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Effects of ginseng dietary supplementation on a high-Fat diet-induced obesity in C57BL/6 Mice

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ABSTRACT

Excess caloric intake increases the amount of adipose tissue and contributes to metabolic disorders in disrupted metabolic homeostasis. This study aimed to investigate the anti-obesity effects of ginseng and the alternation of gut microbiota composition in high-fat diet (HFD)-induced obesity. The results showed that HFD treatment influenced body weight gain, adipose tissue accumulation and biochemical parameter changes. Compared to the HFD group, ginseng supplementation of HFD-fed mice decreased body weight, adipose tissue mass, total cholesterol (T-CHO) and high-density lipoprotein (HDL)/low-density lipoprotein (LDL) ratio. To analysis the alterations of gut microbiota, ginseng in dietary supplements decreased Firmicutes abundance and increased Bacteroidetes abundance. Taken together, these findings suggest ginseng may modulate the energy storage and alter gut microbiota composition.

1. Introduction

Excess intake may cause fat accumulation in adipose tissue and contribute to metabolic disorders in disrupted metabolic homeostasis [1]. Adipose tissue acts as an endocrine organ and secretes various bioactive factors such as hormones, cytokines, adipocytokines and growth factors involved in intestinal homeostasis [2]. Previous studies have reported changes in gut microbiota composition in obesity affect the genes involved in energy metabolism [3]. The gut microbiota might be a promising target for preventive and therapeutic intervention against obesity. The gram-positive Firmicutes and the gram-negative Bacteroidetes are major bacterial phyla in the gut microbiota. Bacteroides species are associated with diets rich in protein and carbohydrate [4]. Several researchers have studied that obesity is associated with a higher Firmicutes/Bacteroidetes ratio in human gut microbiota status [5–7].

The herb *Panax* has various biological effects in the plant roots, leaves, stems, and berries. Ginseng, the root of the plant in the genus *Panax*, has been used in traditional herbal medicine for thousands of years in Asian countries [8]. Ginseng supplementation may provide health benefits for immune function, fight fatigue, neuroprotection, and metabolic parameters [9–12]. According to previous studies, ginseng has been shown to possess anti-obesity effects, including changes in fat absorption, lipogenesis-related genes regulation, lipid metabolism enzyme activities, in a rodent model with high-fat diet (HFD)-induced obesity [13–16].

Several publications have reported the effects of ginseng on metabolic diseases and indicated ginseng may be an effective candidate for preventing metabolic disorders. However, few reports focused on gut microbiota composition in mice receiving HFD supplemented with ginseng. Based on these findings, this study aimed to investigate the inhibitory effect of ginseng on HFD-induced obesity in C57BL/6 mice and the changes of gut microbiota.

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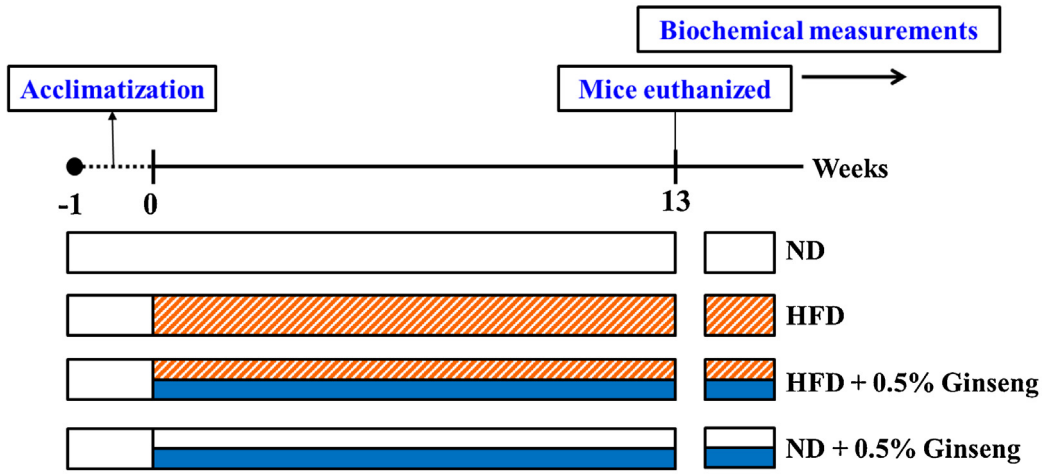
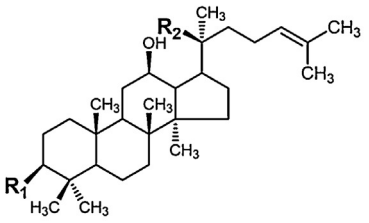
(A)

