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Evaluation of obesity prevention effect of black ginseng on serum, liver, and hypothalamus of mice on a high-fat diet using a metabolomics approach

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Abstract

Black ginseng is being studied to prevent obesity caused by a high-fat diet (HFD). The aim of this study was to evaluate the obesity-preventing effect of black ginseng extract (BGE) in the serum, liver, and hypothalamus of mice on an HFD using metabolomic techniques. Mice were divided into four groups which were respectively fed a normal diet (CTL), an HFD, an HFD with a low concentration of BGE (BGEL), and an HFD with a high concentration of BGE (BGEH) for 8 weeks. Metabolite profiling revealed a clear separation between the BGE diet and HFD groups. Lipid metabolism, including saturated fatty acids and cholesterol, was decreased in the BGEH mice. Specifically, neurotransmitters and intermediates of the tricarboxylic acid cycle were increased in the hypothalamus of BGEH mice. The results suggest the obesity prevention effect of black ginseng in that BGEH inhibits body fat accumulation and restores brain function damaged by HFD.

Keywords Obesity prevention, Black ginseng, Metabolic profile, Hypothalamus, Liver, Serum

Introduction

Obesity is a metabolic disorder in which the high amount of fat accumulated in the body adversely affects health. It is associated with an imbalance of energy metabolism

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in various organs, such as the hypothalamus and liver, which regulate energy expenditure [1–3]. Among the brain tissues, the hypothalamus is essential for maintaining body homeostasis. Its role in regulating energy homeostasis has attracted considerable attention, as it coordinates both food intake and energy metabolism in response to a wide range of nutritional and other signals. It also maintains body weight and energy homeostasis by regulating glucose and lipid metabolism in the liver, lipid oxidation in muscles, and insulin secretion [4, 5]. In particular, the hypothalamus energy homeostasis mechanism is very sensitive to high-fat intake. Therefore, hypothalamic and hepatic metabolite profiling is essential to understanding obesity-related metabolism.

Metabolomics examines metabolites (sugars, nucleic acids, amino acids, lipids, etc.) produced during biological changes. It also studies metabolic pathways through correlations between metabolites. High-performance

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liquid chromatography (HPLC), gas chromatography (GC), mass spectrometry (MS), and nuclear magnetic resonance (NMR) are the main techniques used for metabolite analysis. Among them, GC–MS is widely used for metabolic profiling and provides effective and reproducible results [4]. Moreover, metabolomic technology is being applied in functional food development and efficacy evaluation because it is very useful for tracking environmental and dietary changes through comprehensive metabolite profiling and multivariate statistical analysis [6–8].

Black ginseng is made by steaming and drying raw or white ginseng several times; the transformation to black through the steaming process means that the white ginseng has undergone functional chemical changes [9]. For example, as steaming decomposes starch and polysaccharides, secondary metabolites such as phenolic acid and ginsenosides in the ginseng increase, which are also better digested in the body than fresh ginseng [10, 11]. In particular, black ginseng has a higher content of the ginsenosides Rg3, Rg5, Rk1, Rh1, and Rh2, showing superior pharmacological activity compared to white ginseng and red ginseng [12–14]. Among the various pharmacological effects of black ginseng, the anti-obesity effect is increasingly being subjected to research [15–17]. Most studies to date have investigated the obesity prevention or alleviation effects of black ginseng through gene expression analysis and blood lipid analysis [9, 15–17]. However, obesity is part of a metabolic syndrome, and it is important to study the extensive metabolic flow in blood and tissues (liver and hypothalamus) to validate the obesity-preventing effects of black ginseng.

Therefore, we aimed to evaluate the obesity-preventing effect of black ginseng in the serum, liver, and hypothalamus of mice on a high-fat diet (HFD) using metabolomic techniques. We conducted comprehensive metabolite profiling of the serum, liver, and hypothalamus of mice fed an HFD along with black ginseng supplementation for 8 weeks using GC–MS and multivariate statistical analysis.

Materials and methods

Preparation of black ginseng

Black ginseng extract (BGE) was prepared from the roots of 5-year-old *Panax ginseng* grown in Korea according to the Good Agricultural Practices standards established by the Rural Development Administration (RDA) as previously described [18]. The voucher specimen (NIHHS1901) was placed at the herbarium of the Department of Herbal Crop Research, National Institute of Horticultural and Herbal Science, RDA, Korea. Briefly, the root samples of *Panax ginseng* were prepared via three cycles involving steaming at 95–98 °C for 3–5

h and drying at 50 °C for 24 h for each cycle. The sample was powdered, percolated with water at 80 °C for 4 h, and concentrated using a vacuum evaporator to separate the extract. The powdered BGE was stored at −20 °C. The ginsenosides were identified using HPLC; the main ginsenosides (Rg3, Rg5, and Rk1) together weighed 7.48 mg/g of the BGE sample [19].

Animals, diets, and sample collection

Seven-week-old C57BL/6 male mice were purchased from the Dae Han Bio Link Company, Limited (Eumseong-gun, Chungcheongbuk-do, Korea). The purchased mice were housed in cages with a 12 h light–dark cycle at a controlled temperature (22 °C) and $55 \pm 5\%$ humidity. In total, 24 mice were randomly divided into 4 groups with 2 cages per group and 3 mice per cage. One week prior to the start of the experiment, the mice were acclimated to a normal diet and free access to tap water.

Four groups were treated as follows for 8 weeks: normal diet group (CTL), HFD group, HFD with 400 mg/kg/day BGE (HFD + BGEL), and HFD with 600 mg/kg/day BGE (HFD + BGEH) (n = 6 per group). The experimental feed conditions for BGE were conducted with reference to the conditions of previous studies [20–22]. It has been reported that feeding black or red ginseng along with a high-fat diet at 400 mg/kg/day for more than 4 weeks has an anti-obesity effect or insulin sensitivity improvement. CTL mice were fed a standard diet (D12450B, Research Diets, Inc., New Brunswick, NJ, USA), while the other three groups were fed an HFD (D12492, Research Diets, Inc.) with 60% kcal of fat. CTL and HFD groups were provided with tap water, while the HFD + BGEL and HFD + BGEH groups were given drinking water containing BGE at the concentrations specified for each group. Based on average water consumption and increased body weight, the dose of BGE was adjusted to maintain the desired concentration for 8 weeks.

