the Reitman - Frankel method according to the manufacturer's protocol. The absorbance of a corresponding end product was measured using a microplate reader (Molecular Devices, USA).

4. Biochemical analysis of liver

Hepatic total lipids were extracted according to the method of Folch et al (Folch et al. 1957). Briefly, liver tissue was homogenized in 20 volumes (w/v) of distilled water, and the protein content was measured using the commercial kit (Bio-Rad, USA). Liver homogenate (300 μL) containing equal amounts of protein (1 mg protein/mL distilled water) was incubated in 1.2 mL of methanol-chloroform (1:2, v/v) at 4℃ for 3 h. Thereafter, 240 μL of 0.88% KCl was added for aggregation of non-lipid contents and centrifuged at 1,000g for 15 min at 4℃. The bottom layer was obtained, aliquoted into 100 μL portions, and concentrated by nitrogen gas. The lipid pellets were resuspended in isopropanol. Hepatic cholesterol concentration was determined by the same method as described above using commercial kits (Asan Pharmaceutical Co.). Hepatic TG concentration was determined by TLC method (Ruiz et al. 1997). Before separation, the TLC precoated silica gel plate (SIL G-60F254 25 Aluminum sheets 20×20 cm, MERCK, Germany) was washed overnight with chloroform : methanol : water (60:40:10, v/v/v)

in the same direction as the impregnation. Dried under hot air and activated at 110℃ for 30 min. Portions of 1 uL of each sample reconstructed in isopropanol were spotted 1cm from the edge of the plate. Plate was developed in chambers saturated with mobile phase n-heptane : isopropyl ether : acetic acid (60:40:10, v/v/v) for 60 min. Thoroughly dried under a stream of hot air and lipids are charred by dipping the plate in a solution of 10% cupric sulfate (w/v) in 8% phosphoric acid (v/v) for 10 sec. The TLC plate was dried under hot air once again and immediately heated at 180℃oven for 60-90 sec until the lipid spots became evident. For total bile acid analysis, liver tissues were homogenized in a 75% ethanol homogenizer and incubated for 2 h at 50℃. Homogenate was centrifuged at 10,000 rpm, 4℃ for 10 min. The separated supernatant was transferred to a new tube and measured using a total bile acid assay reagent kit (Bioquant, USA).

5. Analysis of liver histology

Liver tissues fixed by formalin were processed into 4 μm thick paraffin sections and stained with hematoxylin and eosin for histological evaluation. Briefly, paraffin-embedded liver sections were deparaffinized in xylene and rehydrated using graded alcohol. The tissue sections were stained with Harris' hematoxylin for 8 min, washed with 1% HCl in 70% alcohol, dipped in ammonia water for 5 times, and washed with distilled water. The sections were then stained with eosin for 5 - 10 sec, dehydrated in graded alcohol series, cleared with xylene, and coverslipped.

6. Measurement of liver homocysteine

To determine the concentration of liver homocysteine (Hcy), tissues were homogenized in 20 volumes (w/v) of cold homogenizing buffer containing 154 mM KCl, 50 mM Tris-HCl, and 1 mM EDTA (pH 7.4). Homogenates were centrifuged at 600g for 10 min at 4℃ to obtain supernatant. The homogenates, standards and isocratic buffer were prepared as slightly modified method (Araki et al. 1987, Minniti et al. 1998). Each homogenate, 50 μL was mixed with 2.5 μL of 0.625 mM acetylcysteine (Sigma, USA) in 0.9% NaCl with 4 mM EDTA solution as an internal standard. A fresh 2.5 μL of 20% (v/v) tri-n-butylphosphine (Sigma) in dimethylformamide (Sigma) solution was added and samples were incubated at 4℃ for 30 min to allow the reduction of thiols and to decouple them from hepatic proteins. Fifty μL of 10% trichloroacetic acid (Sigma) containing 1 mM EDTA solution was added and incubated for 10 min at room temperature. After samples were centrifuged for 10 min at 13,000 rpm at 4℃, 50 μL of the supernatant was mixed with 50 μL of 1 mg/mL ammonium 6-fluorobenzo-2-oxa- 1,3-diazole-4-sulfonate (Fluka, USA) in 0.125 M potassium tetraborate solution (pH 9.5), and 100 μìL of 0.125 M potassium tetraborate with 4 mM EDTA solution (pH 10.5) then incubated at 60℃ for 60 min. After terminating the reaction, cooling the samples on ice, filtrating with syringe filter, 20 μL of the sample was injected into the HPLC system by autosampler. Hcy standard (Sigma) was dissolved in distilled water and carried through the sample preparation procedure. The analytical column (Luna® C18(2) column 250 x 4.6 mm I.D., 5 μm, Phenomenex) was conditioned with mobile phase at flow rate 1.2 mL/min. Mobile phase was consisted of 0.1 M sodium acetate, 0.1 M acetic acid finally containing 2% methanol and adjusted to pH 4.0 with acetic acid. Then the mobile

phase was filtrated through a 0.45 μm filter. The optimal response was observed using a flourometric detector when the excitation and emission wavelengths are set at 385 and 515 mm, respectively.

7. Total RNA extraction and quantitative real-time PCR analysis

Total RNA of liver tissue was isolated using RNAiso Plus (Takara, Japan). Approximately 50 mg of liver tissue was homogenized with 1 mL of RNAiso Plus and 5 mm sterile stainless steel beads using a Tissue-Lyser system (Qiagen, Netherlands). The homogenate was left in room temperature for 5 min and 200 μL of chloroform was added and vigorously mixed for 15 sec. The mixture was left in room temperature for 3 min and centrifuged at 12,000g for 15 min at 4℃. Four hundred μL of supernatant was transferred to a new tube and 500 μL of isopropanol was added. The mixture was left in room temperature for 10 min and centrifuged at 12,000g for 10 min at 4℃. After discarding the supernatant and rinsing with 1 mL of 75% ethanol, the RNA pellet was centrifuged at 7,500g for 2 min at 4℃. The purified RNA precipitate was resuspended in diethyl pyrocarbonate-treated water, and the concentration and purity of RNA was measured by optical density at 260 nm and 280 nm. cDNA was synthesized using 2 μg of total RNA with the Superscript® Ⅱ Reverse Transcriptase (Invitrogen, USA). All amplification reactions were performed using a StepOne™ Real Time PCR System (Applied Biosystems, USA) using SYBR® Green PCR Master Mix (Applied Biosystems) according to the manufacturer's protocol. The PCR condition was 40 cycles of the following protocol; denaturation at 95℃ for 10 sec; and then annealing and extension at 60℃ for 1 min. Mouse 60S ribosomal protein L19 (*Rpl19*) was used for reference

gene and relative gene expression levels were analyzed using the $2^{-\Delta\Delta Ct}$ method. Primer sequences are shown in Table 2.

Table 2. Quantitative real-time PCR primer sequences

Gene	Sequence (5'-3')	
Asl	Forward	ATC TGT GGA ATG TGG ATG TGC
	Reverse	TTC AGC AAC CTT GTC CAA CC
Nnmt	Forward	GCT GAA GAA GGA ACC AGG AG
	Reverse	CTT GAT TGC ACG CCT CAA C
Rpl19	Forward	TCA GGC TAC AGA AGA GGC TTG C
	Reverse	ATC AGC CCA TCC TTG ATC AGC
Sds	Forward	TGC CAT CAA GGA AGG AAA GC
	Reverse	AAA CAG CTT CAG GGT CTG C

Asl, argininosuccinate lyase; *Nnmt*, nicotinamide N-methyltransferase; *Rpl19*, 60S ribosomal protein L19; *Sds*, serine dehydratase.

8. Total protein extraction and immunoblotting

Equal amounts of liver tissues were homogenized in protein lysis buffer [50 mM Hepes-KOH (pH 7.5), 150 mM NaCl, 1 mM EDTA (pH 8.0), 2.5 mM EGTA (pH 8.0), 1 mM NaF, 10 mM β -glycerophosphate, 0.1 mM Na_3VO_4, 1mM DTT. 0.1% Tween-20, 10% glycerol, protease inhibitor cocktail (Sigma)] using Tissue-Lyser system with 5mm sterile stainless steelbeads. After centrifugation at 10,000g, 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). Equal amounts of protein were loaded into the lanes of polyacrylamide gel, separated by Tris-glycine running buffer system and them transferred to polyvinylidene fluoride membrane using semi-dry electrotransferring unit (Bio-rad) at 15 V for 60 min. Membranes were blocked with nonfat milk or bovine serum albumin (BSA) 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 BSA in TTBS. The specific antibodies used are as follows: anti-phospho Insulin receptor substrate 1 (p-IRS1; #2384, Cell Signaling Technology, USA), anti-β-actin (A5441, Sigma). β-actin 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 visusalized using Immobilon western chemiluminescent using HRP substrate (Millipore, USA) by exposure to X-ray film (Fuji, Japan). Band intensities were quantified using Quantity One software (Bio-rad).

9. Microarray hybridization and data analysis

Total RNA was isolated from using RNAiso Plus (Takara) and RNA purity and integrity were evaluated by Agilent 2100 Bioanalyzer (Agilent Technologies, USA). Transcriptome profiles were analyzed using Affymetrix Mouse Clariom S arrays (Thermo Fisher Scientific, USA). cDNA was synthesized using the GeneChip Whole Transcript Amplification kit (Thermo Fisher Scientific) as described by the manufacturer. The sense cDNA was then fragmented and biotin-labeled with terminated deoxynucleotidyl transferase using the GeneChip WT Terminal labeling kit. Approximately 5.5 μg of labeled DNA target was hybridized to the Affymetrix GeneChip Array and incubated at 45℃ for 16 h. Hybridized arrays were washed and stained on the Affymetrix GeneChip Fluidics Station 450, and scanned on the GeneChip 3000 Scanner. Data were collected using Affymetrix GeneChip Command Console Software. Reliability, maintainability, and availability analysis performed with Affymetrix Power Tools Software. Probe signal value was transformed by logarithm and normalized. Statistical significance of the expression data was determined using one-way analysis of variance (ANOVA). DEGs were selected according to the criteria of |fold change| $\geq$1.5 and false discovery rate (FDR) $<$ 0.05. Functional analysis of DEGs was performed using functional annotation and clustering tool of GeneOntology (GO, http://geneontology.org/), KEGG pathway (http://www.kegg.jp/kegg/pathway.html), and WikiPathways (https://www.wikipathways.org). Heat map was visualized using the PermutMatrix software (http://www.atgc-montpellier.fr/permutmatrix/). R package (www.r-project.org) was used to generate a dot plot.

10. Statistical analysis

All data except microarray data were analyzed using SPSS software v 23 (IBM SPSS Inc., USA). Data were analyzed by one-way ANOVA followed by Duncan's multiple range test. Data were showed as the mean ± SEM and differences were considered significant at $P < 0.05$.

Ⅳ. Results

1. Body and organ weights

Body weight, liver weight and epididymal fat weight of mice were measured (Table 3). Body weight, liver weight and epididymal fat weight of HFD fed groups (HFD7, HFD1) were significantly higher than those of CD groups (CD7, CD1). There were no statistical changes in body weight, liver weight and epididymal fat weight between CD7 and CD1 or HFD7 and HFD1.

Table 3. Body and organ weights

	Diet			
	CD7	CD1	HFD7	HFD1
Initial body weight (g)	21.5 ± 0.37	21.4 ± 0.38	21.4 ± 0.35	21.6 ± 0.36
Final body weight (g)	31.7 ± 1.45^{b}	30.16 ± 1.13^{b}	40.64 ± 2.14^{a}	37.83 ± 1.31^{a}
Liver weight (g)	1.17 ± 0.05^{b}	1.11 ± 0.04^{b}	2.30 ± 0.25^{a}	1.90 ± 0.15^{a}
Relative liver weight (% of body weight)	3.70 ± 0.09^{b}	3.70 ± 0.07^{b}	5.57 ± 0.34^{a}	4.99 ± 0.25^{a}
Epididymal fat weight (g)	0.90 ± 0.16^{b}	0.65 ± 0.17^{b}	1.88 ± 0.22^{a}	1.70 ± 0.17^{a}
Relative epididymal fat weight (% of body weight)	2.72 ± 0.39^{b}	2.06 ± 0.42^{b}	4.52 ± 0.41^{a}	4.43 ± 0.34^{a}

Data are presented as mean ± SEM (n=7-9). Different superscripts are significantly different at $P < 0.05$ (One-way ANOVA followed by Duncan's multiple comparison test). CD7, a control diet with 7 mg PN/kg diet; CD1, a control diet with 1 mg PN/kg diet; HFD7, a high-fat diet with 7 mg PN/kg diet; HFD1, a high-fat diet with 1 mg PN/kg diet.