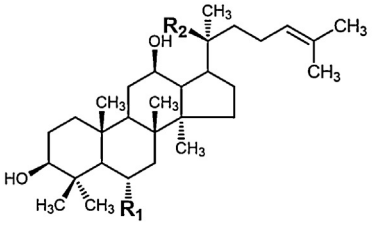


(B)

Compound name	Content (mg/g)
Ginsenoside Rb1	11.42 ± 0.35
Ginsenoside Rb2	1.14 ± 0.03
Ginsenoside Rc	4.71 ± 0.16
Ginsenoside Rd	4.89 ± 0.14
Ginsenoside Re	5.60 ± 0.12
Ginsenoside Rg1	3.33 ± 0.10
Ginsenoside Rg2	6.97 ± 0.28
Ginsenoside Rg3	N.D.
Ginsenoside Rh1	1.04 ± 0.03
Ginsenoside Rh2	N.D.
Total chemicals	39.1 ± 1.21



Ginsenoside	R_1	R_2	Formula	MW
Rb1	-Oglc(2-1)glc	-Oglc(6-1)glc	$C_{54}H_{92}O_{23}$	1109
Rb2	-Oglc(2-1)glc	-Oglc(6-1)ara(pyr)	$C_{54}H_{90}O_{22}$	1079
Rc	-Oglc(2-1)glc	-Oglc(6-1)ara(fur)	$C_{54}H_{90}O_{22}$	1079
Rd	-Oglc(2-1)glc	-Oglc	$C_{48}H_{82}O_{18}$	947



Ginsenoside	R_1	R_2	Formula	MW
Re	-Oglc(2-1)rha	-Oglc	$C_{48}H_{82}O_{18}$	947
Rg1	-Oglc	-Oglc	$C_{42}H_{72}O_{14}$	801
Rg2	-Oglc(2-1)rha	-OH	$C_{42}H_{72}O_{13}$	785
Rh1	-Oglc	-OH	$C_{36}H_{62}O_9$	639

Fig. 1. Illustrates the experimental procedure and the chemical constituents of Ginseng. (A) After acclimatization for 1 week, mice were randomly divided into four groups: normal diet (ND), high-fat diet (HFD), HFD with 0.5% (*w*/*w*) ginseng (HFD + 0.5% Ginseng), and ND with 0.5% (*w*/*w*) ginseng (ND + 0.5% Ginseng). At the termination of study, mice were euthanized by CO_2 asphyxiation for further examination. (B) the chemical structures and its compositions of ginseng.

Table 1
Effects of ginseng on serum biochemical parameters in HFD-fed C57BL/6 mice.

	ND	HFD	HFD + 0.5% Ginseng	ND + 0.5% Ginseng
AST (U/L)	77.8±9.2^a	81.4±6.1^a	103.4±38.6^a	62.5 ±18.4^a
ALT (U/L)	28.1±9.6^a	15.5 ±7.7^a	22.2±5.3^a	18.0±4.6^a
T-CHO (mg/dL)	80.1±16.0^c	183.8 ±7.8^a	152.1±13.9^b	58.4±3.0^d
TG (mg/dL)	77.8 ±13.8^a	96.5 ±10.0^a	73.2±18.4^a	72.5±8.9^a
HDL (mg/dL)	66.5±12.5^c	150.9±0.5^a	125.9±11.6^b	47.8±3.0^d
LDL (mg/dL)	4.8±4.0^c	35.9±2.4^a	23.5±1.6^b	1.7±0.2^c
HDL/LDL	18.5±8.7^b	4.2±0.2^c	5.3±0.4^c	28.4±3.7^a

Data were presented as mean ± SE. The significance of difference among the four groups was analyzed by one way ANOVA analysis of variance and Duncan's multiple range tests. Values in the same rows not sharing the same superscript letters in the same row are significantly different among group. $P < 0.05$, a, b, c, and d significantly different between each group. AST, aspartate aminotransferase; ALT, alanine transaminase; T–CHO, total cholesterol; TG, triglyceride; HDL, high–density lipoprotein; LDL, low–density lipoprotein.