Food and water intake were recorded every other day, and each water bottle was changed. Water intake was monitored by weighing the water bottles. Body weight was measured weekly. Serum and tissue samples were obtained at the end of the 8 weeks. Blood samples were collected by heart punctures and centrifuged at 3000 rpm for 10 min at 4 °C to obtain the serum. Tissue samples (epididymal fat, liver, and hypothalamus) were collected and weighed. The hypothalamus was immediately stored at −80 °C while the liver was lyophilized for 72 h after which it was ground and stored until further analysis.

Methods of extraction and analysis of metabolites

Previously described procedures for extracting and analyzing the serum, liver, and hypothalamus were followed [23, 24]. Briefly, the serum (0.1 mL) was extracted with

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0.3 mL of 3:1 (v/v) methanol: chloroform mixture and 0.03 mL of the internal standard (IS, 2-chloro-L-phenylalanine, 300 µg/mL). The hypothalamus and lyophilized liver (10 mg) were extracted after three rounds of homogenizing for 20 s with 250–300 mg glass beads of size 425–600 µm in 1 mL of 50% methanol (v/v) and 0.03 mL of IS, using a bead beater (Mini Beadbeater 96, BioSpec Products, Bartlesville, OK, USA). The sample extracts were sonicated for 10 min and centrifuged at 13,000×g for 15 min. The supernatant liquid separated into a clean tube was completely dried using a vacuum concentrator (VS-802 F, Visionbionex, Gyeonggi-do, Korea) and a freeze dryer (MCFD8512, IlShinBioBase, Gyeong-gi-do, Korea) for about 4 and 16 h, respectively. The freeze-dried sample was derivatized in 80 µL of methoxyamine hydrochloride in pyridine (20 mg/mL) at 37 °C for 90 min, mixed with 80 µL of *N,O*-bis(trimethylsilyl) trifluoroacetamide and incubated at 60 °C for 60 min. After cooling, 1 µL of the solution was injected into the GC–MS. The GC–MS analysis was performed on an AOC-20i auto-sampler and GCMS-QP2010 Ultra system (both from Shimadzu, Kyoto, Japan). For separation of metabolites from the serum, liver, and hypothalamus, a DB-5 column (30 m × 0.25 mm id, film thickness 1.0 µm, 122-5033, Agilent, Santa Clara, CA, USA) was used with helium as the carrier gas at a constant flow rate of 1.1 mL/min. The oven temperature was initially maintained at 100 °C for 4 min, increased to 320 °C at a rate of 10 °C/min, and finally sustained at 320 °C for 11 min. The split ratio was 1:10, and full scan mode (m/z 45–600) was used. Peak identification of the GC–MS data was conducted by comparing their retention times and mass spectra with standard compounds, MS library (Nist 7.0 and Wiley 9), and in-house library (Additional file 1: Table S1). Peaks with a signal-to-noise ratio > 5 and a Kovats retention index gap of < 20 were selected. Quantitative analysis was performed using the ratio of the analyte peak area to IS peak area.

Statistical analysis
The normalization (unit variance scaling) of identified GC–MS data was performed for principal component analyses (PCA), orthogonal partial least squares discriminant analysis (OPLS-DA), and hierarchical clustering heat map. OPLS-DA was conducted using the soft independent modeling of class analogy (SIMCA) package 14.1 software (Umetrics, Umeå, Sweden). Multi-Experiment Viewer version 4.9.0 (MeV) was used to construct the hierarchical clustering heat map. Box plots were constructed with Prism Graph Pad 8.0 software (GraphPad, San Diego, CA, USA). Analysis of variance (ANOVA) and Student's *t*-test for the serum, liver, and hypothalamus metabolites of the four groups were performed using SAS version 9.4 (SAS Institute, Cary, NC, USA).

Results and discussion
Effect of black ginseng on body and organ weight of mice
In this study, the daily food intake, daily water intake, and body weight were measured to investigate the obesity prevention effect of BGE consumption (Table 1). Eight-week-old male C57BL/6 mice with similar initial body weights were randomly divided into four groups (n = 6 per group): CTL, HFD, HFD + BGEL, and HFD + BGEH. The difference in daily food intake among the four groups was not significant during the experimental period. The daily water intake was not statistically different among the three groups fed the HFD but was significantly lower than in the CTL group ($p < 0.05$). These results are consistent with previous studies that observed that rats fed an HFD were less thirsty during meals than those on a normal diet due to increased salivation [25]. After the 8-week experiments, the HFD group had the highest body weight (46.89 ± 1.64 g), followed by the HFD + BGEL, HFD + BGEH, and CTL groups (Fig. 1). In particular, the average body weights in the HFD + BGEL and HFD + BGEH groups were 43.87 ± 3.20 g and 42.18 ± 1.70 g, respectively, which were significantly lower than that in the HFD group ($p < 0.05$).

In addition, excessive caloric intake causes fat accumulation in the body, leading to obesity. Therefore, the weights of epididymal fat and liver were measured as indicators of the development of obesity [26]. Epididymal fat weight was higher in the HFD, HFD + BGEL, and HFD + BGEH groups than in the CTL group ($p < 0.05$). However, when the other conditions remained the same, there was a difference in the amount of fat accumulated on an HFD diet based on the amount of BGE consumed. The epididymal fat weight of HFD + BGEH mice (2.61 ± 0.19 g) was statistically lower than that of HFD
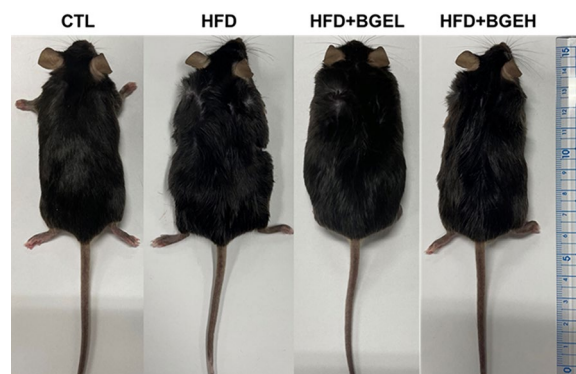