2. Vitamin B6 concentration in serum and the liver

Serum and liver PLP concentrations were measured to determine changes in *in vivo* vitamin B6 metabolism of the mice affected by different amounts of fat content and vitamin B6 in the diet (Figure 3). Relative serum PLP in CD1 and HFD1 were significantly lower compared with CD7 and HFD7, respectively. A comparison of CD7 and HFD7 indicates that ingestion of HFD is the cause of decreased serum PLP levels. Both insufficient intake of vitamin B6 and the HFD consumption lowered the level of PLP, therefore serum concentration was lowest in HFD1 (Figure 3A). Relative hepatic PLP levels were expressed as 'nmol/g liver' and 'nmol/mg protein' (Figure 3B and 3C, respectively). There was no statistical change in relative hepatic PLP level of 'nmol/mg protein' but CD1 and HFD1 tended to have lower levels than CD7 and HFD7, respectively (Figure 3C). Relative hepatic PLP level of 'nmol/g liver' in HFD7 was significantly lower than in CD7 (Figure 3B).

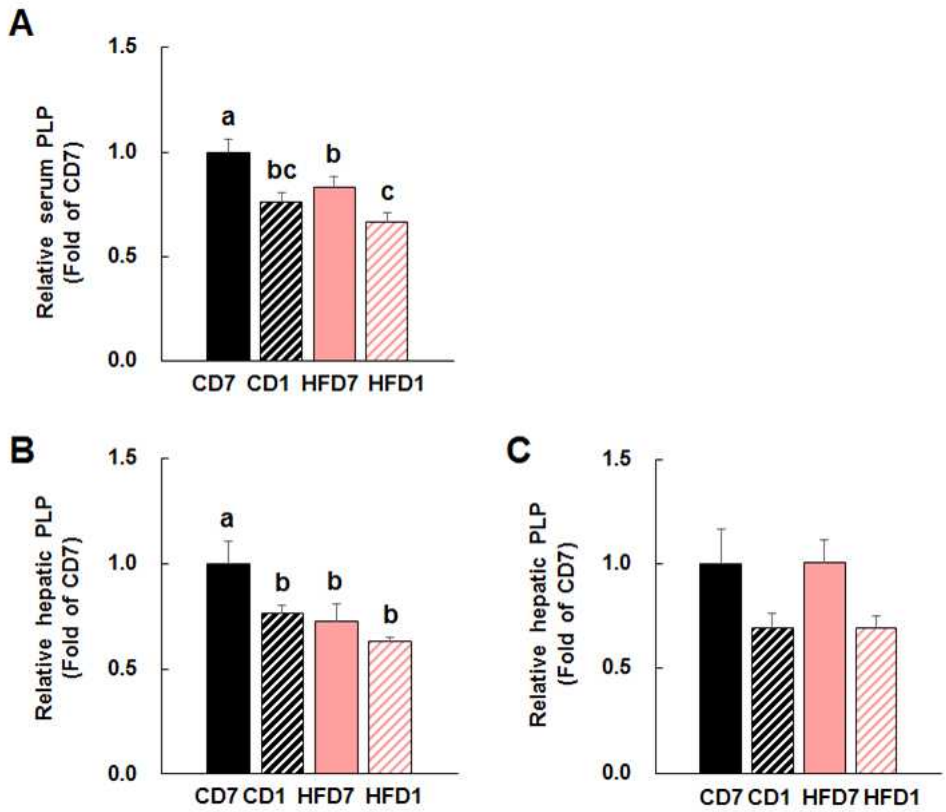

Figure 3. PLP concentrations of serum and the liver

(A) Relative serum PLP level (expressed as umol/L) (n=7-8), (B) Relative hepatic PLP level (expressed as nmol/g liver) (n=3) and (C) Relative hepatic PLP level (expressed as nmol/mg protein) (n=3). Data are presented as mean ± SEM. Different superscripts are significantly different at $P < 0.05$ (One-way ANOVA followed by Duncan's multiple comparison test). CD7, a control diet with 7 mg PN/kg diet; CD1, a control diet with 1 mg PN/kg diet; HFD7, a high-fat diet with 7 mg PN/kg diet; HFD1, a high-fat diet with 1 mg PN/kg diet.

3. Biochemical parameters in serum

Serum glucose, triglyceride (TG), and total cholesterol (TC) were measured in order to confirm the changes in blood parameters by the experimental diet (Table 4). There was no significant difference in serum glucose in CD groups. On the other hand, in HFD groups, the serum glucose level of the HFD1 was significantly lower than that of the HFD7. Serum TG levels were not affected by dietary vitamin B6 but were decreased by HFD. Serum TC concentration was significantly higher in mice fed HFD compared to mice fed CD. No difference in serum total cholesterol levels was observed according to the vitamin B6 content in the diets regardless of whether they were fed CD or HFD.

Serum glutamate-pyruvate transaminase (GPT) level is commonly measured as a biomarker for liver health. Serum GPT was significantly higher in HFD7 compared with CD7, suggesting that HFD consumption damages the liver. The marginal deficiency of vitamin B6 did not affect serum GPT level (Table 4).

4. Biochemical parameters in the liver

Hepatic TG and cholesterol were measured to determine changes in liver lipid contents by ingestion of the experimental diet (Table 4, Figure 4). TG and cholesterol concentrations of the liver were significantly higher in the HFD groups compared to the CD groups. Vitamin B6 deficient diet groups CD1 and HFD1 did not have significantly lower TG levels compared to mice fed CD7 and HFD7. Hepatic cholesterol content was also not altered by marginal vitamin B6 deficiency. Total bile acid content in the liver was not different among four groups, but CD1 tended to have higher levels than CD7 (t-test, P=0.07).

Table 4. Biochemical parameters in serum and the liver

	Diet			
	CD7	CD1	HFD7	HFD1
Serum				
Glucose (mg/dL)	96.0 ± 18.3^{b}	63.4 ± 13.0^{b}	157.7 ± 21.1^{a}	107.4 ± 8.6^{b}
TG (mg/dL)	98.0 ± 11.0^{ab}	111.9 ± 13.8^{a}	60.9 ± 7.5^{c}	72.9 ± 8.0^{bc}
TC (mg/dL)	120.1 ± 7.8^{b}	109.6 ± 6.2^{b}	162.9 ± 12.5^{a}	147.4 ± 7.4^{a}
GPT (IU/L)	19.5 ± 2.6^{b}	20.6 ± 2.7^{b}	58.0 ± 10.0^{a}	46.2 ± 7.2^{a}
Liver				
TG (mg/mg protein)	0.48 ± 0.11^{b}	0.44 ± 0.04^{b}	1.54 ± 0.37^{a}	1.15 ± 0.04^{a}
Cholesterol (mg/mg protein)	0.038 ± 0.003^{b}	0.044 ± 0.002^{b}	0.082 ± 0.004^{a}	0.083 ± 0.005^{a}
Total bile acid (nmol/g liver)	415.8 ± 45.4	582.1 ± 65.3	515.0 ± 66.2	555.5 ± 67.8

Data are presented as mean ± SEM (n=7-9). Different superscripts are significantly different at $P < 0.05$ (One-way ANOVA followed by Duncan's multiple comparison test). CD7, a control diet with 7 mg PN/kg diet; CD1, a control diet with 1 mg PN/kg diet; HFD7, a high-fat diet with 7 mg PN/kg diet; HFD1, a high-fat diet with 1 mg PN/kg diet; TG, triglyceride; TC, total cholesterol; GPT, Glutamate-pyruvate transaminase.

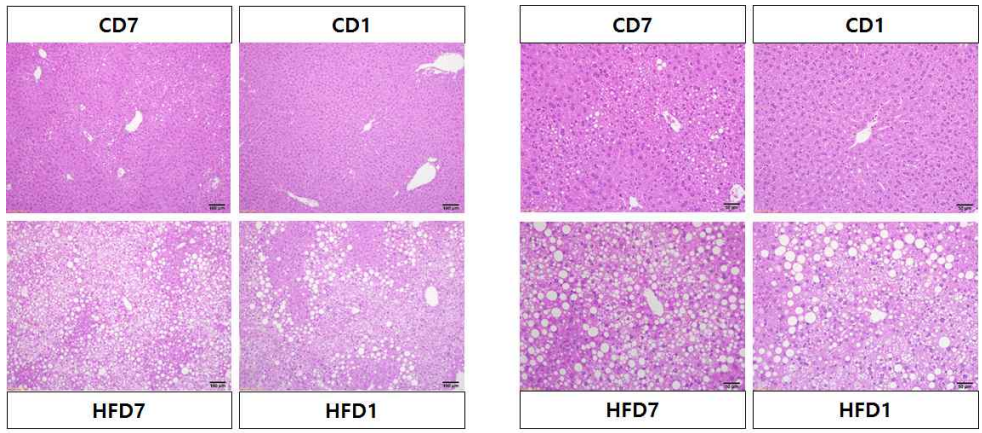

Figure 4. Hematoxylin and eosin staining of the liver

Magnification 100× in left panels and 200× in the right panels (n=4). CD7, a control diet with 7 mg PN/kg diet; CD1, a control diet with 1 mg PN/kg diet; HFD7, a high-fat diet with 7 mg PN/kg diet; HFD1, a high-fat diet with 1 mg PN/kg diet.

5. Effects of marginal vitamin B6 deficiency on hepatic transcriptomes

Global gene expression was analyzed to identify the characteristics of hepatic gene expression profiles related to marginal vitamin B6 deficiency. One-way ANOVA analysis showed that expressions of 4,000 genes were significantly altered, which was remarkably influenced by dietary fat rather than marginal vitamin B6 deficiency (Figure 5).

Figure 6 shows the number of DEGs whose expression has been altered by marginal vitamin B6 deficiency in mice fed a diet with the same fat content. The DEGs between CD7 and CD1 (CD1/CD7) was 108, of which half was upregulated and the other half was downregulated. There were 44 DEGs in HFD1/HFD7 comparison. 26 of them were upregulated and 18 were downregulated. Among 108 DEGs in CD1/CD7 and 43 DEGs in HFD1/HFD7, only three DEGs, *Asl, Nnmt,* and *Sds*, were in the overlapped section between CD1/CD7 and HFD1/HFD7 (Figure 6). These genes were involved in amino acid metabolism. For the expression of the three genes, the microarray results were described with the dot plots and the PCR validation test was shown using the bar graph (Figure 7).

The low number of genes in the overlapped section means that the effect of marginal vitamin B6 deficiency is obviously different depending on the level of dietary fat. This can be seen not only in the venn diagram but also in the KEGG enrichment heatmap (Figure 8). According to the KEGG enrichment heatmap, enrichment results of the pathways except for 'Metabolic pathways' were very different between CD1/CD7 and HFD1/HFD7. 'Jak-STAT signaling pathway' (FDR = 0.005) and 'Steroid biosynthesis' (FDR = 0.015), were significant in CD1/CD7 comparison. On the other hand, comparison of

HFD1/HFD7 shows that ‘Carbon metabolism’ (FDR = 0.001), ‘Biosynthesis of amino acids’ (FDR = 0.008), ‘Alanine, aspartate and glutamate metabolism’ (FDR < 0.001), ‘Cysteine and methionine metabolism’ (FDR = 0.044) and ‘Phenylalanine, tyrosine and tryptophan biosynthesis’ (FDR =0.044) were significant by KEGG pathways analysis.

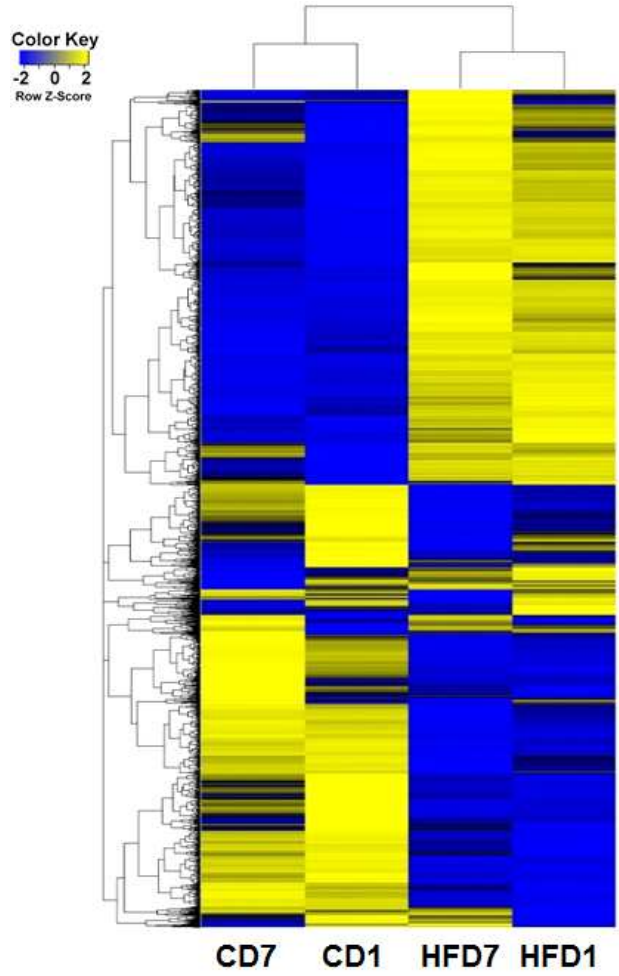

Figure 5. Effects of experimental diets on the global gene expression

Two-dimensional clustering of gene expression profiles. Each cell represents the mean value of gene expression in each group (n=3). The 4,000 DEGs were identified by one-way ANOVA ($P < 0.05$). CD7, a control diet with 7 mg PN/kg diet; CD1, a control diet with 1 mg PN/kg diet; HFD7, a high-fat diet with 7 mg PN/kg diet; HFD1, a high-fat diet with 1 mg PN/kg diet.

