Hypolipidemic effect of Goami-3 rice (Oryza sativa L. cv. Goami-3) on C57BL/6J mice is mediated by the regulation of peroxisome proliferator-activated receptor-α and -γ

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Abstract

We investigated the hypolipidemic effects of Goami-3 rice (GR; Oryza sativa L. cv. Goami-3), a newly developed strain with high levels of amylose and fibers. Diet-induced obese mice were fed three types of isocaloric diets for 8 weeks: a high-fat diet, a high-fat diet with GR or control rice (CR; O. sativa L. cv. Ilpumbyeo). Mice fed GR exhibited a significant reduction in body fat (~23%), total cholesterol (~20%) and triglyceride concentrations (~30%) compared to mice fed CR. The mice fed GR showed induction of peroxisome proliferator-activated receptor (PPAR)-α and inhibition of γ expressions in the liver and adipose tissue. The reduced adiposity of mice fed GC was supported by changes in the expression of genes related to lipid accumulation and hydrolysis in adipose tissues and the plasma concentrations of insulin, adiponectin and leptin. Principal components analysis with gas chromatography–time-of-flight mass spectrometry-based metabolomic data revealed that the average level of specific plasma metabolites in the GR group was statistically different from that in the other groups after 4 weeks. These metabolites included propionic acid, valine, leucine and proline. Based on partial least-squares analysis, the plasma concentrations of valine were inversely correlated with the high-density lipoprotein (HDL) to non-HDL and HDL to total cholesterol ratios. In conclusion, GR feeding for 8 weeks significantly improved dyslipidemia and adiposity in diet-induced obese mice by regulating gene expression of PPARs and its target genes. Key plasma metabolites (including valine) were significantly altered by the hypolipidemic effects of GR.

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1. Introduction

Hyperlipidemia is a medical disorder characterized by elevated levels of lipids (including cholesterol and triglycerides) in the blood. Hyperlipidemia is often associated with obesity, a condition characterized by an excessive accumulation of body fat due to an imbalance between energy intake and expenditure [1]. Both obesity and hyperlipidemia lead to various metabolic disorders including hypertension, insulin resistance and hepatic steatosis [1]. Thus, the management of lipid profiles can be used to improve metabolic and cardiovascular functions. Biologically, lipid profile regulation is associated with a group of closely related transcription factors, i.e., peroxisome proliferator-activated receptors (PPARs) [2].

PPARs are ligand-activated receptors that bind to the promoter region of target genes involved in lipid and glucose metabolism to promote or repress their transcription [3]. PPAR subtypes α, δ and γ, which are encoded by three separate genes, are nutrient sensors with distinct expression patterns. PPARα is predominantly expressed in tissues that metabolize large amounts of fatty acids, such as the liver, kidney, heart and muscle. PPARδ is ubiquitously expressed, whereas the expression of PPARγ is most prevalent in adipose tissue [4]. PPARα and γ have been more widely investigated as targets for therapeutic agents against metabolic diseases due to their prominent roles in lipid metabolism. PPARα plays an important role in fatty acid oxidation, and lipid and lipoprotein metabolism [5], while PPARγ induces adipocyte differentiation and lipid accumulation in adipocytes by modulating genes associated with adipogenesis, lipid uptake and lipid metabolism [6]. The regulation of these genes is strongly
associated with carbohydrate intake, indicating that our diet may play a critical role in lipid metabolism [7].

For many people, rice (Oryza sativa L.) is consumed as a staple crop and provides a carbohydrate and energy source in the form of starch, glucose and fibers. Rice is generally classified as a high-glycemic-index (GI) food due to the high content of carbohydrates, which are rapidly digested and absorbed [8]. This diet can result in obesity and hyperlipidemia through insulinemic responses, which increase carbohydrate metabolism and suppress fat oxidation [9]. In contrast, low-GI food, which generally possesses high levels of dietary fiber, can decrease the risk of obesity and hyperlipidemia. One of the primary actions of dietary fiber is reducing dietary fat and cholesterol uptake in the intestine. In addition, starch (especially in the form of amylose), which is difficult to be hydrolyzed, is known to decrease lipogenesis and steroidogenesis [7]. Thus, a diet with a high composition of dietary fiber and amylose is ideal and may serve as a therapeutic approach for lipid profile management. However, little is known about the detailed molecular pathway behind the hypolipidemic responses to dietary fiber and amylose.