Fig. 1 Photographs of representative mice of each group after experiments lasting 8 weeks

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Table 1 Effect of black ginseng extract on general characteristics of mice fed different experimental diets for 8 weeks (means ± SD)

	CTL	HFD	HFD + BGEL	HFD + BGEH
Food intake (g/day)	2.65 ± 0.24	2.48 ± 0.34	2.45 ± 0.24	2.55 ± 0.29
water intake (g/day)	2.54 ± 0.24	2.27 ± 0.19**	2.20 ± 0.15**	2.30 ± 0.31*
Initial body weight (g)	24.61 ± 0.46	25.09 ± 0.36	24.85 ± 0.71	25.25 ± 0.70
Final body weight (g)	33.78 ± 1.33	46.89 ± 1.64*	43.87 ± 3.20*,#	42.18 ± 1.70*,##
Epididymal fat (g)	1.37 ± 0.13	2.91 ± 0.20*	2.83 ± 0.14*	2.61 ± 0.19*,###
Liver weight (g/100 g B.W)	3.39 ± 0.41	4.86 ± 0.78*	4.05 ± 0.52*,#	3.35 ± 0.22##

BW body weight

*$p < 0.05$ CTL versus HFD, HFD + BGEL, or HFD + BGEH

# $p < 0.05$

$p < 0.01$

$p < 0.001$ HFD versus HFD + BGEL, or HFD + BGEH

mice (2.91 ± 0.20 g, $p < 0.01$). Liver weights decreased in the HFD + BGEL and HFD + BGEH groups compared to that of the HFD group ($p < 0.05$). Notably, the liver weights of HFD + BGEH mice were similar to those of the CTL group. In previous studies, several tea extracts and herbal mixtures significantly reduced liver weight, epididymal fat content, and serum triglycerides in mice fed with a HFD, suggesting that tea supplementation inhibits hepatic lipid accumulation [27, 28]. Therefore, our results combined suggest that BGE intake ameliorates fat accumulation caused by an HFD, and consumption of BGE in high concentrations is more effective in combating obesity than in low concentrations.

Metabolic characteristics according to black ginseng intake in serum, liver, and hypothalamus of mice fed a high-fat diet

Mechanisms acting against obesity are related to amino acid, lipid, and glucose metabolisms [29]. The purpose of this study was to investigate the metabolic differences induced by black ginseng in conditions of obesity. Therefore, metabolic profiling of samples from the serum, liver, and hypothalamus was conducted using GC–MS to identify hydrophilic and lipophilic metabolites involved in various metabolisms. A total of 57, 55 and 57 metabolites were detected in the serum, liver, and hypothalamus of mice, respectively (Additional file 1: Tables S2–S4). First, PCA, an unsupervised statistical method, was performed using serum, liver, and hypothalamus metabolites to visualize differences between metabolic phenotypes [30]. In the score plots of PCA models obtained using serum, liver, and hypothalamus metabolites, there were distinctions between the CTL, HFD, HFD + BGEL, and HFD + BGEH groups (Fig. 2). In addition, there was a difference according to the intake of black ginseng among the three groups (HFD, HFD + BGEL, and HFD + BGEH) that consumed an HFD. A common result of the three

PCA models was that the HFD and HFD + BGEH groups were at either ends, and the HFD + BGEL group was in the middle. This result means that the differences between the HFD and HFD + BGEH groups are the most pronounced. Additionally, there are metabolic changes that depend on whether black ginseng is consumed or not, as well as on the amounts of the black ginseng consumed.

Based on the results of the above statistical analysis, OPLS-DA was performed between the HFD and HFD + BGEH groups to confirm the distinct metabolic changes that occurred after intake of black ginseng at high concentrations in mice with obesity induced by an HFD. As a supervised characterization recognition approach, the OPLS-DA model is established to maximize class separation [30]. The values of R^2Y and Q^2 obtained from the model represent the fit and predictability according to the class, respectively. Values close to 1 indicate a better established model. In our results, the ranges of R^2Y and Q^2 values of the models obtained from serum, liver, and hypothalamus metabolites for the two groups were 0.984–0.998, and 0.847–0.978, respectively.