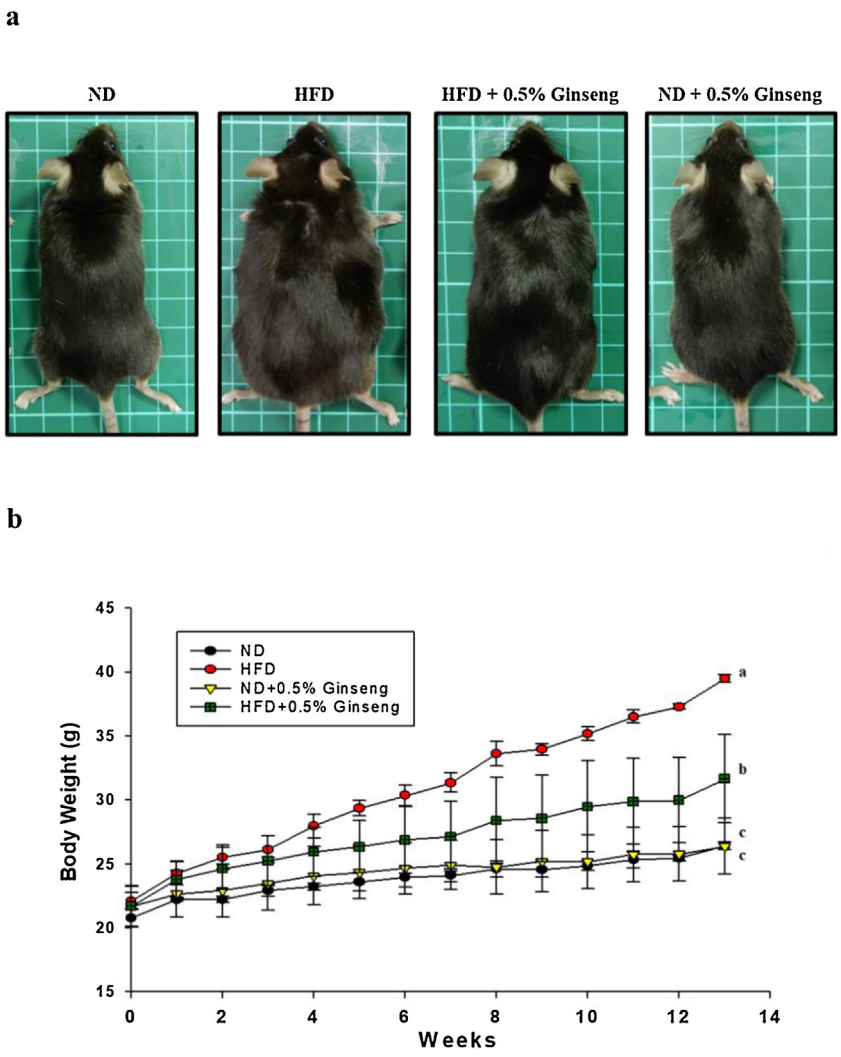


Fig. 2. Effects of ginseng supplementation on body weight in HFD-fed C57BL/6 mice. (a) Representative photographs of mice from each group at the end of week 13. (b) Change in body weight during ginseng exposure over 13 weeks. Data are presented as mean ± SE. The significance of difference among the four groups was analyzed by one way ANOVA analysis of variance and Duncan's multiple range tests. Values in the same rows not sharing the same superscript letters are significantly different among group. $P < 0.05$, a, b, c, and d significantly different between each group.

2. Materials and methods

2.1. Reagents

The ginseng was obtained from Bing Han International Group. The purity of ginseng was determined to be higher than 95% by high-performance liquid chromatography (HPLC).