Metabolic profiling is a promising approach to investigate the relationship between metabolites and metabolic syndrome (e.g., obesity, diabetes, hypertension, and cardiovascular problems) [10–14]. For example, metabolic profiling was performed to identify metabolites contributing to obesity by comparing differences in the metabolic profiles of blood from lean and obese rats [15]. To perform metabolite profiling, diverse analytical methods have been developed using gas chromatography (GC)–mass spectrometry (MS), liquid chromatography (LC)–MS and nuclear magnetic resonance spectrometry [14–18]. Among them, GC coupled to time-of-flight (TOF) MS has high resolution and sensitivity [19,20]. Moreover, TOF-MS can be used for deconvolution processes due to its fast scan rate [21]. The deconvolution of overlapping peaks from complex data sets can be useful in metabolic analysis using TOF-MS [22] because it can efficiently identify and quantify diverse metabolites [23].

In our current study, we investigated the effect of functional rice (O. sativa L. cv. Goami; GR), which has been reinforced with dietary fiber and amylose, on lipid metabolism. The effects of GR on lipid biosynthesis and hepatic steatosis were compared to those of control rice (CR; O. sativa L cv. Ilpumbyeo) using in vitro (HepG2 and 3T3-L1 cell lines) and in vivo (C57BL/6j mice) models. To examine the mechanism behind the reduced lipid biosynthesis and risk of hepatic steatosis by GR, we conducted target analysis and detailed metabolic profiling. The target analysis focused mainly on PPARs, which are key regulators of energy homeostasis. To our knowledge, this is the first detailed study on the effect of rice with enhanced fiber and amylose via PPAR-mediated target analysis and metabolic profiling.

2. Materials and methods

2.1. Rice

Goami-3 rice (GR) is a newly bred rice strain by National Institute of Crop Science of the Rural Development Administration (Suwon, Korea). Rice extracts (80%) were prepared in dimethyl sulfoxide for cell experiments. For animal feeding, an isocaloric diet was formulated with 20% (w/w) rice powder based on an AIN-92 standard chow diet. The composition of the diet is shown in Supplemental Table 2.

2.2. Cell culture and treatments

HepG2 and 3T3-L1 cells were grown in Dulbecco’s modified Eagle’s medium (DMEM; HyClone, Logan, UT, USA) supplemented with 10% heat-inactivated fetal bovine serum and 1% penicillin/streptomycin at 37°C in 5% CO₂. Adipocyte differentiation was induced by dexamethasone (1 μM), insulin (10 μg/ml) and 3-

| Table 1: Concentration of plasma lipids for the NC, CR and GR groups. |
|--------------------------|--------------------------|--------------------------|
|                         | 0 weeks                  | 4 weeks                  | 8 weeks                  |
|                         | TC (mg/dl)               | CR (mg/dl)               | GR (mg/dl)               |
| NC                      | 125 ± 8*                 | 161 ± 9*                 | 174 ± 9*                 |
| CR                      | 124 ± 12*                | 167 ± 9*                 | 198 ± 9*                 |
| GR                      | 128 ± 3*                 | 134 ± 3*                 | 159 ± 3*                 |
| Glucose (mg/dl)         | 73 ± 18*                 | 365 ± 28*                | 413 ± 8*                 |
| CR                      | 86 ± 19*                 | 224 ± 10*                | 203 ± 11*                |
| GR                      | 94 ± 10*                 | 215 ± 17*                | 192 ± 13*                |
| TG (mg/dl)              | 114 ± 7*                 | 67 ± 7*                  | 64 ± 2*                  |
| CR                      | 120 ± 8*                 | 90 ± 3*                  | 57 ± 3*                  |
| GR                      | 125 ± 3*                 | 58 ± 6*                  | 40 ± 2*                  |
| HDL-C (mg/dl)           | 103 ± 9*                 | 126 ± 6*                 | 141 ± 7*                 |
| CR                      | 93 ± 11*                 | 146 ± 3*                 | 186 ± 6*                 |
| GR                      | 100 ± 3*                 | 119 ± 2*                 | 147 ± 5*                 |
| Non-HDL-C (mg/dl)       | 19 ± 3*                  | 29 ± 2*                  | 31 ± 2*                  |
| CR                      | 31 ± 1*                  | 21 ± 1*                  | 16 ± 1*                  |
| GR                      | 29 ± 5*                  | 15 ± 2*                  | 12 ± 1*                  |

Values are expressed as the mean ± S.E.M.

Table entries within the same column followed by different lowercase superscript letters are significantly different (P < 0.05).