In addition, the variable importance in the projection (VIP) plot of the OPLS-DA mod-el accounts for metabolites contributing to the differences between the two groups. Metabolites with a VIP value of 1 or higher are significant contributors [31]. In the VIP plot of the OPLS-DA model derived from serum metabolites of the HFD and HFD + BGEH groups, 25 metabolites, comprising 12 lipids, 7 amino acids, and 6 organic acids, had VIP values greater than 1.0 and were statistically significant ($p < 0.05$) (Fig. 3). Specific changes in serum metabolites resulting from obesity-preventing effects of BGE were observed in lipid and amino acids metabolism. First, the levels of 12 lipids, i.e., saturated fatty acids (decanoic, lauric, myristic, palmitic, and stearic acids), unsaturated fatty acids (palmitoleic,
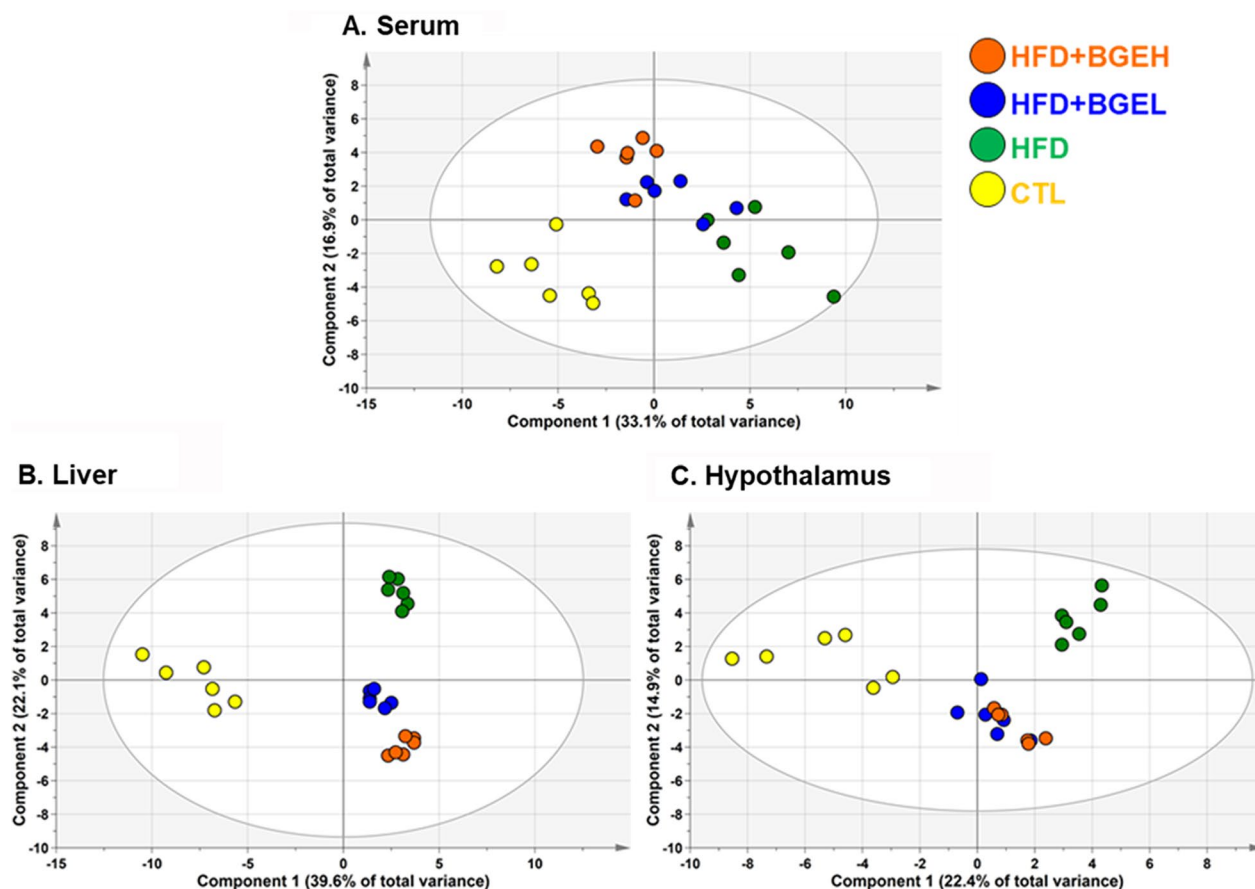
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Fig. 2 Score plots from PCA models derived from metabolites in **A** serum, **B** liver, and **C** hypothalamus of mice fed the CTL diet or HFD, HFD + BGEL, or HFD + BGEH for 8 weeks. CTL, normal diet; HFD, high-fat diet; HFD + BGEL, HFD with 400 mg/kg/day black ginseng extract; HFD + BGEH, HFD with 600 mg/kg/day black ginseng extract

oleic, linoleic, and arachidonic acids), cholesterol, glycerol, and *O*-phosphoethanolamine (O-PE), were lower in the serum of HFD + BGEH mice than in HFD mice. An HFD contains many triglycerides where three fatty acids are bound to glycerol. Therefore, triglycerides are broken down into one glycerol and three fatty acids in the body and absorbed. These three fatty acids are two saturated fatty acids and one unsaturated fatty acid, mainly linoleic, palmitic, and stearic acids [32]. This means that an HFD increases the concentration of saturated fatty acids, linoleic acid, and glycerol in the body. In previous studies, consuming an HFD for 8 weeks was found to increase the concentration of blood lipids (free fatty acids and cholesterol) and body fat, leading to obesity and various diseases (for example, diabetes, hypertension, and brain disease) [33–35]. In addition, it has been reported that total cholesterol and triglycerides decreased in the serum of mice fed HFD supplemented with black ginseng, and total fecal weight and fecal fat excretion increased in the mice on an HFD