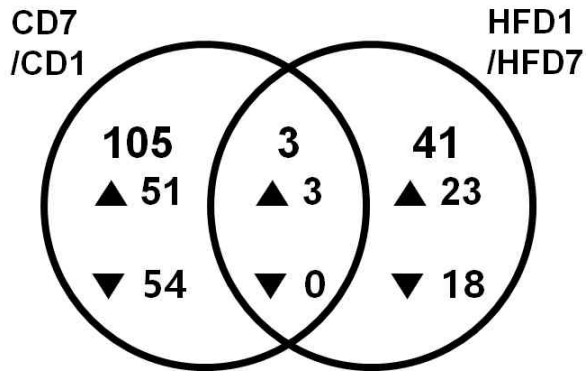

Figure 6. Venn diagram representing the effect of vitamin B6 deficiency

CD7, a control diet with 7 mg PN/kg diet; CD1, a control diet with 1 mg PN/kg diet; HFD7, a high-fat diet with 7 mg PN/kg diet; HFD1, a high-fat diet with 1 mg PN/kg diet.

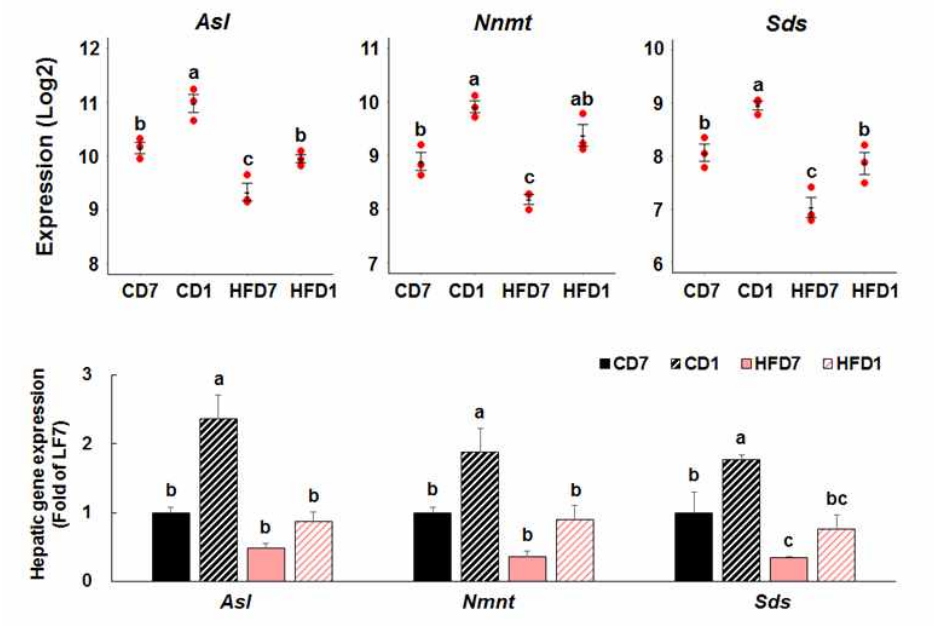

Figure 7. Gene expressions of venn diagram in overlapped section

Expressions of genes (*Asl, Nnmt* and *Sds*) from microarray were presented as dot plots and PCR validation data were presented as bar graphs. Different superscripts are significantly different at $P < 0.05$ (One-way ANOVA followed by Duncan's multiple comparison test). *Asl*, argininosuccinate lyase; *Nnmt*, nicotinamide N-methyltransferase; *Sds*, serine dehydratase; CD7, a control diet with 7 mg PN/kg diet; CD1, a control diet with 1 mg PN/kg diet; HFD7, a high-fat diet with 7 mg PN/kg diet; HFD1, a high-fat diet with 1 mg PN/kg diet.

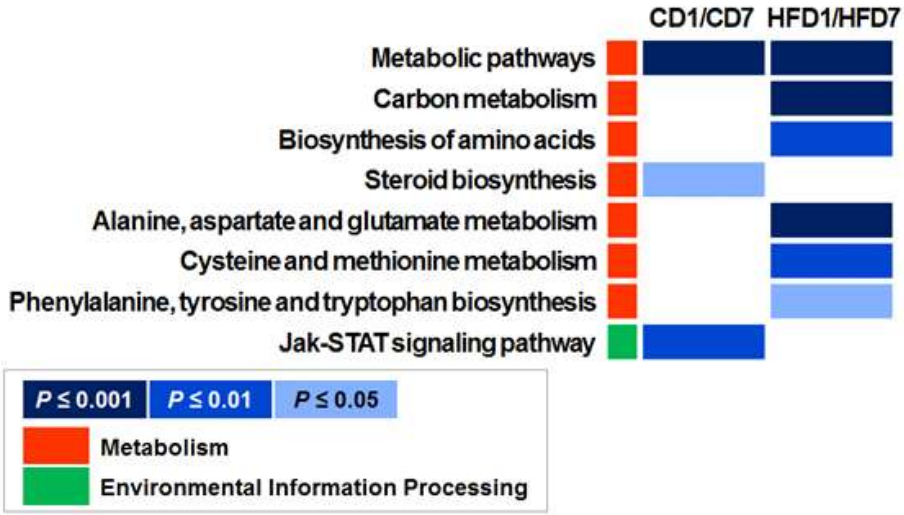

Figure 8. KEGG enrichment heatmap representing the effect of vitamin B6 deficiency

Comparison of vitamin B6 deficiency between CD and HFD using KEGG enrichment heat map. CD7, a control diet with 7 mg PN/kg diet; CD1, a control diet with 1 mg PN/kg diet; HFD7, a high-fat diet with 7 mg PN/kg diet; HFD1, a high-fat diet with 1 mg PN/kg diet; STAT, signal transducer and activator of transcription.

6. Effects of margianl vitamin B6 deficiency on hepatic transcriptomes in mice fed a control diet

6.1 Upregulated DEGs of CD1/CD7 comparison

The DEGs between CD7 and CD1 (CD1/CD7) were 108, of which half was upregulated and the other half was downregulated (Figure 6). In the enrichment analysis of the upregulated DEGs, GO terms related to cholesterol metabolism such as 'Sterol metabolic process', 'Organic hydroxyl compound metabolic process' and 'Steroid metabolic process' were significant (Table 5). This result indicates that the expression of genes related to cholesterol metabolism is upregulated in CD1 compared to CD7. The top 10 GO terms were described in Table 5 and the 11 gene expressions included in the top 10 GO terms were visualized with a heatmap (Figure 9).

GO term-based enrichment analysis is limited because it was performed by selecting the fold change (FC) ≥ 1.5 DEGs. Therefore, WikiPathway and KEGG pathway were used to identify all of the genes involved in cholesterol metabolism. As a result, expressions of *Hmgcr, Idi1, Fdps, Lss, Cyp51, Msmo1* and *Nsdhl* were significantly higher in CD1 compared to CD7 (Figure 10). However, the content of liver cholesterol was not different between CD7 and CD1 (Table 4), so we assumed that cholesterol was converted to bile acid. Among the genes included in the Top 10 GO term, there was *Cyp39a1* involved in bile acid synthesis, so the total bile acid content was expected to be affected. The content of hepatic total bile acid was not significantly different between CD7 and CD1 but showed an increasing tendency in CD1 (Table 4; t-test, P=0.07). Among genes involved in bile acid synthesis, *Cyp39a1* expression was significantly higher in CD1 than in CD7. There was no significant difference between CD7 and CD1 but *Cyp7a1, Cyp7b1,* and *Cyp8b1* expression

were higher in CD1 than in CD7 (FC 1.49, 1.21, 1.19 respectively; Data not shown).

Table 5. Top 10 enriched GO terms of upregulated DEGs in CD1/CD7

Category	Term	FDR	Fold enrichment
Biological process	GO:0016126~ Sterol biosynthetic process	5.24E-05	47.1
	GO:0006695~ Cholesterol biosynthetic process	1.49E-03	43.8
	GO:1902653~ Secondary alcohol biosynthetic process	1.60E-03	42.8
	GO:0016125~ Sterol metabolic process	2.35E-06	27.7
	GO:0008203~ Cholesterol metabolic process	5.13E-05	25.3
	GO:1902652~ Secondary alcohol metabolic process	6.36E-05	24.2
	GO:0006694~ Steroid biosynthetic process	1.70E-04	19.8
	GO:0008202~ Steroid metabolic process	1.48E-05	14.4
	GO:1901615~ Organic hydroxy compound metabolic process	1.23E-05	11.3
	GO:0044283~ Small molecule biosynthetic process	5.21E-05	9.3

GO terms are indicated in descending order of fold enrichment. FDR, false discovery rate; CD7, a control diet with 7 mg PN/kg diet; CD1, a control diet with 1 mg PN/kg diet.

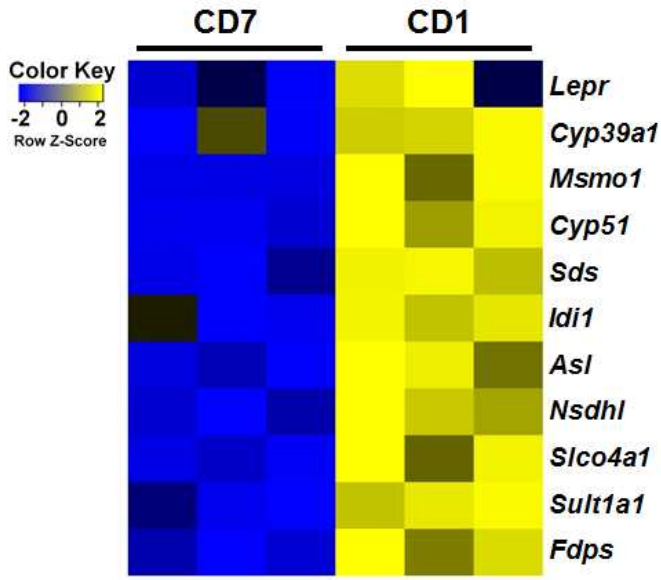

Figure 9. Heatmap of upregulated DEGs in Top 10 GO terms of CD1/CD7 comparison

Each cell represents the single sample value of gene expression. Gene names of each gene symbol are showed in Appendix 1. CD7, a control diet with 7 mg PN/kg diet; CD1, a control diet with 1 mg PN/kg diet.

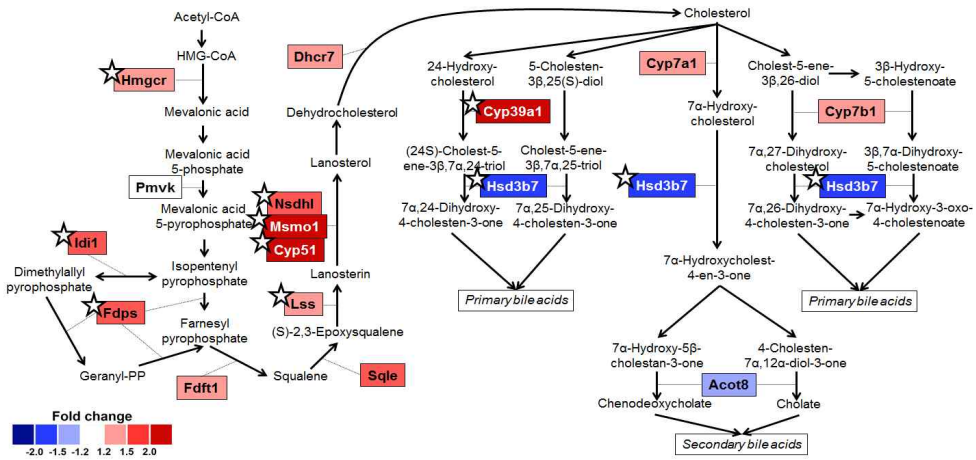

Figure 10. Cholesterol and bile acid biosynthetic metabolism of CD1/CD7 comparison

Gene expression profile in cholesterol and bile acid pathway. Significant changes are marked with ☆. (Modified from KEGG pathway and WikiPathway) Gene names of each gene symbol are showed in Appendix 2. CD7, a control diet with 7 mg PN/kg diet; CD1, a control diet with 1 mg PN/kg diet.