2.3. Lipid staining and cellular lipid quantification

3T3-L1 adipocytes were stained using 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI; Molecular Probes, Carlsbad, CA, USA) according to the manufacturer’s instructions or stained with Oil Red O as described previously [24]. The stained cells were visualized using an Eclipse Ti inverted microscope (Nikon, Tokyo, Japan) for image analysis. Cellular lipids were extracted at room temperature with 2 ml of hexane/isopropanol mixture (2:1, v:v). The organic solvent was removed by vacuum centrifugation, and the lipids were resuspended in 100 ml of 95% ethanol. Cellular triglyceride (TG) was quantified enzymatically with an automatic analyzer (Cobas C111; Roche, Basel, Switzerland). Total cholesterol (TC) levels were measured using the Amplex Red Cholesterol Assay Kit (Invitrogen, Carlsbad, CA, USA). The free fatty acid (FFA) content in cell culture medium was quantified using Free Fatty Acid Quantification Kit (Biovision, Mountain View, CA, USA).

2.4. Transfection and luciferase assays

HepG2 cells were seeded in 24-well plates at 1 × 10⁵ cells/well. The following day, the cells were cotransfected with the pG3-PPRE3-Tk-luc reporter vector, which contains the firefly luciferase gene under control of a peroxisome proliferator response element, with an expression vector encoding full-length human PPARα and PPARγ using Hilymax (Dojindo Laboratories, Rockville, MD, USA). The medium was removed after 4 h and was replaced with high-glucose DMEM. After 18 h, cells were treated with 10 μM fenofibrate, 10 μM troglitazone, 10 μM C20662, CR (0.4, 2.0 and 10 μg/ml) and GR (0.4, 2.0 and 10 μg/ml) for 24 h and lysed in Firefly Luciferase Lysis Buffer (Biotium, Inc., Hayward, CA, USA). The luciferase activity in the cell lysate was measured using a Firefly Luciferase Assay Kit (Biotium) according to the manufacturer’s protocol [25].

2.5. Animals and diets

Animal care and handling were performed according to protocols approved by the Animal Experimentation and Ethics Committee of Korea University (Protocol No. KUIACUC-20090420-4). C57BL/6j male mice (8 weeks old) were purchased from Samtako (Seoul, Korea) and maintained in a temperature–controlled (25°C) specific-pathogen-free facility on a 12-h light/dark cycle. The mice were fed an AIN-93G-based high-fat diet (40% of the total calories from fat) for 5 weeks to induce obesity prior to subsequent feeding experiments. The mice were divided into three groups, with one

isobutyl-1-methylxanthine (0.5 mM) 2 days postconfluence. The medium was replaced with fresh medium containing 10 μg/ml of insulin after 72 h.

2.6. Lipid staining and cellular lipid quantification

3T3-L1 adipocytes were stained using 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI; Molecular Probes, Carlsbad, CA, USA) according to the manufacturer’s instructions or stained with Oil Red O as described previously [24].
group of mice continuously fed a high-fat diet for 8 weeks while another two groups of mice were fed CR- and GR-isocaloric diets. The diet composition of each group is shown in Supplemental Table 2. Body weight was measured twice per week, and food intake was monitored once every 2 weeks. Blood samples were collected retroorbitally after 12 h of fasting. After 8 weeks of feeding, the mice were killed, and blood was collected by cardiac puncture and tissue samples including liver, epididymal fat (EF), mesenteric fat (MF) and perirenal fat (PF) were collected. The organ weights were determined immediately prior to snap freezing and storage at −80°C [26].

2.6. Plasma analysis and histology

Plasma lipid and glucose analyses were performed every 4 weeks during the feeding period (0, 4 and 8 weeks) [41]. Blood samples were collected retroorbitally after 12 h of fasting. Plasma TG, TC and high-density lipoprotein cholesterol (HDL-C) concentrations were determined using Cobas111, and non-HDL cholesterol was calculated by subtracting HDL cholesterol from TC values. Plasma glucose concentrations were measured using a portable glucose meter, Plasma insulin (Alpco, Salem, NH, USA), leptin (Millipore, Bedford, MA, USA) and adiponectin (Invitrogen, Carlsbad, CA, USA) concentrations were measured using an enzyme-linked immunosorbent assay kit according to the manufacturer’s instructions [27]. Liver and EF were fixed in 4% formaldehyde and stained with hematoxylin and eosin (H&E), and tissue images were obtained using a microscope. Adipocyte size was assessed using software analysis (Axio Imager M1; Carl-Zeiss, Oberkochen, Germany).