supplemented with black ginseng [16]. This suggested that a diet that includes black ginseng is effective in combating obesity through suppression of fat digestion. Thus, in our results, the high concentration of lipids in the HFD group indicates excessive fat accumulation, whereas BGEH in the diet inhibited fat absorption. Next, among major amino acids, alanine, leucine, isoleucine, and proline increased in the serum of HFD mice. Also, glycerol-3-phophatate (G3P), dihydroxyacetone, and dihydroxyacetone phosphate (DHAP) were raised in the HFD group. Previous studies have reported that an HFD for 8 weeks or longer increases insulin resistance. As a result, the body's glucose balance is disturbed, and gluconeogenesis is activated in the liver [36]. In this process, amino acids such as alanine, leucine, and isoleucine, as well as intermediates of gluconeogenesis (G3P, dihydroxyacetone, and DHAP) are elevated. In other words, our results suggest that fat accumulation and problems with insulin regulation occurred in mice on an HFD for 8 weeks. In contrast,
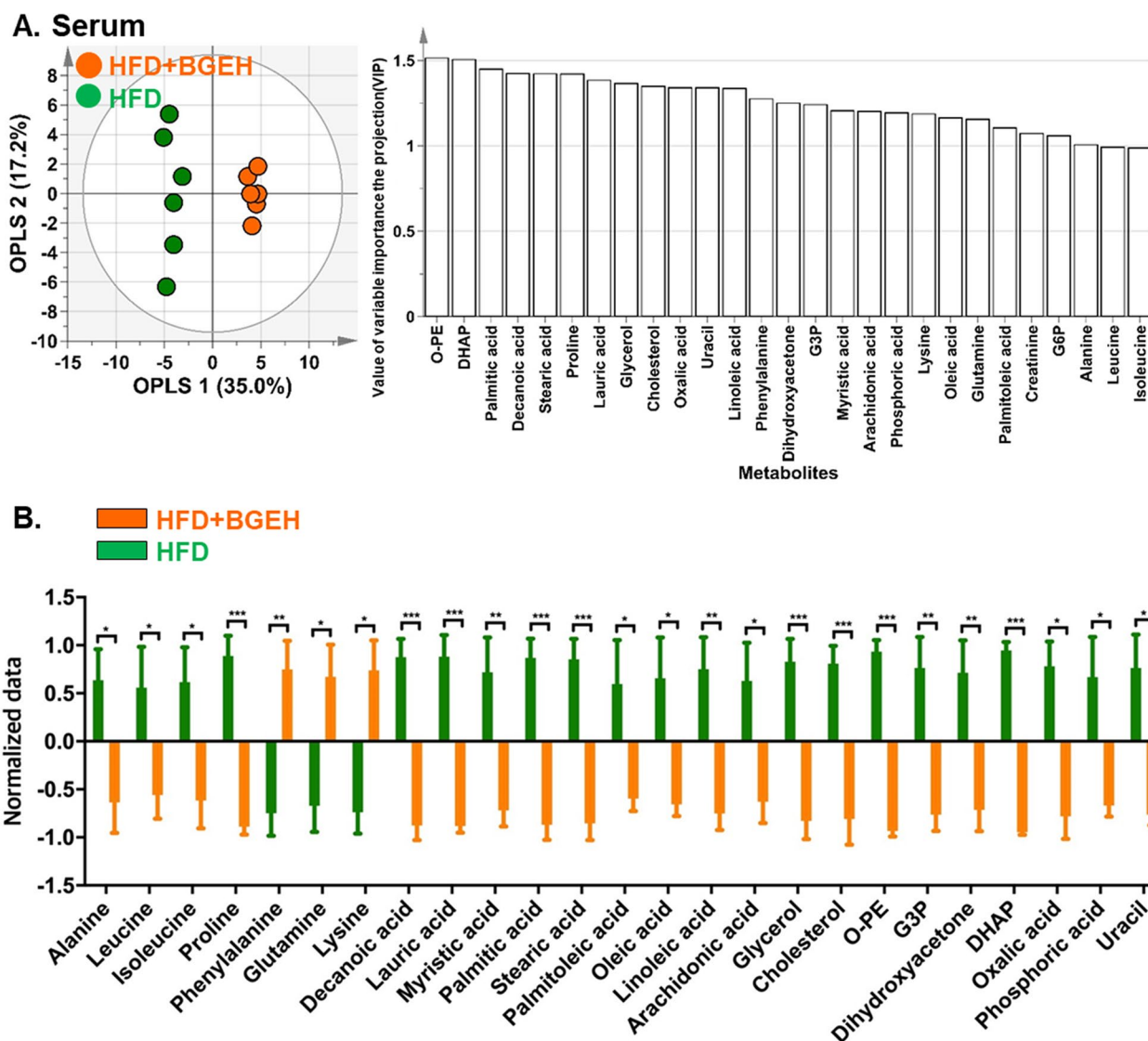
Fig. 3 A Score and VIP plots of OPLS-DA models created from 57 metabolites detected in the serum of mice. OPLS-DA model represents regression between HFD and HFD + BGEH mice. **B** Serum metabolites with VIP values > 1 and $p < 0.05$ are shown in the normalized graph with unit variance scaling. HFD: high-fat diet; HFD + BGEH: HFD with 600 mg/kg/day black ginseng extract; G3P: glycerol 3-phosphate; G6P: glucose 6-phosphate; O-PE: *O*-phosphoethanolamine; DHAP: dihydroxyacetone phosphate. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$

BGEH consumption reduced the absorption of body fat even when fat intake was high, thereby reducing the negative metabolic effects of HFD.

We identified the metabolite differences between the HFD and HFD + BGEH mice through the OPLS-DA model of liver metabolites (Fig. 4A). A total of 26 metabolites were statistically significant (VIP values > 1.0 and $p < 0.05$) (Fig. 4B). Among these metabolites, 3 amino acids (valine, isoleucine, and threonine), 13 lipids (decanoic acid, lauric acid, myristic acid, palmitic acid, stearic acid, palmitoleic acid, oleic acid, arachidonic acid, glycerol, cholesterol, O-PE, and monostearin), phosphoric