6.2 Downregulated DEGs of CD1/CD7 comparison

Of the 108 DEGs in CD1/CD7 comparison, the remaining 54 genes were downregulated (Figure 6). As a result of GO term-based enrichment analysis of downregulated DEGs, Table 6 was obtained. The GO terms associated with immune responses such as 'Response to interferon-beta', 'Regulation of viral process', 'Response to cytokine', and 'Innate immune response' were significant. The expression of the genes in the Top 10 GO terms were visualized as a heatmap (Figure 11).

Table 6. Top 10 enriched GO terms of downregulated DEGs in CD1/CD7

Category	Term	FDR	Fold enrichment
Biological process	GO:0035456~ Response to interferon-beta	4.28E-03	31.8
	GO:0034341~ Response to interferon-gamma	2.44E-03	18.4
	GO:0031331~ Positive regulation of cellular catabolic process	2.23E-03	11.9
	GO:0050792~ Regulation of viral process	1.68E-04	10.2
	GO:0043903~ Regulation of symbiosis, encompassing mutualism through parasitism	3.10E-04	9.3
	GO:0009896~ Positive regulation of catabolic process	7.87E-03	9.0
	GO:0043900~ Regulation of multi-organism process	1.82E-03	7.0
	GO:0034097~ Response to cytokine	6.42E-04	5.8
	GO:0045087~ Innate immune response	2.99E-03	5.4
	GO:0002682~ Regulation of immune system process	3.45E-03	3.7

GO terms are indicated in descending order of fold enrichment. FDR, false discovery rate; CD7, a control diet with 7 mg PN/kg diet; CD1, a control diet with 1 mg PN/kg diet.

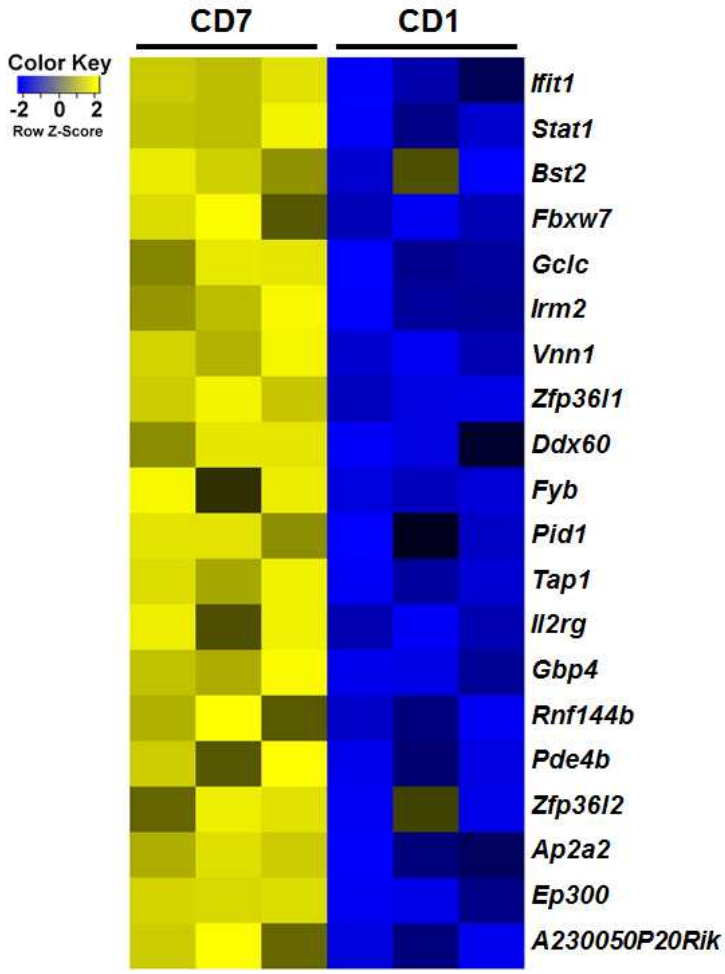

Figure 11. Heatmap of downregulated DEGs in Top 10 GO terms of CD1/CD7 comparison

Each cell represents the single sample value of gene expression. Gene names of each gene symbol are showed in Appendix 3. CD7, a control diet with 7 mg PN/kg diet; CD1, a control diet with 1 mg PN/kg diet.

7. Effects of marginal vitamin B6 deficiency on hepatic transcriptomes in mice fed a high-fat diet

7.1 Upregulated DEGs of HFD1/HFD7 comparison

As described above, 44 DEGs were observed in HFD1/HFD7 (Figure 6). Twenty-six of them showed higher expression and the remaining 18 showed lower expression in HFD1 than in HFD7. GO term-based enrichment analysis was performed with 26 DEGs that were upregulated. The terms related to organic acids and amino acids such as 'Cellular amino acid catabolic process', 'Small molecule biosynthetic process', 'Organic acid biosynthetic process' and 'Alpha-amino acid metabolic process' were significant. Also, in the category of molecular function, 'Pyridoxal phosphate binding' was significant (Table 7). There were 7 genes constituting GO terms corresponding to the biological process. Of the 7 genes, *Sds, Lao1, Agxt, Got1* and *Asl* were associated with amino acid metabolism (Figure 12).

The GO term analysis included only DEGs with FC $\geq$ 1.5, so we identified the overall amino acid metabolism. All of the genes constituting 'Alanine, aspartate and glutamate metabolism' and 'Cysteine and methionine metabolism', which are significantly shown in the KEGG enrichment heatmap (Figure 8), are expressed in Figure 13. Among the genes involved in 'Alanine, aspartate and glutamate metabolism', expressions of *Agxt, Asl, Got1, Cps1* and *Ppat* were significantly higher in HFD1 compared to HFD7 (Figure 13A).

Among the 37 genes involved in 'Cysteine and methionine metabolism', expressions of *Sds, Cth, Got1, Agxt2, Mat2a, Tat* and *Lao1* were significantly upregulated in HFD1 compared to HFD7. There was no gene whose expression was significantly decreased in HFD1 compared to HFD7 (Figure 13B). Since Hcy is a one representative metabolite of 'Cysteine and methionine metabolism', the

effect of marginal vitamin B6 deficiency on this metabolism was investigated by measuring the Hcy concentration in the liver (Figure 14). Vitamin B6 deficient diet groups CD1 and HFD1 showed greater increase than those of CD7 and HFD7, respectively.

Table 7. Top 10 enriched GO terms of upregulated DEGs in HFD1/HFD7

Category	Term	FDR	Fold enrichment
Biological process	GO:0009063~ Cellular amino acid catabolic process	7.97E-04	50.9
	GO:0006520~ Cellular amino acid metabolic process	9.85E-04	21.0
	GO:0046395~ Carboxylic acid catabolic process	1.22E-02	20.2
	GO:1901605~ Alpha-amino acid metabolic process	1.58E-02	18.4
	GO:0046394~ Carboxylic acid biosynthetic process	1.79E-03	18.0
	GO:0016054~ Organic acid catabolic process	1.73E-02	17.9
	GO:0016053~ Organic acid biosynthetic process	2.34E-03	16.8
	GO:0044283~ Small molecule biosynthetic process	1.71E-03	11.2
	GO:0019752~ Carboxylic acid metabolic process	2.14E-02	6.5
Molecular function	GO:0030170~ Pyridoxal phosphate binding	1.65E-02	51.1

GO terms are indicated in descending order of fold enrichment. FDR, false discovery rate; HFD7, a high-fat diet with 7 mg PN/kg diet; HFD1, a high-fat diet with 1 mg PN/kg diet.

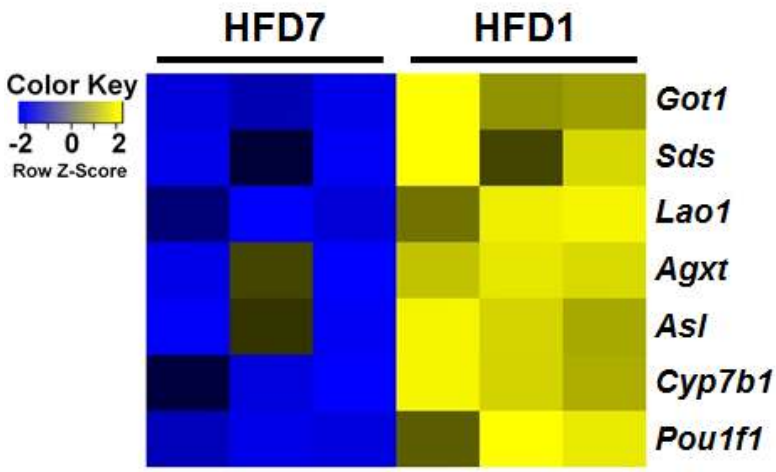

Figure 12. Heatmap of upregulated DEGs in Top 10 GO terms of HFD1/HFD7 comparison

Each cell represents the single sample value of gene expression. Gene names of each gene symbol are showed in Appendix 4. HFD7, a high-fat diet with 7 mg PN/kg diet; HFD1, a high-fat diet with 1 mg PN/kg diet.

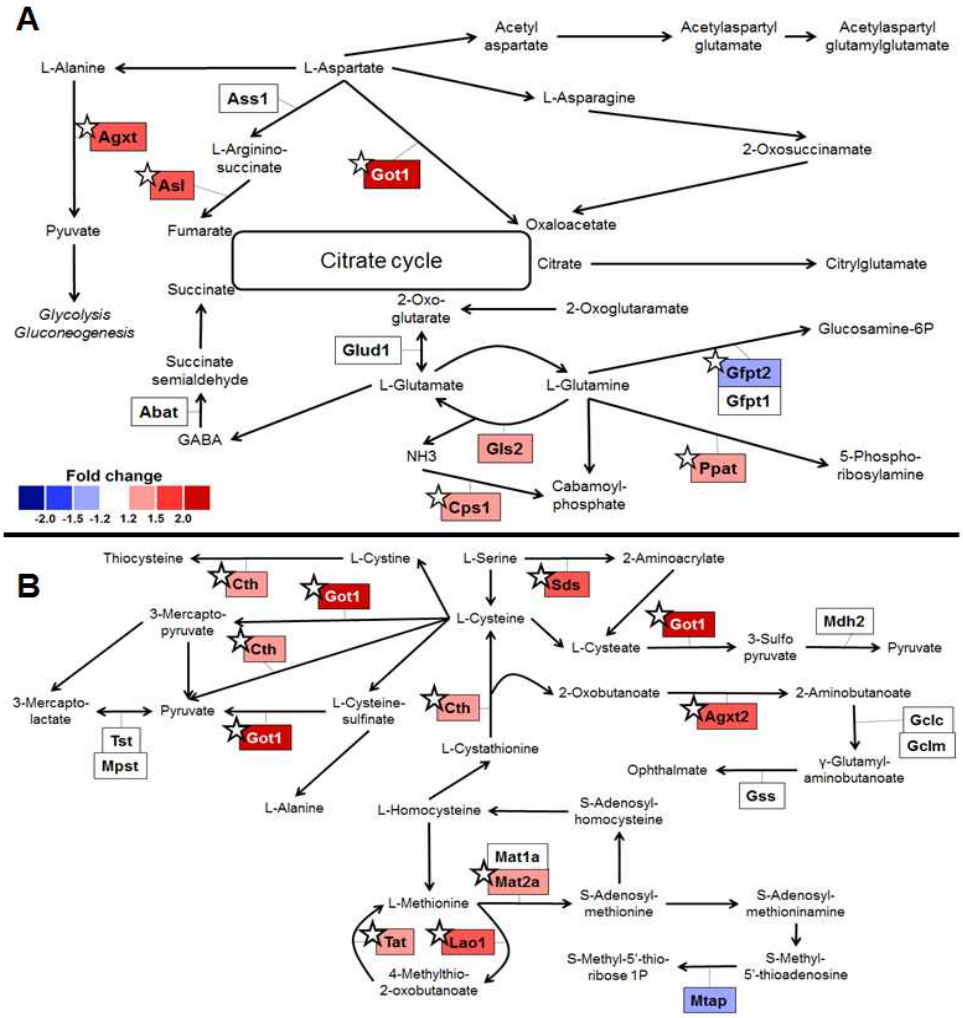

Figure 13. Amino acid metabolism of HFD1/HFD7 comparison

(A) Alanine, aspartate and glutamate metabolism and (B) Cysteine and methionine metabolism are presented. Significant changes are marked with ☆. (Modified from KEGG pathway) Gene names of each gene symbol are showed in Appendix 5 and 6. HFD7, a high-fat diet with 7 mg PN/kg diet; HFD1, a high-fat diet with 1 mg PN/kg diet.