2.7. RNA preparation and quantitative polymerase chain reaction (qPCR)

qPCR was performed as described previously, with minor modifications [24]. Briefly, RNA was extracted from cultured cells or animal tissues with a phenol and guanidine isothiocyanate reagent (RNAiso Plus; Takara Bio Inc, Shiga, Japan). cDNA synthesis was then performed according to the standard procedure with reverse transcriptase (Mbiotech, Seoul, Korea) at 42°C for 60 min and 70°C for 15 min. The synthesized cDNA was amplified by real-time PCR (iCycler iQ5; Bio-Rad, Hercules, CA, USA) using PCR premix solution containing SYBR Green (SYBR Premix Ex TaqII; Takara, USA) concentrations. Amplification was performed as described previously, with minor modifications [24].

2.8. Metabolomic analysis with GC–TOF-MS

Aliquots (30 μl) of plasma were extracted with cold methanol (500 μl) after being spiked with each of internal standard compounds (Sigma-Aldrich, St. Louis, MO, USA), i.e., 2-deoxy-o-ribose [200 ppm (w/v) in water] for carbohydrates, heptadecanoic acid [100 ppm (w/v) in hexane] for lipids, tropic acid [50 ppm (w/v) in acetone] for organic acids, norleucine [200 ppm (w/v) in water] for amino acids. Prior to centrifugation, the extract was subjected to vortexing for 1 min and incubated on ice for 10 min. The extract supernatant (400 μl) was dried using a Centri-Vap (Labconco Corp., Kansas City, MO, USA) for 10 h. The dried sample was derivatized using 50 μl of N,N-bis(trimethylsilyl)trifluoroacetamide containing 1% trimethylchlorosilane (Supelco, Bellefonte, PA, USA) and 50 μl of acetonitrile. The samples were reacted using the Centri-Vap at 70°C for 1 h and cooled for 10 min before GC–TOF-MS analysis. All solvents were obtained from J.T. Baker (Phillipsburg, NJ, USA). Subsequently, GC–TOF-MS analysis was performed on a 6890N GC (Agilent Technologies, Palo Alto, CA, USA) interfaced with a Pegasus III TOF mass spectrometer (Leco, St. Joseph, MI, USA) using electron ionization at 70 eV. The GC was equipped with a DB-5MS column (30-m length × 0.25-mm id. × 0.25-μm film thickness; J&W Scientific, Folsom, CA, USA). The flow rate of helium, a carrier gas, was 1.0 ml/min. The derivatized sample (2 μl) was injected in splitless mode. The oven temperature program was as follows: initial temperature of 85°C for 5 min raised to 205°C at 8°C/min, which was held for 5 min and then elevated to 300°C at 8°C/min, then maintained for 5 min. The data acquisition rate was 20 scans/s in the mass range 45–550 m/z. The injector and detector transfer line temperatures were 230°C and 250°C, respectively. Metabolites were positively identified by comparing their retention times and mass spectra with those of authentic reference compounds. When the authentic standard compounds were not available, metabolites were tentatively identified based on Wiley 7n mass spectral database (Hewlett-Packard, Palo Alto, CA, USA; 1995), NIST05 MS Library and MS search Program V.2.0d (NIST, 2005). The relative levels of metabolites in the samples (n = 5) were determined by comparing their peak areas to those of the internal standard compound.

Fig. 1. Reporter gene assay results for PPARα and PPARγ in cells stimulated with CR or GR extracts (A) and expression of PPARγ and its target genes (B). Gene expression was measured using qPCR. Values are shown as the means ± S.E.M. *P < .05; **P < .01; ***P < .005: significant difference between groups treated with CR and GR at the corresponding concentrations.
2.9. Statistical analysis

In vitro and in vivo data were shown as means ± S.E.M., unless expressed otherwise. Student’s t test was performed to compare two groups. Statistical significance levels were preset at \( P < .05 \). For metabolic profiling, analysis of variance was performed using SPSS (version 12.0; SPSS Inc., Chicago, IL, USA) to estimate statistical significance among the metabolites (e.g., amino acids, organic acids, carbohydrates and lipids) found in the plasma of diet-induced obese mice fed the control, CR or GR. When the samples exhibited significantly different peak areas for the metabolites at the significance level \( P < .05 \), Duncan’s multirange test was employed. Multivariate statistical analysis (e.g., principal components analysis [PCA] and partial least-squares regression [PLSR]) was performed using SIMCA-P+ 11.0 (Umetrics, Umeå, Sweden). PCA was used for visualization and interpretation of the data matrix. PLSR analysis was applied to assess correlations between metabolites and antiobesity parameters of tested rice. Metabolites with variable importance in the projection (VIP) values >1.0 were considered to be compounds related to antiobesity activity.