acid, G3P, DHAP, and dihydroxyacetone exhibited decreases in the HFD + BGEH group. These findings were the same as for the differences in serum metabolites of HFD and HFD + BGEH mice. Lipid metabolism and gluconeogenesis occur in the liver, and there are many reports of hepatic metabolic changes related to an HFD [37, 38]. An HFD is known to be the main cause of fatty liver, and fatty liver is accompanied by complications of diabetes and hyperlipidemia [39, 40]. That is, the changes to the liver metabolites brought about by the BGEH in the diet in this study suggest that the BGEH can improve liver health impaired by obesity. In addition,
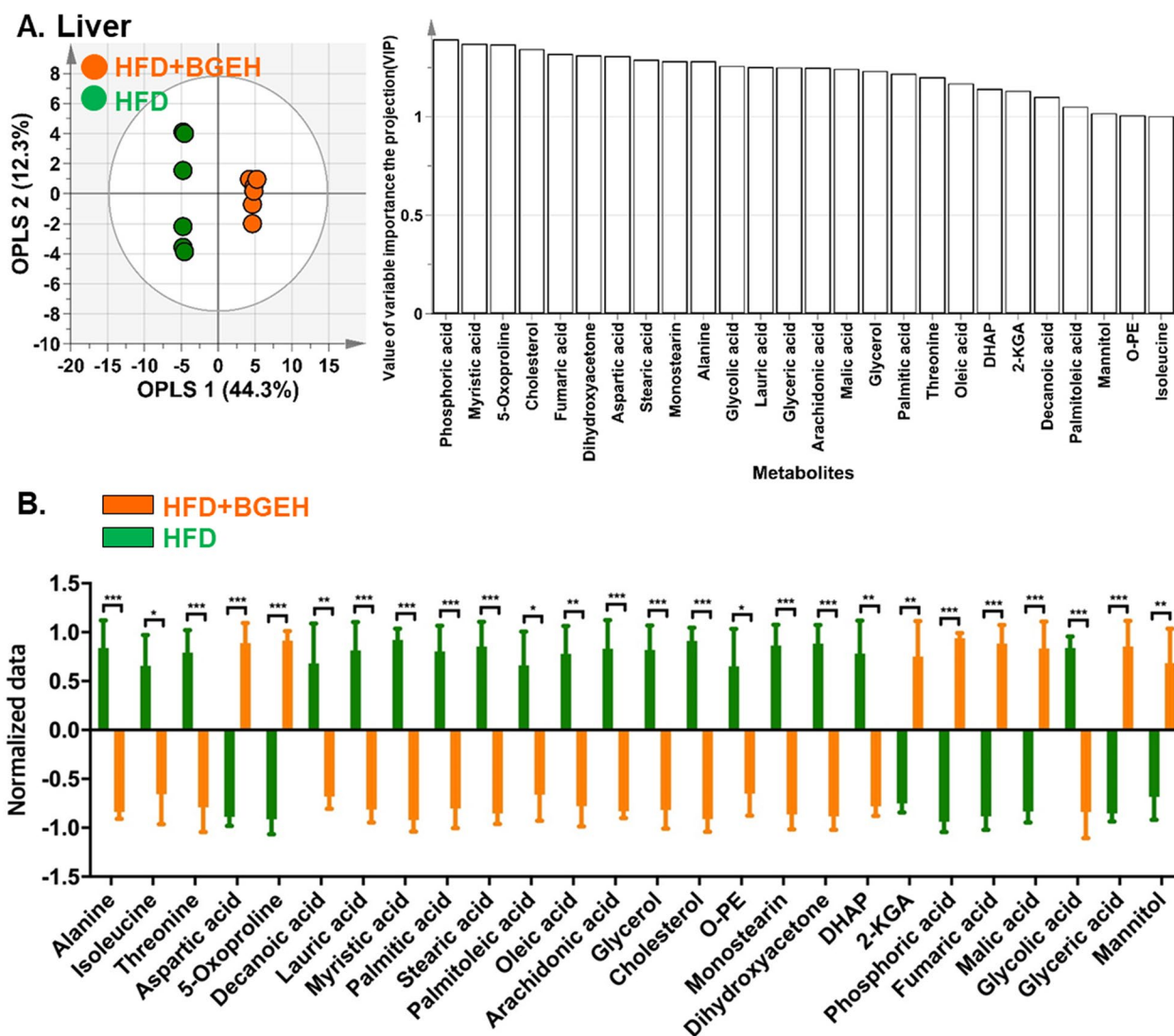
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Fig. 4 A Score and VIP plots of OPLS-DA models created from 55 metabolites detected in the liver of mice. OPLS-DA model represents regression between HFD and HFD + BGEH mice. **B** Liver metabolites with VIP values > 1 and $p < 0.05$ are shown in the normalized graph with unit variance scaling. HFD: high-fat diet; HFD + BGEH: HFD with 600 mg/kg/day black ginseng extract; 2-KGA: 2-ketoglutaric acid; O-PE: *O*-phosphoethanolamine; DHAP: dihydroxyacetone phosphate. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$

in our results, 5-oxoproline increased in the liver of HFD + BGEH mice. 5-Oxoproline, an intermediate metabolite of glutathione, has been reported to be significantly reduced in the liver of obese mice because it is closely related to antioxidant function [41, 42]. A high-fat and high-cholesterol diet causes oxidative stress, which causes oxidative damage to body tissues [43]. Our results suggest that the liver of HFD mice suffered oxidative damage due to high fat and that BGEH in the diet prevented this damage. Thus, the metabolite differences in the liver reflect the significant reduction in liver weights of the groups on diets that include BGE compared to that of the HFD group.

In the VIP plot of the OPLS-DA model derived from hypothalamus metabolites of the HFD and HFD + BGEH groups, 21 metabolites were identified as important contributors (Fig. 5A). These metabolites had statistical significance ($p < 0.05$) and included lipids, amino acids, and pyrimidine nucleobases (Fig. 5B). Compared to the HFD group, the HFD + BGEH group showed a decrease in eight lipids, namely decanoic acid, myristic acid, palmitic acid, stearic acid, palmitoleic acid, linoleic acid, glycerol, and cholesterol. High levels of saturated fatty acids, linoleic acid, and cholesterol are known biomarkers that trigger brain inflammation [33, 44]. In other words, our results implied that brain inflammatory metabolism
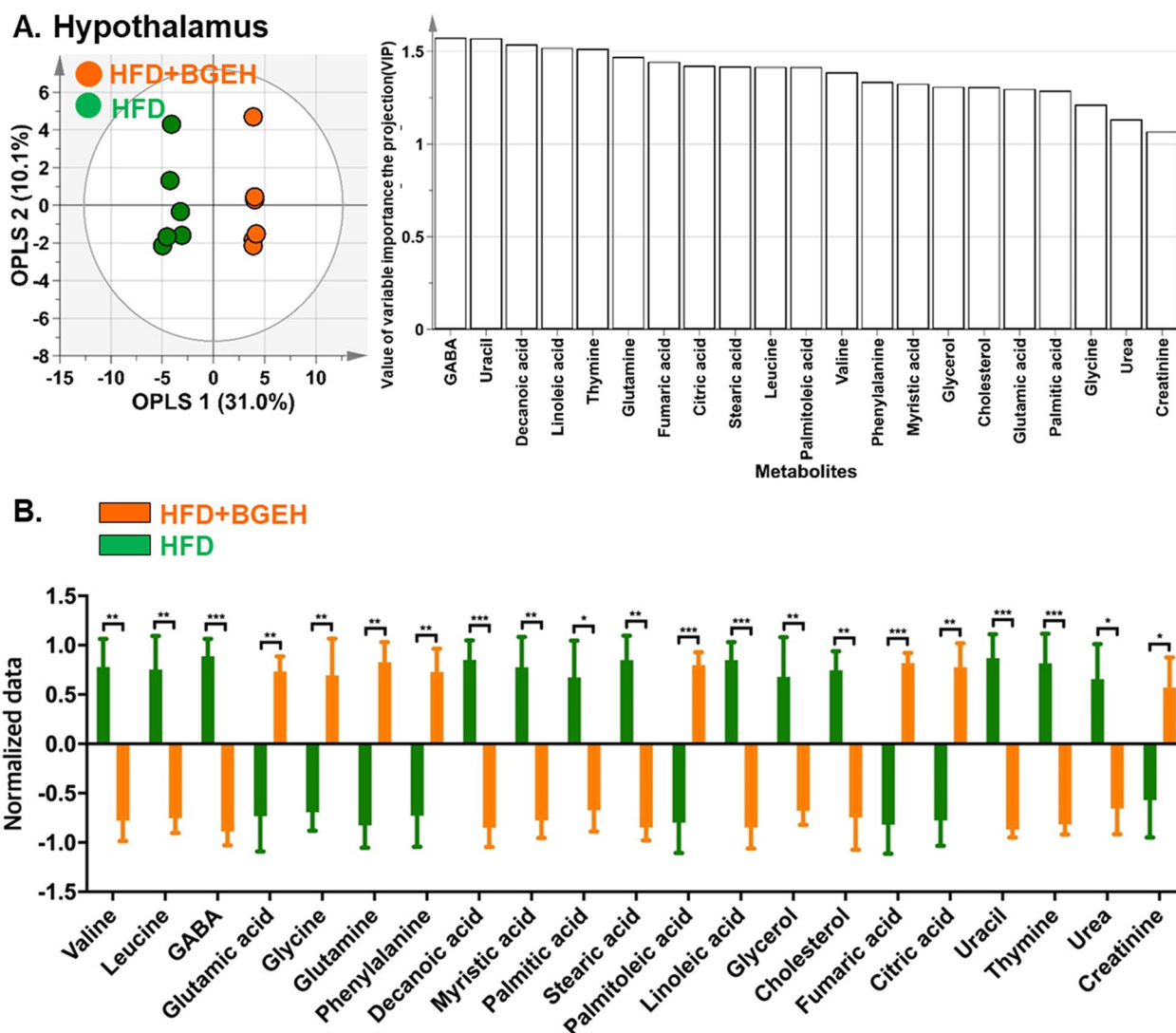
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A. Hypothalamus