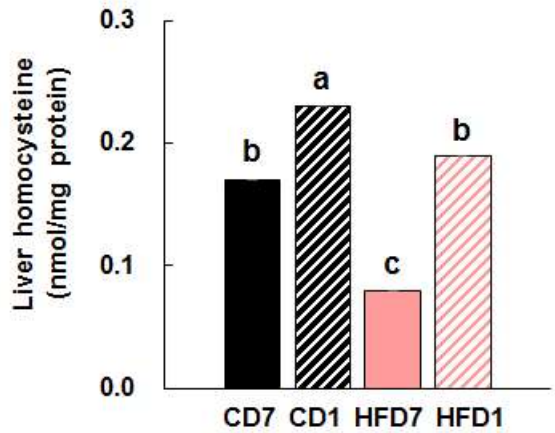

Figure 14. Homocysteine concentration of the liver

Data are presented as mean ± SEM (n=7-9). Different superscripts are significantly different at $P < 0.05$ (One-way ANOVA followed by Duncan's multiple comparison test). CD7, a control diet with 7 mg PN/kg diet; CD1, a control diet with 1 mg PN/kg diet; HFD7, a high-fat diet with 7 mg PN/kg diet; HFD1, a high-fat diet with 1 mg PN/kg diet.

7.2 Downregulated DEGs of HFD1/HFD7 comparison

Downregulated DEGs of HFD1/HFD7 are presented in Table 8. GO term-based enrichment analysis of those DEGs showed no significant GO term in HFD1/HFD7.

Expressions of *Fasn* involved in fatty acid synthesis and *Gck* involved in carbohydrate metabolism are lower in HFD1 than in HFD7. The genes involved in protein metabolism are *Lox* and *Mmp2*, which are related to collagen formation. *Lox* and *Mmp2* expressions were lower in the HFD1 group than in the HFD7 group.

Gck can mediate phosphorylation of glucose to glucose-6-phosphate (G6P), which is the first step of glycogen synthesis and glycolysis. We measured the phosphorylated insulin receptor substrate 1 (p-IRS1) protein by western blotting to identify lowered hepatic glycogen indirectly. IRS1 is a signaling adapter protein that transmits signals from the insulin. When insulin resistance occurs, more serine phosphorylated IRS1 is produced (Copps et al. 2012). The expressions of p-IRS1 of HFD1 was higher than that of HFD7 (Figure 15).

Table 8. Gene lists of downregulated DEGs in HFD1/HFD7

Gene symbol	Fold change	FDR correction *P* value	Description
Ly6c2	-2.16	2.57E-02	lymphocyte antigen 6 complex, locus C2
St3gal6	-1.82	2.05E-02	ST3 beta-galactoside alpha-2,3-sialyltransferase 6
Lox	-1.77	4.05E-02	lysyl oxidase
Ly6c1	-1.75	4.29E-02	lymphocyte antigen 6 complex, locus C1
Rsad2	-1.73	2.58E-02	radical S-adenosyl methionine domain containing 2
Sirpb1a	-1.73	3.11E-02	signal-regulatory protein beta 1A
Zfp536	-1.71	2.95E-02	zinc finger protein 536
Sytl2	-1.68	4.58E-02	synaptotagmin-like 2
Gck	-1.66	1.78E-02	glucokinase
Asb7	-1.63	8.77E-03	ankyrin repeat and SOCS box-containing 7
Frs3	-1.61	2.83E-02	fibroblast growth factor receptor substrate 3
Trim34b	-1.59	1.87E-02	tripartite motif-containing 34B
Mmp2	-1.56	4.97E-02	matrix metallopeptidase 2
Fasn	-1.56	2.16E-02	fatty acid synthase
Adgb	-1.55	1.19E-04	androglobin
Rasgrp3	-1.55	2.74E-02	RAS, guanyl releasing protein 3
Itgbl1	-1.54	1.22E-02	integrin, beta-like 1
1700049L16Rik	-1.53	3.08E-02	hematological and neurological expressed 1-like pseudogene

GO terms are indicated in ascending order of fold change. FDR, false discovery rate; HFD7, a high-fat diet with 7 mg PN/kg diet; HFD1, a high-fat diet with 1 mg PN/kg diet.

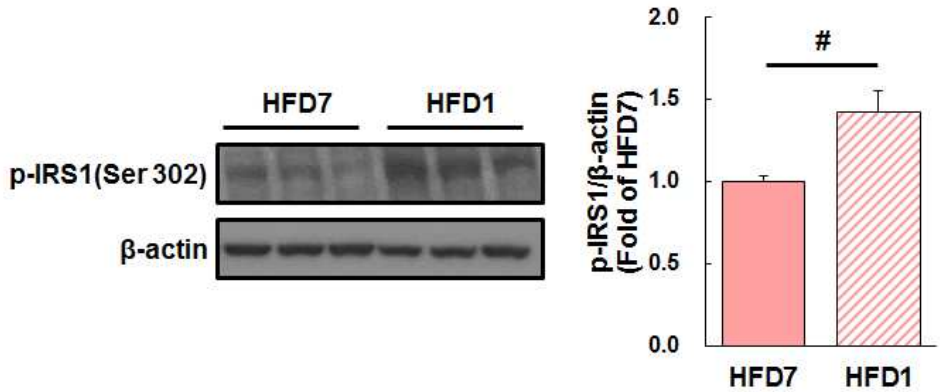

Figure 15. Effect of marginal vitamin B6 deficiency on regulation of p-IRS1 in the liver of HFD groups

Protein levels of p-IRS1 (ser 302) was determined by western blotting. Data are presented as mean ± SEM (n=3). # $P < 0.05$ (Student's t-test). HFD7, a high-fat diet with 7 mg PN/kg diet; HFD1, a high-fat diet with 1 mg PN/kg diet.

8. Effects of dietary fat on vitamin B6 metabolism

To investigate whether dietary fat regulates vitamin B6 metabolism, we examined the expression of genes related with vitamin B6. As a result, expressions of *Pdxk, Pdxp,* and *Pnpo*, which convert B6 vitamers to each other, were not significantly different among four groups (Data not shown). On the other hand, the expressions of *Aox1* and *Aox3*, which convert pyridoxal to 4-pyridoxate, a form excreted in urine, were significantly changed. Expressions of *Aox1* and *Aox3* were significantly lower in the CD1 group compared to the other groups. In HFD groups, *Aox1* expression was higher than CD groups, but the difference was not significant (Figure 16). Among DEGs in GO term 'Pyridoxal phosphate binding', *Agxt2, Mocos, Sptlc2,* and *Sptlc3* were upregulated and the remaining 17 genes except for *Pygb* were downregulated in the HFD7 group compared to the CD7 group, implicating the overall down-regulation of pathways involving PLP as a cofactor in response to a high-fat consumption (Figure 17).

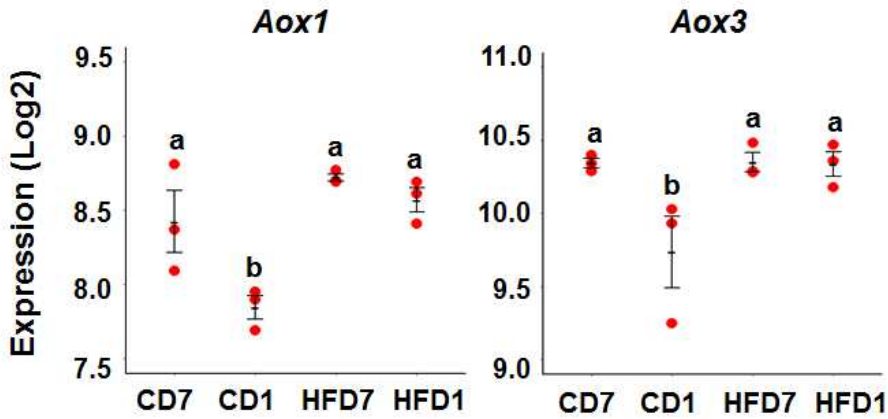

Figure 16. Effects of dietary fat content on gene expression of vitamin B6 catabolism

Hepatic mRNA levels of *Aox1* and *Aox3* involved in vitamin B6 catabolism were analyzed by microarray and presented as a dot plot (n=3). Different superscripts are significantly different at $P < 0.05$ (One-way ANOVA followed by Duncan's multiple comparison test). *Aox*, aldehyde oxidase; CD7, a control diet with 7 mg PN/kg diet; CD1, a control diet with 1 mg PN/kg diet; HFD7, a high-fat diet with 7 mg PN/kg diet; HFD1, a high-fat diet with 1 mg PN/kg diet.

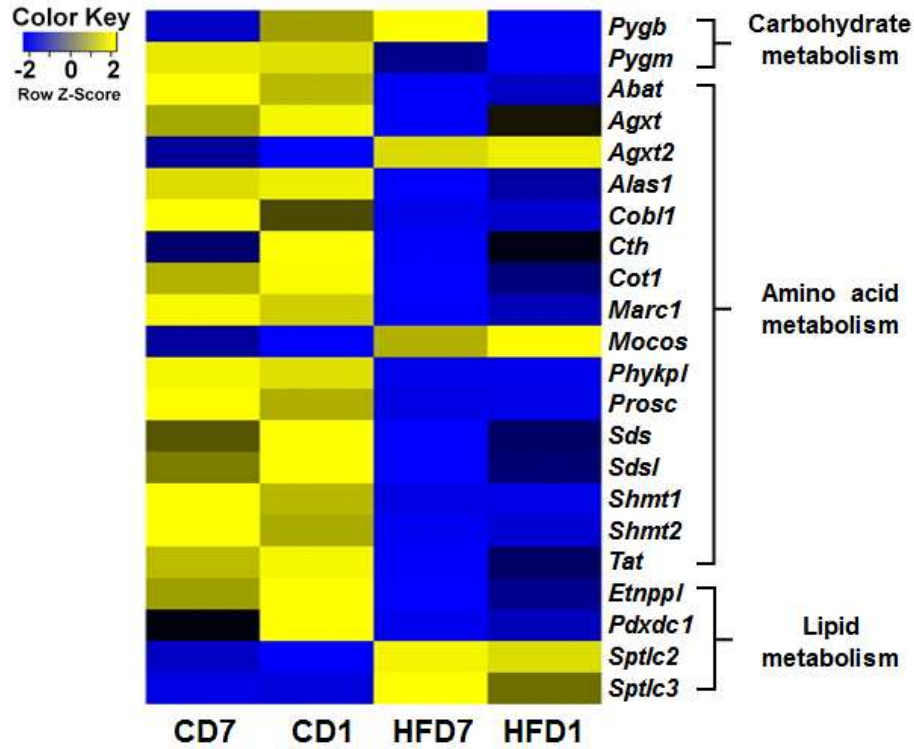

Figure 17. Heatmap of genes involved in GO term 'Pyridoxal phosphate binding'

Each cell represents the mean value of gene expression in each group (n=3). Gene names of each gene symbol are showed in Appendix 7. CD7, a control diet with 7 mg PN/kg diet; CD1, a control diet with 1 mg PN/kg diet; HFD7, a high-fat diet with 7 mg PN/kg diet; HFD1, a high-fat diet with 1 mg PN/kg diet.

Ⅴ. Discussion

Vitamin B6 is essential for human health because it serves as a coenzyme in many metabolic reactions. Many studies suggest that PLP has protective effects against many chronic diseases (Maessen et al. 2016, Bird 2018). Patients with obesity, T2DM, fatty liver or metabolic syndrome have lower vitamin B6 status than healthy individuals (Aasheim et al. 2008, Odum et al. 2012, Nix et al. 2015, Liu et al. 2016), suggesting an important role for vitamin B6 in metabolic regulation. Several marginal vitamin B6 deficiency studies have reported changes in metabolites, but few studies have performed gene expression profiling in marginal deficiency models. There is a study that has performed microarray analysis of vitamin B6 deficiency in skeletal muscle (Suidasari et al. 2017), but to the best of our knowledge, our study is the first to report on gene expression in the liver of vitamin B6-deficient animals.

Obese patients have lower vitamin B6 status than normal subjects. In this study, we examined the effects of high-fat diet-induced obesity on vitamin B6 status and metabolism by measuring PLP concentration and gene expression associated with vitamin B6 metabolism. Although the concentration of PLP in HFD7 was lower than that of CD7, the expression of *Aox1* and *Aox3* did not change. Therefore, it is difficult to conclude that *Aox* plays a critical role in lowering the PLP concentration of HFD7. Several studies have found that animals with chronic inflammatory disease such as rheumatoid arthritis showed decreased plasma PLP levels without increase in the excretion of vitamin B6. These studies have hypothesized that plasma PLP concentration was reduced because of the increased utilization of vitamin B6 at inflammatory sites (Chiang et al. 2005). Since obesity

is a systemic inflammatory state, it is necessary to examine the effects of high-fat diet-induced obesity on vitamin B6 excretion and *in vivo* distribution.