3. Results

3.1. Transactivation of PPAR-α and PPAR-γ by GR stimulation

Luciferase reporter gene assays were performed to examine the effect of GR on PPAR activity (Fig. 1A). Ethanolic extracts (80%, w/v) from CR and GR were used for in vitro experiments. In the PPARα assay, GR strongly increased PPARα activity compared to the control, fenofibrate and CR groups. Transactivation of PPARα significantly by 2.6-, 2.7- and 3-fold compared with the CR \( (P < .05) \) upon stimulation with 0.4, 2 and 10 \( \mu \)g/ml of GR. In contrast, PPARγ activity decreased in the GR group compared to troglitazone and CR groups. Troglitazone and CR increased PPARγ activity by more than four- and twofold, respectively. However, the GR treatment group only showed 1.8- and 1.7-fold changes at 2 and 10 \( \mu \)g/ml, respectively, which were significantly lower than those of CR \( (P < .01 \) and \( P < .05 \), respectively).

3.2. Cellular lipids and gene expression in cultured hepatocytes and adipocytes, and gene expression in cultured hepatocytes

The mRNA levels of PPARγ, sterol regulatory element-binding protein 1c (SREBP1c) and fatty acid synthase (FAS) were analyzed using qPCR in HepG2 cells after GR stimulation for 24 h (Fig. 1B). PPARγ expression was down-regulated in GR compared to CR by 32% at 10 \( \mu \)g/ml \( (P < .01 \) and \( P < .05 \), respectively). The effects of GR on hepatic lipid concentrations were also examined. TG, TC and FFA concentrations were measured in HepG2 cells after 24-h treatment (Fig. 2A). The lipid levels in hepatocytes stimulated with GR were comparable with the levels in cells incubated with the PPARγ antagonist GW9662. In 3T3-L1 adipocytes, TC was significantly reduced in GR \( (P < .05 \) vs. CR; Fig. 2B). We confirmed the reduced lipid accumulation in adipocytes with GR using microscopy with Oil Red O and DiI staining (Fig. 2C) and found that cellular lipids in GR-stimulated 3T3-L1 adipocytes were comparable to cells treated with the PPARγ agonist GW9662.

3.3. Body weight of C57BL/6j mice and weight of liver and adipose tissues

The body weight of mice increased by 0.24, 0.29 and 0.28 g/day upon receiving a 40% fat, CR or GR diet, respectively. The weight gain
corresponded to the food intake in the three groups (Supplemental Table 4). The liver weights were slightly increased in rice groups, but the difference was not significant (15% vs. NC; Fig. 3A). Notably, white adipose tissue weight was significantly reduced in the GR group (Fig. 3A). The EF weights were lowest in the GR group (2.7, 2.5 and 1.7 g in NC, CR and GR, respectively). The weight of PF and MF showed similar trends with EF. Moreover, the GR feeding group had markedly reduced adipocyte size, which was confirmed by microscopic analysis of adipose tissue and H&E staining (Fig. 2B). The average adipocyte size was significantly reduced in GR compared to the NC and CR groups (P < .05). Liver steatosis developed after receiving a high-fat diet, but the signs of fatty liver were reduced in the GR feeding group, as demonstrated by the decreased size and number of lipid droplets (Fig. 2C). Hepatic TG concentrations also significantly decreased in GR compared with CR (−15%, P < .05; Fig. 3C).

3.4. Plasma glucose and the lipid profile

Oral administration of GR for 8 weeks effectively decreased the concentration of plasma TG, being 30%–38% lower than that in CR and NC (P < .05). Plasma TC in the GR group was significantly reduced by 20% compared to that in CR (P < .05). Conversely, the HDL-C/non-HDL-C ratio was elevated by 14.7% in the GR group compared to CR (P < .05). The plasma glucose concentrations were similar between the GR and CR groups (Table 1).