2.2. Animals and experimental procedure

Five-week-old male C57BL/6 mice were purchased from the BioLASCO Experimental Animal Center (Taiwan Co., Ltd, Taipei, Taiwan). All experimental protocols used in these animal experiments were approved by Institutional Animal Care and Use Committee (IACUC) of National Taiwan University. Mice were housed under a controlled atmosphere, 25 ± 1 °C, humidity 50%, a 12 h light - 12 h dark cycle, and provided with food and water at all times. The mice were randomly divided into four groups: normal diet (ND), HFD (50% calories from fat), HFD with 0.5% (*w*/*w*) ginseng (HFD + 0.5% ginseng), and ND with 0.5% (*w*/*w*) ginseng (ND + 0.5% ginseng) ($n = 3$ per group). All mice were allowed free access to diet and water throughout the experiment. Food intake and water consumption were recorded every other day, and all animals were weighed weekly. The experimental procedure is illustrated in Fig. 1. At the termination of study, mice were euthanized by CO_2 asphyxiation for further examination. The adipose tissues, liver, spleen, and kidney were also immediately collected and weighed.

2.3. Biochemical analysis

Plasma samples were separated by centrifugation at 1000 × g for 15 min at 4 °C. The blood tests for aspartate aminotransferase (AST), alanine transaminase (ALT), total cholesterol (T-CHO), triglyceride (TG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) were measured using a Hitachi 780 biochemical Automatic Analyzer (Hitachi, Tokyo, Japan) at the National Laboratory Animal Center (NLAC, Taipei, Taiwan).

2.4. Gut microbiota analysis

Stool samples were collected to analyze bacterial community profiles of the gut microbiota. Microbial DNA was isolated form