B.

Fig. 5 **A** Score and VIP plots of OPLS-DA models created from 57 metabolites detected in the hypo-thalamus of mice. OPLS-DA model represents regression between HFD and HFD+BGEH mice. **B** Hypothalamus metabolites with VIP values > 1 and $p < 0.05$ are shown in a normalized graph with unit variance scaling. HFD: high-fat diet; HFD+BGEH: HFD with 600 mg/kg/day black ginseng extract; GABA: 4-aminobutyric acid. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$

decreased due to the inhibition of fat absorption following consumption of the diet that includes BGEH. In the case of the hypothalamus, glutamine, glycine, glutamic acid, and 4-aminobutyric acid (GABA) involved in neurotransmission were markedly changed (Fig. 6A); among them, all except GABA were detected at higher concentrations in the HFD+BGEH group than in the HFD group. The hypothalamus is an important part of the brain that regulates body and energy metabolism homeostases, which are affected by brain inflammation and neurotransmission [45]. The relationship between glutamine, glutamic acid, and GABA in the hypothalamus has been reported in many studies. Glutamine is

a precursor to glutamic acid and GABA. Glutamic acid is used in the synthesis of GABA and is also involved in the synthesis of α-ketoglutarate, an intermediate in the TCA cycle [46–48]. A recent study reported that an HFD inhibits the breakdown of GABA and that abnormally increased GABA leads to impaired nervous system function [49]. GABA is known to have anti-obesity effects when ingested, but when locally infused in the brain, it adversely affects the nervous system [49]. Therefore, our findings suggest that the hypothalamus of mice on an HFD was impaired in brain function, including neurotransmission. In contrast, fumaric acid and citric acid, as intermediates of the tricarboxylic acid (TCA) cycle,
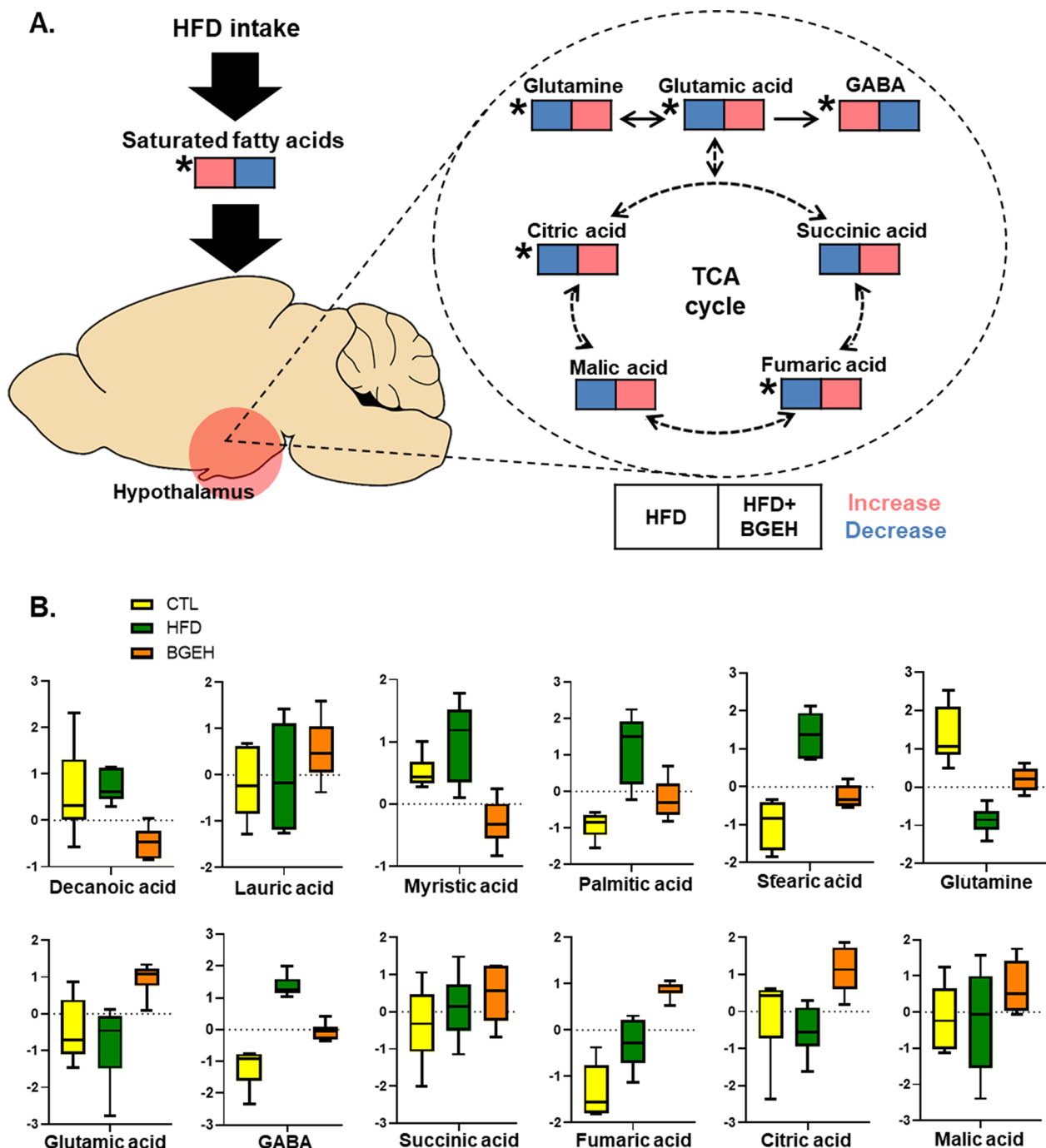
Fig. 6 A Metabolic pathway and **B** box plots of metabolites in the hypothalamus affected by BGEH combined with an HFD. Data normalized to UV scaling with *p* < 0.05 are presented as box plots. CTL: normal diet; HFD: high-fat diet; HFD + BGEH: HFD with 600 mg/kg/day black ginseng extract; GABA: 4-aminobutyric acid; **p* < 0.05