3.5. Plasma insulin, leptin and adiponectin

The major hormones that regulate lipid accumulation were measured in plasma (Fig. 3D). Plasma insulin and leptin levels were significantly reduced in mice fed GR compared to those fed CR and NC (P < .05). The insulin and leptin concentrations in the GR group were 61% and 19% lower, respectively, than those in the CR feeding group. In contrast, the plasma adiponectin concentration in the GR group was significantly higher than that in the CR group (P < .05).

3.6. Lipid metabolism gene expression in mouse liver and adipose tissue

The effects of GR on lipid accumulation and adipocyte differentiation were examined by monitoring the transcription of key lipid metabolism genes in mouse tissues (Fig. 4). The hepatic mRNA expression of PPARα in the GR group was 1.8-fold up-regulated compared to that in CR (P < .05). Similar trends were observed for the mRNA expression of PPARα in adipose tissue, confirming the self-
regulation of PPARα by its activation. PPARα target genes include lipoprotein lipase (LPL; TG hydrolysis in plasma), acetyl-CoA synthase (ACS; fatty acid uptake), acyl-CoA oxidase (ACOX), carnitine palmitoyl-transferase 1 (CPT1; β-oxidation) and malonyl-CoA decarboxylase (MCD; fatty acid synthesis) [42].

The mRNA level of LPL in adipose tissue increased by 1.2-fold compared to that in CR (P < .05 vs. CR). ACS, ACOX and CPT1 mRNA expression in liver tissue of GR fed mice was up-regulated with an increase of 1.4-, 1.3- and 1.6-fold, respectively, compared to that of CR (P < .05), suggesting that CR feeding may induce TG hydrolysis in
lipoprotein and the subsequent uptake and oxidation of fatty acids in the liver.

PPARγ of the GR group was slightly reduced in the liver and significantly reduced in adipose tissue compared with that in the CR group. We further analyzed the representative PPARγ target genes. The expression levels of SREBP1c, adipose differentiation related protein (ADRP) and FAS in the liver and C/EBPα in adipose tissue were quantified. FAS mRNA expression was reduced by GR feeding ($P < .01$) and was 2.2-fold lower compared to that in CR. The mRNA expression levels of ADRP, C/EBPα and SREBP1c also significantly decreased in the GR compared to the CR group ($P < .05$).

### 3.7. Plasma metabolic profiling by GC–TOF-MS

GC–TOF-MS analysis revealed a total of 25 metabolites, including 4 carbohydrates (mannose, glucose, ribitol and galactose), 6 lipids (propionate, butyrate, palmitate, linoleate, stearate and cholesterol), 5 organic acids (acetate, oxalate, succinate, malate and citrate) and 10 amino acids (alanine, valine, leucine, isoleucine, proline, glycine, serine, threonine, aspartic acid and phenylalanine) in the plasma of mice fed the control, CR and GR diets (Table 1). Among the carbohydrates, the plasma levels of mannose and glucose at the end of the experimental period (8 weeks) were higher in CR than GR. In particular, the group of mice fed GR began to show lower levels of glucose compared to those fed CR from 4 weeks ($P < .05$). For the lipids, the cholesterol concentration was significantly higher in the CR group compared to the GR group from 4 to 8 weeks ($P < .05$), while the levels of other lipids as well as organic acids were similar between the CR and GR groups. Among the amino acids, the level of plasma threonine was statistically lower in mice fed GR than in those fed CR at 4 weeks. All other amino acid levels were similar between the groups.

### 3.8. PCA of plasma metabolites

The complex GC–TOF-MS data sets were visually analyzed using PCA score plotting, including all metabolite data collected from the plasma samples of the control, CR and GR groups (Fig. 5). Significant differences were observed among all three groups between 0 and 8 weeks in principal component 1 (PC1) by 32.1%. The GR group was separated from the control and CR groups at 4 weeks in PC2 by 12.6%. The PCA score plot combining PC1 and PC2 explained 44.7% of the total variance. The major metabolites involved in the separation along PC1 were cholesterol, mannose, glucose and galactose (Fig. 6A), while palmitate, valine, leucine and proline were the major compounds contributing to the separation by PC2 (Fig. 6B). All groups were apparently shifted from the left to the right direction in the score plot according to the feeding period (Fig. 5). Both the control and CR groups were shifted from the left to the right in the score plot. From the 4-week period, the difference became significant according to the type of diets and rice cultivars. All three groups were statistically distinguished based on PCA score plotting after 8 weeks, suggesting that rice diets (especially the CR diet) induced significant changes in metabolomic profiling.