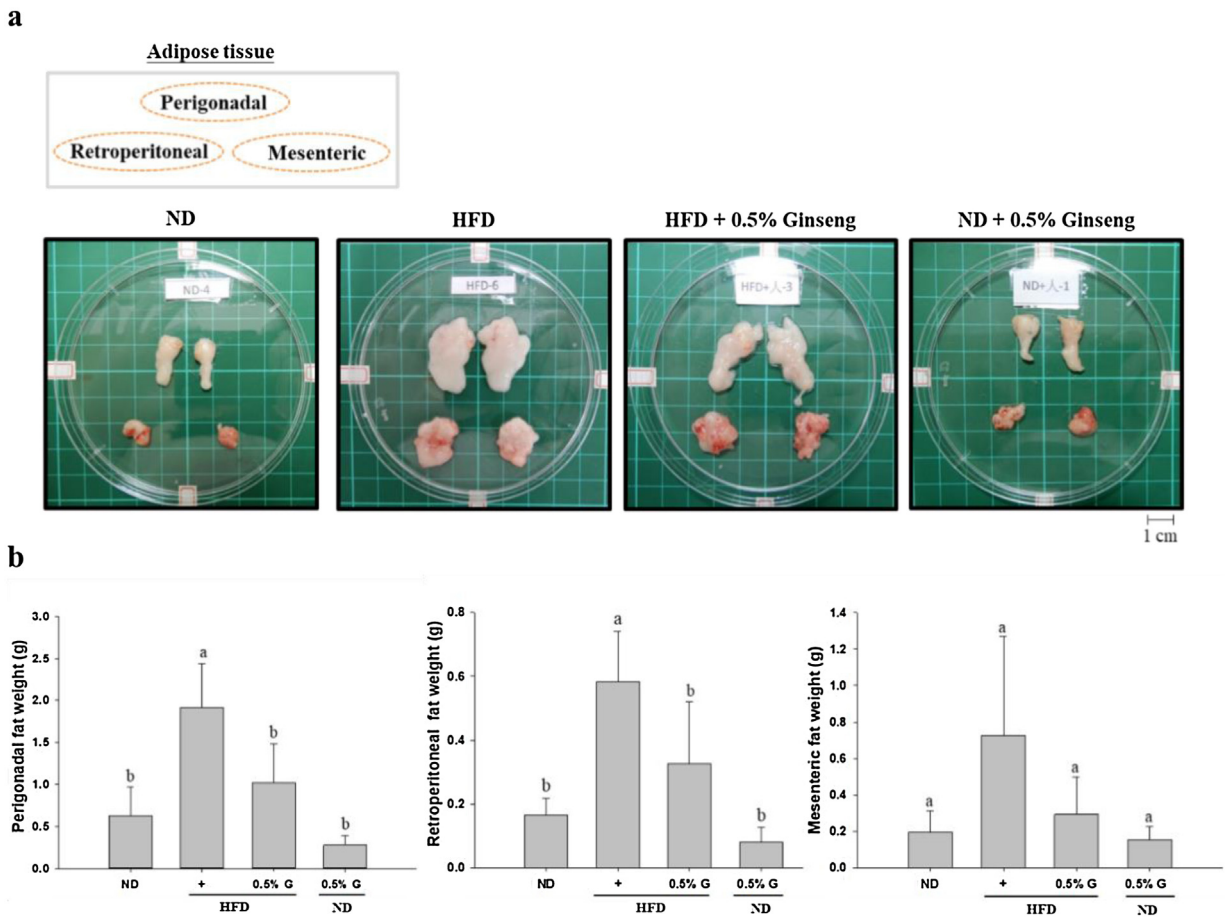


Fig. 3. Effects of ginseng supplementation on white adipose tissue in HFD-fed C57BL/6 mice. (a) Representative photographs of white adipose tissue sections. (b) The weight of perigonadal, retroperitoneal, and mesenteric adipose tissues. Data are presented as mean ± SE. The significance of difference among the four groups was analyzed by one way ANOVA analysis of variance and Duncan's multiple range tests. Values in the same rows not sharing the same superscript letters are significantly different among group. $P < 0.05$, a, b, c, and d significantly different between each group.

stool samples using the innuSPEED Stool DNA kit (Analytik Jena AG, Jena, Germany) according to the manufacturer's instructions. The purified DNA was eluted from the spin column, and the concentration was measured by NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE, USA). The primer sequences to amplify the V4 region of 16S rDNA were used universal primers 515 F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). The PCR conditions were as follows: initial denaturation at 94 °C for 2 min., 20 cycles of 94 °C for 20 s, 54 °C for 30 s, and 72 °C for 1 min; and a final extension at 72 °C for 3 min using Agena PCR kit (Agena, San Diego, CA, USA). The amplicons were purified with Ampure XP beads and quantified using the Quant-iT Pico Green ds DNA assay kit (Invitrogen, USA). The library was sequenced using an Illumina Hiseq 2500 platform and 250 bp paired-end reads were generated. Operational taxonomy units were picked using the UCLUST method and sequences with ≥ 97% identities.

2.5. Statistical analysis

All data were expressed as mean value ± standard error (SE) for the indicated number of independently performed experiments. Statistical differences were performed using student's t-tests, one-way analysis of variance (ANOVA), and Duncan's multiple range tests using the SAS statistical software package (version 8.0). Differences of a P-value < 0.05 were considered statistically significant.

3. Results and discussion

3.1. Effects of ginseng supplementation on body weight in HFD-fed C57BL/6 mice

In this work, five-week-old male C57BL/6 mice which have similar initial weight were randomly divided into four groups as follows: receiving ND, HFD, HFD + 0.5% ginseng, and ND + 0.5% ginseng ($n = 3$ per group). HFD-fed mice gained weight until the end of the study and the average body weight of the HFD group was higher than other groups delivered at 13 weeks. In contrast, the HFD + 0.5% ginseng group gained slightly weight compared to HFD group. There was no significant difference in the body weight between the ND group and the ND + 0.5% ginseng group. In the HFD + 0.5% ginseng group, the average body weight was significantly lower than that in the HFD group and higher than that in the ND + 0.5% ginseng group (Fig. 2).

3.2. Effects of ginseng supplementation on adipose size in HFD-fed C57BL/6 mice

Excessive caloric intake may contribute to the development of obesity, adipocyte tissue dysfunction and metabolic consequences