were increased in the HFD + BGEH group (Fig. 6A). This is consistent with previous findings that an HFD decreased energy circulation and that green tea and ginseng extracts increased energy expenditure in obese rats [4, 50]. Also, uracil and thymine, as pyrimidine

nucleobases, were increased in the HFD group. Increased levels of uracil and thymine are known biomarkers associated with impaired deoxyribonucleic acid (DNA) repair function [4, 23]. Therefore, the results for uracil and thymine, along with GABA in the HFD group,

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suggest brain dysfunction. Furthermore, in the box plots of hypothalamus metabolites between CTL, HFD, and HFD + BGEH groups, glutamic acid and intermediates of TCA cycle were increased in the HFD + BGEH group compared to CTL group ($p < 0.05$) (Fig. 6B). Saturated fatty acids increased in the hypothalamus of HFD mice compared to CTL group. However, compared to the HFD group, the HFD + BGEH group showed a decrease. In the HFD + BGEH group, saturated fatty acids were similar to those in the CTL group or showed a tendency to slightly increase (Fig. 6B). Overall, the changes in hypothalamic metabolites in this study suggest that a diet that includes BGEH increases energy metabolism activity and has obesity-preventing effects by alleviating brain function damaged by an HFD.

In conclusion, metabolomic approaches were used to investigate the obesity-preventing effects of BGE on the serum, liver, and hypothalamus of mice fed an HFD. An HFD diet along with BGE for 8 weeks reduced body weight, adipose tissue, and liver weights compared to that of mice on HFD alone. In addition, metabolic changes were confirmed using metabolite profiling and multivariate analysis. It was confirmed that se-rum, liver, and hypothalamus from the HFD + BGEH group showed a decrease in lipids metabolism, with saturated fatty acids, glycerol, and cholesterol showing similar trends ($p < 0.05$). In the liver of the HFD + BGEH mice, the level of 5-oxoproline—known as an oxidative stress-relief biomarker—was elevated ($p < 0.001$). These results indicate that a diet that includes black ginseng has obesity prevention effects by inhibiting fat absorption. Furthermore, the levels of glycine, glutamine, glutamic acid, fumaric acid, and citric acid were increased in the hypothalamus of HFD + BGEH mice ($p < 0.05$), demonstrating that black ginseng diet relieved metabolic brain damage caused by obesity and activated energy metabolism. Therefore, the obesity-preventing effect of black ginseng in the serum, liver, and hypothalamus of mice fed an HFD was successfully evaluated for the first time using metabolomic techniques. The application of metabolomics in our study also suggests that metabolic phenotypes could be a useful tool to verify the obesity-preventing effects of medicinal plants. Further studies should explore the metabolic responses with multiple organs involved in obesity prevention.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13765-023-00829-8.

Additional file 1: Table S1. Relative retention times (RRTs) and GC–MS data of hydrophilic and lipophilic metabolites in the serum, liver and hypothalamus of mice. **Table S2.** Composition and content (ratio/g) of hydrophilic and lipophilic metabolites in serum of mice fed different experimental diets for 8 weeks. **Table S3.** Composition and content

(ratio/g) of hydrophilic and lipophilic metabolites in liver of mice fed different experimental diets for 8 weeks. **Table S4.** Composition and content (ratio/g) of hydrophilic and lipophilic metabolites in hypothalamus of mice fed different experimental diets for 8 weeks.

Author contributions

Conceptualization, JKK, and JGK; investigation, SUP, and K-HI; resources, YJK, DYL, and HRY; data curation, SUP, and K-HI; writing—original draft preparation, YJK, DYL, and JKK; writing—review and editing, JKK, and JGK; visualization, YJK, and DYL; project administration, JKK, and JGK. All authors read and approved the final manuscript.

Availability of data and materials

All study data are included in the article and Additional file. Data will be made available on request.

Declarations

Ethics approval and consent to participate

All animal care and experimental procedures were performed according to protocols approved by the Institutional Animal Care and Use Committee (IACUC) of the Incheon National University (Authorization Number: INU-ANIM-2021-05).

Competing interests

All authors have no cmpeting interests to declare.

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