### 3.9. PLSR analysis

This analysis examined the correlations between data collected from GC–TOF-MS and lipid parameters of animal studies (e.g., HDL, non-HDL, BW, glucose, TG, TC, HDL/TC and HDL/non-HDL ratio). Partial least square could assess the relationship between a descriptor matrix $x$ (metabolites of GC–TOF-MS data) and a response matrix $y$ (lipid parameters). Metabolites with VIP values over 1 were considered strong contributors to explain the selection of $y$. The total variance in the PLS loading biplot explained 44.0% (PLS component 1: 31.5%, PLS component 2: 12.6%, PLS component 3: 10.0%).
component 2: 12.5%). Minor but significant changes were seen in the same groups according to the experimental feeding duration based on PLS1 (Fig. 7). All groups at week 0 were positioned on the left side in the plot (negative PLS1 values), while those groups were shifted to the right side after 8 weeks (positive PLS1 values) along PLS1. The metabolites with VIP scores exceeding 1.0, which contributed to PLS1 for the classification, included alanine, cholesterol, glucose, mannose, galactose, palmitate, butyrate acetate and glycine (Fig. 8A).

Fig. 7. The correlation plot of the relationship between plasma metabolites (▲) of GC–TOF-MS data and parameters involved in obesity activities (■). The control, CR and GR mice groups examined using GC–TOF-MS are represented by an abbreviation with a green (■); for example, 0 (week)_1 (replicate #).

Fig. 8. The value of VIP selected by PLSR analysis (VIP values > 1.0). (A) VIP scores based on PLS1; (B) VIP scores along PLS2.
Overall, PLS2 revealed differences among groups at 4 and 8 weeks. In particular, mice fed GR (negative PLS2 values) were distinguished from others after 4 weeks along PLS2. Moreover, the GR group was clearly separated from the control group after 8 weeks along PLS2. The metabolites with VIP scores higher than 1.0 were alanine, cholesterol, butyrate, glucose, mannose, galactose, ribitol, palmitate, valine, glycerine and acetate along PLS2 (Fig. 8B).

The major metabolites, which correlated with lipid parameters, were chosen based on the correlation coefficient values exceeding 0.1. HDL/non-HDL ratio and HDL/TC ratio (the lipid parameters) were found near the GR group at 8 weeks. Palmitate (16:0) \( r = -0.141 \), proline \( r = -0.138 \), leucine \( r = -0.133 \), valine \( r = -0.154 \) and oleate \( r = -0.125 \) were inversely correlated with the HDL/non-HDL ratio. In addition, valine was negatively correlated with the HDL/TC ratio \( r = -0.116 \) and positively associated with TG \( r = 0.114 \). Palmitate \( r = 0.120 \), valine \( r = 0.107 \), leucine \( r = 0.103 \) and proline \( r = 0.116 \) were non-HDL concentrations.

4. Discussion

Carbohydrate consumption has shifted to include relatively higher GI foods (rapidly absorbed carbohydrates). In parallel with this dietary shift, an increase has occurred in the prevalence of obesity, hyperlipidemia and hepatic steatosis. Recent studies have demonstrated that diets high in rapidly absorbed carbohydrates induced hepatic fat deposition, total body adiposity, hyperinsulinemia and higher plasma triacylglycerol concentrations [28,39]. In contrast, low GI food including those rich in dietary fiber can potentially ameliorate lipid metabolism and homeostasis [29]. This suggests that reducing the intake of high-GI foods and increasing the intake of low-GI foods are crucial to avoid metabolic disorders. Note that rice, which is consumed almost daily in many places, is classified as a high-GI food. Therefore, our current study evaluated the effect of a newly developed strain of rice, Goami-3 rice (GR with enhanced amylose fibers), on lipid metabolism and responsible biological mechanism of action compared to conventional rice.

In animal feeding studies, the plasma cholesterol concentrations from mice fed GR decreased compared to those fed CR. Although body weight and food intake were not altered in GR-fed mice, the size and number of lipid droplets and TG concentrations in liver tissue were significantly lower in the GR group along with reductions in TC levels. The weight of adipose tissues was also significantly lower in the GR-fed group compared to the other groups. This suggested that Goami rice may regulate lipid homeostasis in liver and adipose tissue in vivo.

Altered concentrations of adipokines, leptin and adiponectin corroborated the metabolic benefits of GR-feeding in mice. The reduction in plasma leptin (a sensor for fat mass) was consistent with the reduction of fat in mice fed GR in line with the study by Islam and coworkers [32], which improved leptin sensitivity [31,33,40].