In the CD groups, marginal vitamin B6 deficiency suppressed *Aox* expression, but in the HFD groups marginal vitamin B6 deficiency showed no effect on *Aox* expression. The expression of *Aox1* and *Aox3* in CD1 was significantly lower than that of CD7, suggesting that vitamin B6 excretion is suppressed by marginal vitamin B6 deficiency when the control diet is consumed. However, there was no change in *Aox* expression in HFD1, suggesting that the mechanism which maintains vitamin B6 level does not work optimally in vitamin B6 deficient mice. It seems that high-fat diet damages vitamin B6 metabolism in the liver.

In this study, relative serum PLP levels of CD1 (0.76), a vitamin B6 deficient diet, were significantly lower than CD7 (1.00). A previous study in which mice fed a vitamin B6 deficient diet (1 mg PN/kg diet) for a long period (22 weeks) lowered relative plasma PLP (0.79) to a level similar to our study (0.76) (Lu et al. 2008). On the other hand, providing a vitamin B6 deficient diet for a short period results in a very low relative plasma PLP (0.15) (Masisi et al. 2012). *Aox* expression in this study was lower in CD1 than in CD7. These results suggest that long-term intake of vitamin B6-deficient diets can reduce excretion of vitamin B6 and thereby alleviate vitamin B6 deficiency.

In our study, we investigated the mRNA expression levels of genes involved in cholesterol and bile acid biosynthesis, using transcriptome analysis of hepatic tissue. Previous studies have shown that the incorporation rate of hepatic acetate to cholesterol increases with vitamin B6 deficiency (Shah et al. 1960, Lupien et al. 1969, Hinse et

al. 1971, Okada et al. 1977). However, the results of studies on cholesterol levels are not consistent (Shah et al. 1960, Williams et al. 1966, Lupien et al. 1969). Also, vitamin B6 does not act as a coenzyme in cholesterol synthesis. Therefore, it was necessary to examine the gene expression levels of cholesterol-metabolizing enzymes. In the present study, the expression of several genes involved in cholesterol synthesis was significantly higher in the CD1 than in the CD7 without changes in serum and hepatic cholesterol levels. In a previous study, plasma concentrations of bile acids including glycocholic acid, glycoursodeoxycholic acid and murocholic acid were increased in moderately vitamin B6-deficient rats (0.7 mg PN/kg diet) (Mayengbam et al. 2016a). Thus, we assumed that cholesterol would be converted to bile acid. We found that total bile acid content in the liver did not differ among the groups, but CD1 had higher levels than CD7, although there was no statistical difference (t-test, P=0.07). This observation suggests that the cholesterol content was not changed but the bile acid content tends to increase because cholesterol was converted into bile acid. Not all cholesterol is converted to bile acid; cholesterol can also be converted to steroid hormones. Also, 7-dehydrocholesterol which is converted to cholesterol by *Dhcr7,* may be converted to vitamin D by ultraviolet light. We measured the expression of genes related to steroid hormones and vitamin D synthesis using microarray. There was no difference in gene expression between CD1 and CD7 (Data not shown). This finding implies that cholesterol is more likely to be converted to bile acid than steroid hormone or vitamin D. Bile acid, although synthesized in the liver, is secreted into the intestines. Reabsorbed bile acid enters the blood stream. In order to confirm the exact concentration of bile acid *in vivo*, not only the liver but also

the serum bile acid levels should have been measured. Since PLP does not act as a coenzyme for cholesterol synthesis, further studies should clarify the mechanism by which vitamin B6 may be involved in cholesterol enzyme expression.

In the HFD groups, the expression of genes related to cholesterol or bile acid biosynthesis did not change under marginal vitamin B6 deficiency. Additional hepatic cholesterol synthesis did not occur because the mice in the HFD groups have already consumed high amounts of cholesterol (Stange et al. 1985). It is possible that a high hepatic cholesterol content caused by a high-cholesterol intake masked the effect of increased cholesterol biosynthesis in vitamin B6 deficiency. This may be the reason why the expression of the genes involved in cholesterol biosynthesis was not significantly different in the HFD1/HFD7 comparison.

Vitamin B6 is a micronutrient vital for normal human immunity. Vitamin B6 deficiency suppresses antibody and cytokine production (Meydani et al. 1991, Doke et al. 1997, Inubushi et al. 2000). In this study, many of downregulated genes in the CD1/CD7 comparison were immune related. This observation indicates that the immune response was weakened by marginal vitamin B6 deficiency in the CD groups.

In the HFD1/HFD7 comparison, the expression of genes involved in the metabolism of carbohydrates, proteins, and lipids was changed. Previous studies on vitamin B6 deficiency have shown changes in the concentration of amino acids and amino acid derivatives. These changes are characterized by an increase in the concentration of cystathionine in humans and animals (Davis et al. 2006, Gregory et al. 2013, Mayengbam et al. 2016b). In our study, we did not measure the concentration of amino acids or their metabolites. However, we

could investigate the gene expression of genes related to cystathionine metabolism using microarray. *Cth* expression in HFD1 is higher than in HFD7. The increased need to degrade cystathionine may explain the increased *Cth* expression in HFD1.

HFD1 had lower serum glucose and *Gck* expression than HFD7. *Gck* is a major enzyme in carbohydrate metabolism, producing G6P, the first step in glycolysis and glycogen synthesis (Agius 2008). The protein levels of p-IRS1 were measured by western blot to confirm that the low expression of *Gck* was due to insulin resistance. The expression of p-IRS1 of HFD1 was higher than that of HFD7, suggesting that hepatic glycogen accumulation of HFD1 may not be sufficient, because of insulin resistance. Since glycogen phosphorylase is a vitamin B6-dependent enzyme (Helmreich 1992), there is a possibility that glycogen is not degraded, due to decreased activity of the enzyme upon vitamin B6 deficiency. Declined degradation of glycogen and inadequate glycogen storage induced by insulin resistance may not have elevated the serum glucose concentration during the 12 hours of fasting in the HFD1 group.

Of the DEGs of HFD1/HFD7, *Fasn* is associated with triglyceride metabolism. Expression of *Fasn* involved in fatty acid synthesis was lower in HFD1 than in HFD7, but the levels of hepatic triglyceride did not differ between the two groups. Marginal deficiency of vitamin B6 resulted in differences in *Fasn* expression in the HFD groups, but did not affect the actual triglyceride content in the liver. Some previous studies show that vitamin B6 deficiency leads to fatty liver without obesity (Kitagawa et al. 2015, Kitagawa et al. 2017, Mayengbam et al. 2015). However, additional triglyceride accumulation was not observed in our study. We used a marginal vitamin B6 deficiency model, unlike previous studies which used absolute vitamin

B6 deficiency models. We suggest that marginal deficiency does not appear to be critical enough to cause fatty liver.

In conclusion, the functional effects of marginal vitamin B6 on cholesterol metabolism and immune response were observed in mice fed control diets. The functional effects of marginal vitamin B6 deficiency on amino acid metabolism were observed in mice fed a high-fat diet, by transcriptome profiling of the liver. These results indicate that high-fat diet-induced obesity may regulate metabolic processes associated with marginal vitamin B6 deficiency.

References

Aasheim, E. T., D. Hofso, J. Hjelmesaeth, K. I. Birkeland and T. Bohmer (2008). "Vitamin status in morbidly obese patients: a cross-sectional study." *Am J Clin Nutr* 87(2): 362-369.

Agius, L. (2008). "Glucokinase and molecular aspects of liver glycogen metabolism." *Biochem J* 414(1): 1-18.

Albersen, M., M. Bosma, N. V. Knoers, B. H. de Ruiter, E. F. Diekman, J. de Ruijter, W. F. Visser, T. J. de Koning and N. M. Verhoeven-Duif (2013). "The intestine plays a substantial role in human vitamin B6 metabolism: a Caco-2 cell model." *PLoS One* 8(1): e54113.

Araki, A. and Y. Sako (1987). "Determination of free and total homocysteine in human plasma by high-performance liquid chromatography with fluorescence detection." *J Chromatogr* 422: 43-52.

Bird, R. P. (2018). "The Emerging Role of Vitamin B6 in Inflammation and Carcinogenesis." *Adv Food Nutr Res* 83: 151-194.

Buchet, R., J. L. Millan and D. Magne (2013). "Multisystemic functions of alkaline phosphatases." *Methods Mol Biol* 1053: 27-51.

Chew, S. C., G. L. Khor and S. P. Loh (2011). "Association between dietary folate intake and blood status of folate and homocysteine in Malaysian adults." *J Nutr Sci Vitaminol (Tokyo)* 57(2): 150-155.

Chiang, E. P., D. E. Smith, J. Selhub, G. Dallal, Y. C. Wang and R. Roubenoff (2005). "Inflammation causes tissue-specific depletion of vitamin B6." *Arthritis Res Ther* 7(6): R1254-1262.

Choi, E. Y. and Y. O. Cho (2008). "Vitamin B6 deficiency can reduce fuel storage and utilization in physically trained rats." *Int J Vitam Nutr Res* 78(2): 64-69.

Coburn, S. P. (1994). "A critical review of minimal vitamin B6 requirements for growth in various species with a proposed method of calculation." *Vitam Horm* 48: 259-300.

Coburn, S. P. (1996). "Modeling vitamin B6 metabolism." *Adv Food Nutr Res* 40: 107-132.

Coburn, S. P. (2015). "Vitamin B-6 Metabolism and Interactions with TNAP." *Subcell Biochem* 76: 207-238.

Copps, K. D. and M. F. White (2012). "Regulation of insulin sensitivity by serine/threonine phosphorylation of insulin receptor substrate proteins IRS1 and IRS2." *Diabetologia* 55(10): 2565-2582.

Davis, S. R., E. P. Quinlivan, P. W. Stacpoole and J. F. Gregory, 3rd (2006). "Plasma glutathione and cystathionine concentrations are elevated but cysteine flux is unchanged by dietary vitamin B-6 restriction in young men and women." *J Nutr* 136(2): 373-378.

Davis, S. R., J. B. Scheer, E. P. Quinlivan, B. S. Coats, P. W. Stacpoole and J. F. Gregory, 3rd (2005). "Dietary vitamin B-6 restriction does not alter rates of homocysteine remethylation or synthesis in healthy young women and men." *Am J Clin Nutr* 81(3): 648-655.

DeRatt, B. N., M. A. Ralat, O. Kabil, Y. Y. Chi, R. Banerjee and J. F. Gregory, 3rd (2014). "Vitamin B-6 restriction reduces the production of hydrogen sulfide and its biomarkers by the transsulfuration pathway in cultured human hepatoma cells." *J Nutr* 144(10): 1501-1508.

Doke, S., N. Inagaki, T. Hayakawa and H. Tsuge (1997). "Effect of vitamin B6 deficiency on an antibody production in mice." *Biosci Biotechnol Biochem* 61(8): 1331-1336.

Eliot, A. C. and J. F. Kirsch (2004). "Pyridoxal phosphate enzymes: mechanistic, structural, and evolutionary considerations." *Annu Rev Biochem* 73: 383-415.

Fabbrini, E., S. Sullivan and S. Klein (2010). "Obesity and nonalcoholic fatty liver disease: biochemical, metabolic, and clinical implications." *Hepatology* 51(2): 679-689.

Folch, J., M. Lees and G. H. Sloane Stanley (1957). "A simple method for the isolation and purification of total lipides from animal tissues." *J Biol Chem* 226(1): 497-509.

Gregory, J. F., 3rd (1980). "Determination of pyridoxal 5'-phosphate as the semicarbazone derivative using high-performance liquid chromatography." *Anal Biochem* 102(2): 374-379.

Gregory, J. F., 3rd, Y. Park, Y. Lamers, N. Bandyopadhyay, Y. Y. Chi, K. Lee, S. Kim, V. da Silva, N. Hove, S. Ranka, T. Kahveci,K. E. Muller, R. D. Stevens, C. B. Newgard, P. W. Stacpoole and D. P. Jones (2013). "Metabolomic analysis reveals extended metabolic consequences of marginal vitamin B-6 deficiency in healthy human subjects." *PLoS One* 8(6): e63544.

Guggenheim, K. and E. J. Diamant (1957). "Carbohydrate metabolism in pyridoxine-deficient rats." *J Biol Chem* 224(2): 861-869.

Helmreich, E. J. (1992). "How pyridoxal 5'-phosphate could function in glycogen phosphorylase catalysis." *Biofactors* 3(3): 159-172.