Plasma adiponectin concentration is generally inversely correlated with adiposity and body fat [36,38] and exhibits potent lipid-lowering properties [37]. Our current study also demonstrated that the elevation of adiponectin in the GR group corresponded to the reduction of body fat. Mice fed GR showed significant reductions in plasma insulin concentrations compared to the CR group, perhaps because GR contains higher amounts of dietary fiber, which limit the demand for insulin secretion in mice. Collectively, the altered hormone levels could have contributed to the reduction in insulin sensitivity and body fat contents of mice fed GR in this study, suggesting that GR can potentially prevent or treat lipid-related metabolic syndrome via hormonal regulation.

PPARs are nuclear receptors and transcription factors that play an important role in the regulation of cellular lipid and glucose metabolism. In the reporter gene assay, GR extracts transactivated PPARα while inhibiting PPARγ transactivation, suggesting that phytochemicals other than amylose in GR extracts may have cellular lipid- and glucose-regulating effects. The gene expression of PPARα and PPARγ showed similar trends in both cultured cells and mouse livers. Inhibition of PPARγ was critical to reduce adipocyte differentiation and adipogenesis [30]. Along with the reduction in PPARγ transactivation, stimulation of adipocytes with GR also reduced lipid accumulation and adipocyte differentiation compared to CR in our present study. The expression of C/EBPα showed similar trends, supporting the inhibition of adipogenesis with GR compared with CR.

Activation of PPARα activity by GR was indeed beneficial as this transcription factor elevated β-oxidation, and the induction of MCD, CPT1 and ACOX gene expression supported PPARα activation with GR and reduced lipid accumulation in mouse livers [34,35]. Inhibition of PPARγ also lowered lipid accumulation by reducing lipogenic gene expression, including SREBP-1, FAS and ADRP, in either cultured hepatocytes stimulated with GR or livers of mice fed GR. This was also supported by the reduction in TG and TC in hepatocytes and the reduced lipid accumulation in cells. Overall, our results suggest that GR can possibly reduce the risk of hepatic steatosis.

Metabolite profiling was performed in the current study. The plasma concentrations of mannose and glucose were more significantly lowered in GR than CR. Both mannose and glucose are common hexoses used as energy sources in mammals [43], and mannose is a major substrate for protein glycosylation and can be formed from glucose [44]. Dietary fiber is known to be hypoglycemic by hindering α-glucosidase activity, thus delaying intestinal glucose uptake [45] and improving glucose metabolism [45,46]. Similarly, high-amylose rice delayed absorption of dietary carbohydrates such as mannose and glucose [47], suggesting that the intake of high-amylose GR could reduce plasma monosaccharide concentrations.

Based on PCA analysis, the GR group was distinguished from the control and CR groups after 4 weeks by specific metabolites such as palmitate and three amino acids (Val, Leu and Pro). Based on PLSR analysis, the HDL/non-HDL ratio, HDL/TC ratio and plasma lipid parameters were similar to the GR group at 8 weeks. Palmitate, oleate, proline, leucine and valine were inversely associated with the HDL/TC ratio, whereas valine was negatively correlated with the HDL/TC ratio. Those metabolites could be mostly involved in the dyslipidemic status of mice. For example, increased plasma concentrations of free fatty acids cause dyslipidemia, obesity and insulin resistance [50]. Plasma branched-chain amino acid (BCAA) concentrations were increased in obese individuals [48,49] since elevated BCAA catabolic flux may increase gluconeogenesis and glucose intolerance in obesity via glutamate transamination of glutamate to alanine [51]. Therefore, the metabolic effect of GR may be operated through increased plasma concentrations of branched chain amino acids.

In conclusion, GR improves metabolic syndrome by activating PPARα and inhibiting PPARγ, which enhance FFA oxidation and suppress lipid accumulation and adipocyte differentiation in both hepatocytes and adipocytes. In addition, GR intake may reduce obesity by lowering glucose, mannose and cholesterol levels in plasma. Thus, our current results suggest that rice with enhanced fiber and amylose may reduce the risks of hepatic steatosis and adiposity in in vitro and in vivo models (mice) [34,35,41,42].

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jnutbio.2013.06.008.

References

Lu H, Dunn WB, Shen H, Kell DB, Liang Y. Comparative evaluation of software for

Jia Y, Bhuiyan MJ, Jun HJ, Lee JH, Hoang MH, Lee HJ, et al. Ursolic acid is a PPAR-