Hinse, C. M. and P. J. Lupien (1971). "Cholesterol metabolism and vitamin B6. III. The stimulation of hepatic cholesterogenesis in the vitamin B6-deficient rat." *Canadian journal of biochemistry* 49(8): 933-935.

Huang, S. C., J. C. Wei, P. T. Lin, D. J. Wu and Y. C. Huang (2012). "Plasma pyridoxal 5'-phosphate is not associated with inflammatory and immune responses after adjusting for serum albumin in patients with rheumatoid arthritis: a preliminary study." *Ann Nutr Metab* 60(2): 83-89.

Inubushi, T., M. Okada, A. Matsui, J. Hanba, E. Murata and N. Katunuma (2000). "Effect of dietary vitamin B6 contents on antibody production." *Biofactors* 11(1-2): 93-96.

Kim, Y. N. and Y. O. Cho (2014). "Evaluation of vitamin B6 intake and status of 20-to 64-year-old Koreans." *Nutrition research and practice* 8(6): 688-694.

Kitagawa, E., T. Yamamoto, M. Fujishita, Y. Ota, K. Yamamoto, T. Nakagawa and T. Hayakawa (2017). "Choline and betaine ameliorate liver lipid accumulation inducedby vitamin B6 deficiency in rats." *Biosci Biotechnol Biochem* 81(2): 316-322.

Kitagawa, E., T. Yamamoto, K. Yamamoto, T. Nakagawa and T. Hayakawa (2015). "Accumulation of lipid in rat liver was induced by vitamin B(6) deficiency and was ameliorated by supplemental phosphatidylcholine in the diet." *Biosci Biotechnol Biochem* 79(8): 1320-1326.

Kjeldby, I. K., G. S. Fosnes, S. C. Ligaarden and P. G. Farup (2013).

"Vitamin B6 deficiency and diseases in elderly people--a study in nursing homes." *BMC Geriatr* 13: 13.

Leklem, J. E. (1990). "Vitamin B-6: a status report." *J Nutr 120 Suppl* 11(4): 1503-1507.

Lima, C. P., S. R. Davis, A. D. Mackey, J. B. Scheer, J. Williamson and J. F. Gregory, 3rd (2006). "Vitamin B-6 deficiency suppresses the hepatic transsulfuration pathway but increases glutathione concentration in rats fed AIN-76A or AIN-93G diets." *J Nutr* 136(8): 2141-2147.

Lin, P. T., C. H. Cheng, Y. P. Liaw, B. J. Lee, T. W. Lee and Y. C. Huang (2006). "Low pyridoxal 5'-phosphate is associated with increased risk of coronary artery disease." *Nutrition* 22(11-12): 1146.

Liu, Z., P. Li, Z. H. Zhao, Y. Zhang, Z. M. Ma and S. X. Wang (2016). "Vitamin B6 Prevents Endothelial Dysfunction, Insulin Resistance, and Hepatic Lipid Accumulation in Apoe (-/-) Mice Fed with High-Fat Diet." *J Diabetes Res* 2016: 1748065.

Lu, T., Y. Xu, E. S. Monttinen and N. Kato (2008). "Supplementing vitamin B6 to a low vitamin B6 diet exaggerates UVB-induced skin tumorigenesis in DMBA-treated hairless mice." *J Nutr Sci Vitaminol (Tokyo)* 54(3): 262-265.

Lumeng, L., R. E. Brashear and T. K. Li (1974). "Pyridoxal 5'-phosphate in plasma: source, protein-binding, and cellular transport." *J Lab Clin Med* 84(3): 334-343.

Lumeng, L., A. Lui and T. K. Li (1980). "Plasma content of B6 vitamers and its relationship to hepatic vitamin B6 metabolism." *J Clin Invest* 66(4): 688-695.

Lupien, P. J., C. M. Hinse and M. Avery (1969). "Cholesterol metabolism and vitamin B6. I. Hepatic cholesterogenesis and pyridoxine deficiency." *Can J Biochem* 47(6): 631-635.

Maessen, D. E., O. Brouwers, K. H. Gaens, K. Wouters, J. P. Cleutjens, B. J. Janssen, T. Miyata, C. D. Stehouwer and C. G. Schalkwijk (2016). "Delayed Intervention With Pyridoxamine Improves Metabolic Function and Prevents Adipose Tissue Inflammation and Insulin Resistance in

High-Fat Diet-Induced Obese Mice." *Diabetes* 65(4): 956-966.

Marzio, A., C. Merigliano, M. Gatti and F. Verni (2014). "Sugar and chromosome stability: clastogenic effects of sugars in vitamin B6-deficient cells." *PLoS Genet* 10(3): e1004199.

Masisi, K., S. Suidasari, P. Zhang, Y. Okazaki, N. Yanaka and N. Kato (2012). "Comparative study on the responses of concentrations of B(6)-vitamers in several tissues of mice to the dietary level of pyridoxine." *J Nutr Sci Vitaminol (Tokyo)* 58(6): 446-451.

Mayengbam, S., J. D. House and M. Aliani (2016a). "Investigation of vitamin B(6) inadequacy, induced by exposure to the anti-B(6) factor 1-amino D-proline, on plasma lipophilic metabolites of rats: a metabolomics approach." *Eur J Nutr* 55(3): 1213-1223.

Mayengbam, S., S. Raposo, M. Aliani and J. D. House (2015). "Oral exposure to the anti-pyridoxine compound 1-amino D-proline further perturbs homocysteine metabolism through the transsulfuration pathway in moderately vitamin B(6) deficient rats." *J Nutr Biochem* 26(3): 241-249.

Mayengbam, S., S. Raposo, M. Aliani and J. D. House (2016b). "A Vitamin B-6 Antagonist from Flaxseed Perturbs Amino Acid Metabolism in Moderately Vitamin B-6-Deficient Male Rats." *J Nutr* 146(1): 14-20.

Meydani, S. N., J. D. Ribaya-Mercado, R. M. Russell, N. Sahyoun, F. D. Morrow and S. N. Gershoff (1991). "Vitamin B-6 deficiency impairs interleukin 2 production and lymphocyte proliferation in elderly adults." *Am J Clin Nutr* 53(5): 1275-1280.

Minniti, G., A. Piana, U. Armani and R. Cerone (1998). "Determination of plasma and serum homocysteine by high-performance liquid chromatography with fluorescence detection." *J Chromatogr A* 828(1-2): 401-405.

Morris, M. S., M. F. Picciano, P. F. Jacques and J. Selhub (2008). "Plasma pyridoxal 5′-phosphate in the US population: the National Health and Nutrition Examination Survey, 2003-2004." *Am J Clin Nutr* 87(5): 1446-1454.

Na, Y. M., H. A. Park, J. H. Kang, Y. G. Cho, K. W. Kim, Y. I. Hur, Y. N.

Kim and S. H. Lee (2011). "Obesity, obesity related disease, and disability." *Korean J Fam Med* 32(7): 412-422.

Nix, W. A., R. Zirwes, V. Bangert, R. P. Kaiser, M. Schilling, U. Hostalek and R. Obeid (2015). "Vitamin B status in patients with type 2 diabetes mellitus with and without incipient nephropathy." *Diabetes Res Clin Pract* 107(1): 157-165.

Odum, E. P. and V. C. Wakwe (2012). "Plasma concentrations of water-soluble vitamins in metabolic syndrome subjects." *Niger J Clin Pract* 15(4): 442-447.

Okada, M. and T. Iwami (1977). "Effect of pyridoxine deficiency on cholesterogenesis in rats fed different levels of protein." *J Nutr Sci Vitaminol (Tokyo)* 23(6): 505-512.

Percudani, R. and A.Peracchi (2009). "The B6 database: a tool for the description and classification of vitamin B6-dependent enzymatic activities and of the corresponding protein families." *BMC Bioinformatics* 10: 273.

Popkin, B. M., G. Keyou, F. Zhai, X. Guo, H. Ma and N. Zohoori (1993). "The nutrition transition in China: a cross-sectional analysis." *Eur J Clin Nutr* 47(5): 333-346.

Qian, B., S. Shen, J. Zhang and P. Jing (2017). "Effects of Vitamin B6 Deficiency on the Composition and Functional Potential of T Cell Populations." *J Immunol Res* 2017: 2197975.

Reeves, P. G., F. H. Nielsen and G. C. Fahey, Jr. (1993). "AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet." *J Nutr* 123(11): 1939-1951.

Ribaya, J. D. and S. N. Gershoff (1977). "Effects of vitamin B6 deficiency on liver, kidney, and adipose tissue enzymes associated with carbohydrate and lipid metabolism, and on glucose uptake by rat epididymal adipose tissue." *J Nutr* 107(3): 443-452.

Ronnenberg, A. G., M. B. Goldman, I. W. Aitken and X. Xu (2000). "Anemia and deficiencies of folate and vitamin B-6 are common and vary with season in Chinese women of childbearing age." *J Nutr* 130(11):

2703-2710.

Ruiz, J. I. and B. Ochoa (1997). "Quantification in the subnanomolar range of phospholipids and neutral lipids by monodimensional thin-layer chromatography and image analysis." *J Lipid Res* 38(7): 1482-1489.

Shah, S. N., P. V. Johnston and F. A. Kummerow (1960). "The effect of pyridoxine on cholesterol metabolism." *J Nutr* 72: 81-86.

She, Q.-B., T. Hayakawa and H. Tsuge (1994). "Effect of vitamin B6 deficiency on linoleic acid desaturation in the arachidonic acid biosynthesis of rat liver microsomes." *Bioscience, biotechnology, and biochemistry* 58(3): 459-463.

She, Q. B., T. Hayakawa and H. Tsuge (1995). "Alteration in the phosphatidylcholine biosynthesis of rat liver microsomes caused by vitamin B6 deficiency." *Biosci Biotechnol Biochem* 59(2): 163-167.

Sokolovic, M., A. Sokolovic, D. Wehkamp, E. Ver Loren van Themaat, D. R. de Waart, L. A. Gilhuijs-Pederson, Y. Nikolsky, A. H. van Kampen, T. B. Hakvoort and W. H. Lamers (2008). "The transcriptomic signature of fasting murine liver." *BMC Genomics* 9: 528.

Stange, E. F. and J. M. Dietschy (1985). "The origin of cholesterol in the mesenteric lymph of the rat." *J Lipid Res* 26(2): 175-184.

Suidasari, S., S. Uragami, N. Yanaka and N. Kato (2017). "Dietary vitamin B6 modulates the gene expression of myokines, Nrf2-related factors, myogenin and HSP60 in the skeletal muscle of rats." *Exp Ther Med* 14(4): 3239-3246.

Suzuki, K. and M. Okada (1982). "Role of glucose on fatty liver formation in pyridoxine-deficient rats." *J Nutr Sci Vitaminol (Tokyo)* 28(4): 367-375.

Suzuki, K. and M. Okada (1984). "Studies on the origin of triglyceride in fatty liver caused by pyridoxine-deficiency in rats." *Nutrition Research* 4(4): 691-699.

Trakatellis, A., A. Dimitriadou, M. Exindari, J. Scountzou, G. Koliakos, D. Christodoulou, N. Malissiovas, A. Antoniadis and T. Polyzoni (1992). "Effect of pyridoxine deficiency on immunological phenomena." *Postgrad Med J* 68 Suppl 1: S70-77.

Tucker, L. A. and M. J. Kano (1992). "Dietary fat and body fat: a multivariate study of 205 adult females." *Am J Clin Nutr* 56(4): 616-622.

Ubbink, J. B., W. J. Serfontein and L. S. de Villiers (1985). "Stability of pyridoxal-5-phosphate semicarbazone: applications in plasma vitamin B6 analysis and population surveys of vitamin B6 nutritional status." *J Chromatogr* 342(2): 277-284.

Ueland, P. M., A. McCann, O. Midttun and A. Ulvik (2017). "Inflammation, vitamin B6 and related pathways." *Mol Aspects Med* 53: 10-27.

Williams, M. A., D. J. McIntosh and I. Hincenbergs (1966). "Changes in fatty acid composition in liver lipid fractions of pyridoxine-deficient rats fed cholesterol." *J Nutr* 88(2): 193-201.

Ye, X., J. E. Maras, P. J. Bakun and K. L. Tucker (2010). "Dietary intake of vitamin B-6, plasma pyridoxal 5′-phosphate, and homocysteine in Puerto Rican adults." *J Am Diet Assoc* 110(11): 1660-1668.

Zhao, M., M. A. Ralat, V. da Silva, T. J. Garrett, S. Melnyk, S. J. James and J. F. Gregory III (2013). "Vitamin B-6 restriction impairs fatty acid synthesis in cultured human hepatoma (HepG2) cells." *American J Physiol Endocrinol Metab* 304(4): E342.

Appendices

Appendix 1. Gene names of upregulated DEGs in Top 10 GO terms of CD1/CD7

Gene symbol	Gene name
Lepr	leptin receptor
Cyp39a1	cytochrome P450, family 39, subfamily a, polypeptide 1
Msmo1	methylsterol monoxygenase 1
Cyp51	cytochrome P450, family 51
Sds	serine dehydratase
Idi1	isopentenyl-diphosphate delta isomerase
Asl	argininosuccinate lyase
Nsdhl	NAD(P) dependent steroid dehydrogenase-like
Slco4a1	solute carrier organic anion transporter family, member 4a1
Sult1a1	sulfotransferase family 1A, phenol-preferring, member 1
Fdps	farnesyl diphosphate synthetase

Appendix 2. Gene names involved in cholesterol and bile metabolism

Gene symbol	Gene name
Hmgcr	3-hydroxy-3-methylglutaryl-Coenzyme A reductase
Pmvk	phosphomevalonate kinase
Idi1	isopentenyl-diphosphate delta isomerase
Fdps	farnesyl diphosphate synthetase
Fdft1	farnesyl diphosphate farnesyl transferase 1
Sqle	squalene epoxidase
Lss	lanosterol synthase
Cyp51	cytochrome P450, family 51
Msmo1	methylsterol monoxygenase 1
Nsdhl	NAD(P) dependent steroid dehydrogenase-like
Dhcr7	7-dehydrocholesterol reductase
Cyp39a1	cytochrome P450, family 39, subfamily a, polypeptide 1
Cyp7b1	cytochrome P450, family 7, subfamily b, polypeptide 1
Hsd3b7	hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 7
Cyp8b1	cytochrome P450, family 8, subfamily b, polypeptide 1
Slc27a1	solute carrier family 27 (fatty acid transporter), member 1
Acot8	acyl-CoA thioesterase 8

Significantly changed genes were sorted from KEGG pathway.

Appendix 3. Gene names of downregulated DEGs in Top 10 GO terms of CD1/CD7

Gene symbol	Gene name
Ifit1	interferon-induced protein with tetratricopeptide repeats 1
Stat1	signal transducer and activator of transcription 1
Bst2	bone marrow stromal cell antigen 2
Fbxw7	F-box and WD-40 domain protein 7
Gclc	glutamate-cysteine ligase, catalytic subunit
Irgm2	immunity-related GTPase family M member 2
Vnn1	vanin 1
Zfp36l1	zinc finger protein 36, C3H type-like 1
Ddx60	DEAD (Asp-Glu-Ala-Asp) box polypeptide 60
Fyb	FYN binding protein
Pid1	phosphotyrosine interaction domain containing 1
Tap1	transporter 1, ATP-binding cassette, sub-family B (MDR/TAP)
Il2rg	interleukin 2 receptor, gamma chain
Gbp4	guanylate binding protein 4
Rnf144b	ring finger protein 144B
Pde4b	phosphodiesterase 4B, cAMP specific
Zfp36l2	zinc finger protein 36, C3H type-like 2
Ap2a2	adaptor-related protein complex 2, alpha 2 subunit
Ep300	E1A binding protein p300
A230050P20Rik	RIKEN cDNA A230050P20 gene

Appendix 4. Gene names of upregulated DEGs in Top 10 GO terms of HFD1/HFD7

Gene symbol	Gene name
Got1	glutamic-oxaloacetic transaminase 1, soluble
Sds	serine dehydratase
Lao1	L-amino acid oxidase 1
Agxt	alanine-glyoxylate aminotransferase
Asl	argininosuccinate lyase
Cyp7b1	cytochrome P450, family 7, subfamily b, polypeptide 1
Pou1f1	POU domain, class 1, transcription factor 1

Appendix 5. Gene names involved in alanine, aspartate and glutamate metabolism

Gene symbol	Gene name
Agxt	alanine-glyoxylate aminotransferase
Ass1	argininosuccinate synthetase 1
Asl	argininosuccinate lyase
Got1	glutamic-oxaloacetic transaminase 1, soluble
Abat	4-aminobutyrate aminotransferase
Glud1	glutamate dehydrogenase 1
Gls2	glutaminase 2 (liver, mitochondrial)
Cps1	carbamoyl-phosphate synthetase 1
Gfpt1	glutamine fructose-6-phosphate transaminase 1
Gfpt2	glutamine fructose-6-phosphate transaminase 2
Ppat	phosphoribosyl pyrophosphate amidotransferase

Significantly changed genes were sorted from KEGG pathway.

Appendix 6. Gene names involved in cysteine and methionine metabolism

Gene symbol	Gene name
Sds	serine dehydratase
Cth	cystathionase (cystathionine gamma-lyase)
Got1	glutamic-oxaloacetic transaminase 1, soluble
Mdh2	malate dehydrogenase 2, NAD (mitochondrial)
Agxt	alanine-glyoxylate aminotransferase
Agxt2	alanine-glyoxylate aminotransferase 2
Gclc	glutamate-cysteine ligase, catalytic subunit
Gclm	glutamate-cysteine ligase, modifier subunit
Gss	glutathione synthetase
Tst	thiosulfate sulfurtransferase, mitochondrial
Mpst	mercaptopyruvate sulfurtransferase
Mat1a	methionine adenosyltransferase I, alpha
Mat2a	methionine adenosyltransferase II, alpha
Tat	tyrosine aminotransferase
Lao1	L-amino acid oxidase 1

Significantly changed genes were sorted from KEGG pathway.

Appendix 7. Gene names of DEGs related to GO-Term 'Pyridoxal phosphate binding'

Gene symbol	Gene name
Pygb	brain glycogen phosphorylase
Pygm	muscle glycogen phosphorylase
Abat	4-aminobutyrate aminotransferase
Agxt	alanine-glyoxylate aminotransferase
Agxt2	alanine-glyoxylate aminotransferase 2
Alas1	aminolevulinic acid synthase 1
Ccbl1	cysteine conjugate-beta lyase 1
Cth	cystathionase (cystathionine gamma-lyase)
Got1	glutamic-oxaloacetic transaminase 1, soluble
Marc1	mitochondrial amidoxime reducing component 1
Mocos	molybdenum cofactor sulfurase
Phykpl	5-phosphohydroxy-L-lysine phospholyase
Prosc	proline synthetase co-transcribed
Sds	serine dehydratase
Sdsl	serine dehydratase-like
Shmt1	serine hydroxymethyltransferase 1 (soluble)
Shmt2	serine hydroxymethyltransferase 2 (mitochondrial)
Tat	tyrosine aminotransferase
Etnppl	ethanolamine phosphate phospholyase
Pdxdc1	pyridoxal-dependent decarboxylase domain containing 1
Sptlc2	serine palmitoyltransferase, long chain base subunit 2
Sptlc3	serine palmitoyltransferase, long chain base subunit 3

국문초록

경도의 비타민 B6의 결핍이 대조군과 비만 마우스의 전사체 발현에 미치는 영향

서울대학교 대학원
식품영양학과
엄현지

비타민 B6는 다양한 식품에 함유되어 있어 섭취량이 부족하기 어려운 영양소임에도 불구하고 비타민 B6의 결핍은 전 세계적으로 매우 흔하다. 또한 비만, 지방간, 대사 증후군 및 제2형 당뇨병 환자는 건강한 사람에 비해 비타민 B6 영양상태가 낮은 경향이 있다. 활성형 비타민 B6인 피리독살 5′-인산 (PLP) 은 아미노기 전이반응, 탈탄산반응, 라세미화, 제거반응 등을 통해 아미노산, 탄수화물 및 지질 대사 등에 조효소로써 관여한다. 이렇듯 비타민 B6는 매우 다양한 대사에 관여하므로, 비타민 B6의 결핍에 의해 체내에서 여러 가지 변화가 일어날 수 있다. 여러 선행 연구들에서는 사람 또는 동물에게 부족한 비타민 B6 식사를 제공함으로써 경도의 비타민 B6 결핍증을 유발하였다. 이러한 경도의 비타민 B6 결핍 선행 연구들에서는 혈중 및 간의 아미노산 및 아미노산 대사물의 농도 변화에 초점을 맞추어 연구를 진행하였고, 간에서의 전사체 분석을 통해 대사의 변화를 확인한 연구는 없었다. 또한 비만과 관련된 여러 질환자들의 비타민 B6 영양상태가 정상인에 비해 낮은 경향이 있는 것으로 보아 비만, 제2형 당뇨병, 지방간 등의 질환들이 비타민 B6 영양상태를 저하시키는 요인이라고 추측하고 있다. 그럼에도 불구하고 고지방식

이로 비만 및 비만과 관련된 질환을 유도한 동물 모델에서 비타민 B6 영양상태를 확인한 연구가 존재하지 않았다. 따라서 본 연구에서는 고지방식이로 인해 유도된 비만 마우스와 정상식이를 섭취한 마우스에서의 경도 비타민 B6 결핍이 간 내 전사체 발현에 미치는 영향을 알아보고자 하였다. 더불어 고지방식이로 인해 유도된 비만에서의 비타민 B6 영양상태 및 비타민 B6 대사를 확인하고자 하였다. 이를 확인하기 위하여 수컷 C57BL/6J 마우스에게 권장량의 비타민 B6 (피리독신 7 mg/kg diet) 또는 부족한 양의 비타민 B6 (피리독신 1 mg/kg diet) 를 지질 함량이 정상인 식이 (CD) 또는 고지방식이 (HFD) 와 함께 제공하였다. 16주 동안의 사육 후, 네 군의 간에서 RNA를 추출하여 마이크로어레이를 통해 전사체 발현을 확인하였고, 혈청 및 간에서 생화학 지표들을 측정하였다. 비타민 B6 영양상태를 반영하는 혈청 및 간 PLP 농도가 비타민 부족 섭취와 고지방식이 섭취에 의해 낮아졌다. 혈청 분석 결과, 혈청 지질은 경도 비타민 B6 결핍에 의해 변하지 않았으나 혈당은 HFD7에 비해 HFD1에서 유의적으로 낮았다. 간 콜레스테롤 농도는 고지방식이에 의해 유의적으로 증가하였고 경도 비타민 B6 결핍에 의해서는 변하지 않았다. 간의 총 담즙산 농도는 모든 군에서 유의적인 차이는 없었으나, CD7에 비해 CD1에서 높은 경향을 보였다. 네 군에 대한 전반적인 전사체 분석 결과에 따르면, DEG (differentially expressed gene) 들의 발현 변화는 경도 비타민 B6 결핍보다 식이 내 지질 함량에 의해 크게 변하였다. 본 연구에서는 비타민 B6 결핍에 의한 간 전사체 발현을 확인하고자 하였으므로 CD7군과 CD1군의 비교조합 (CD1/CD7)과 HFD1/HFD7의 DEG 들에 대한 추가적인 분석을 실시하였다. CD1/CD7에서 상향조절된 유전자들에 대한 GO (Gene Ontology) 기반 기능분석을 실시한 결과에서는 스테롤 대사 과정과 관련된 GO term들이 확인되었다. 콜레스테롤 합성에 관여하는 *Hmgcr, Cyp51* 및 *Msmo1*와 담즙산 생합성에 관여하는 *Cyp39a1*의 발현이 CD7에 비해 CD1에서 유의적으로 높았다. 동일 비교조합에서 하향조절된 유전자들의 기능분석 결과에서는 면역 반응과 관련

된 GO term들을 얻었다. HFD1/HFD7에서 상향조절된 유전자들에 대한 기능분석 결과에서는 아미노산 대사와 관련된 GO term들이 확인되었다. 동일 비교조합에서 하향조절된 유전자들에서는 의미 있는 기능분석 결과를 얻을 수 없었으나 글루코키나아제 (*Gck*)의 발현이 HFD7에 비해 HFD1에서 유의적으로 낮았다. 비타민 B6 대사와 관련된 유전자들 중에서는 *Aox*의 발현만이 유의적으로 변하였는데, CD1의 *Aox* 발현이 나머지 세 군에 비해 유의적으로 낮았다. 결론적으로 CD군들에서는 경도 비타민 B6 결핍에 의해 콜레스테롤 대사 및 면역 반응이 영향을 받은 반면, HFD군들에서는 아미노산 대사가 영향을 받은 것으로 나타났다. 또한 CD군들에서는 경도의 비타민 B6 결핍에 의해 *Aox*의 발현이 유의적으로 낮아진 반면 HFD군들에서는 변하지 않았다. 이는 비타민 B6가 관여하는 아미노산, 지질, 탄수화물 등의 여러 대사들이 비만의 유무에 따라 차별적으로 조절될 수 있음을 보여준다.

주요어: 경도의 비타민 B6 결핍, 아미노산 대사, 고지방식이, 면역, 스테롤 대사, 전사체
학번: 2016-21668