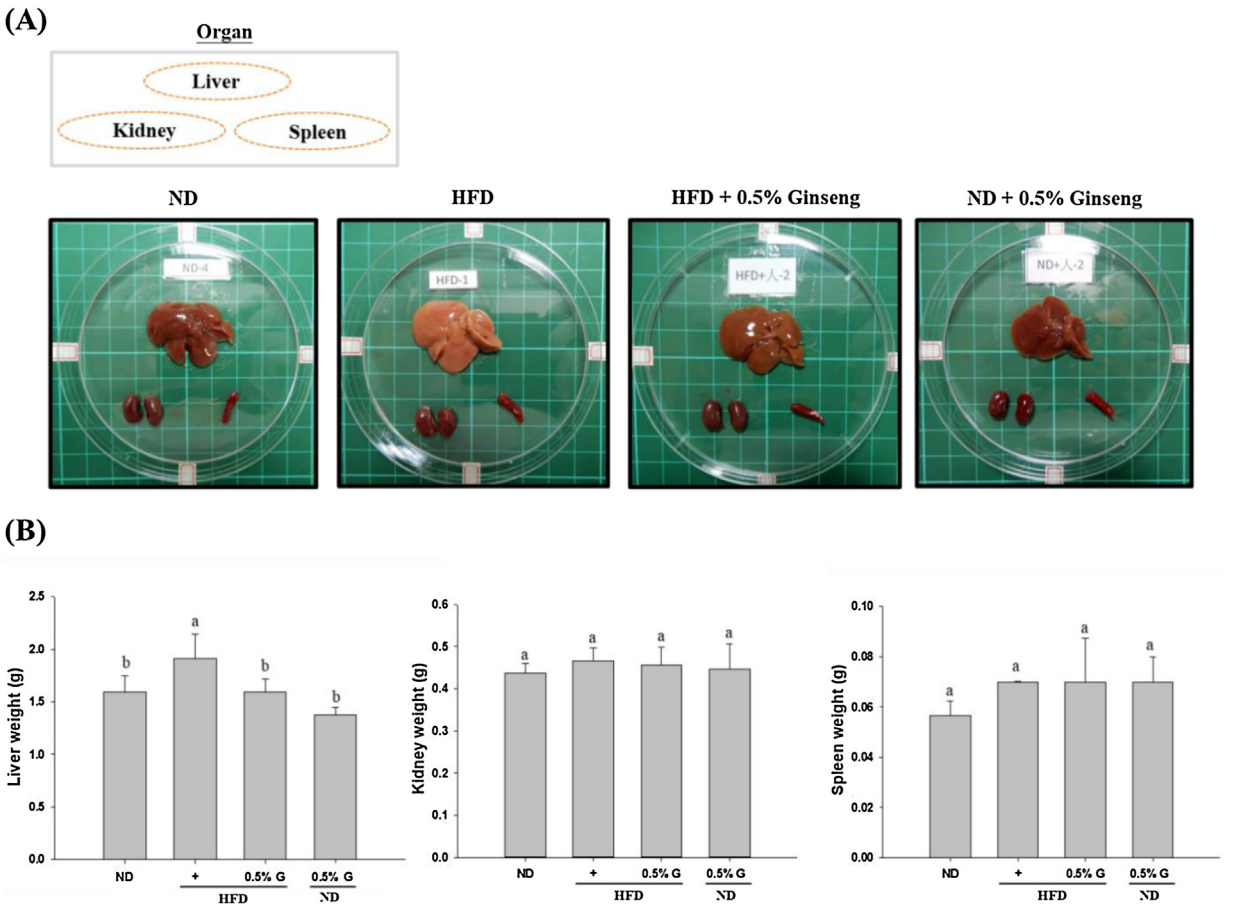


Fig. 4. Effects of ginseng supplementation on organ weight in HFD-fed C57BL/6 mice. (a) Representative photographs of liver, kidney, and spleen sections. (b) The weight of liver, kidney, and spleen section. Data are presented as mean ± SE. The significance of difference among the four groups was analyzed by one way ANOVA analysis of variance and Duncan's multiple range tests. Values in the same rows not sharing the same superscript letters are significantly different among group. $P < 0.05$, a, b, c, and d significantly different between each group.

[17,18]. As depicted in Fig. 3A, the size of adipose tissue was obviously larger in HFD-fed mice compared to ND-fed mice. Therefore, we confirmed these results by evaluating the weight of adipose tissue. Visceral adipose tissue is primarily positioned at perigonadal, retroperitoneal, and mesenteric regions. The weight of perigonadal and retroperitoneal adipocytes in the HFD group was significantly greater than the other groups. The HFD + 0.5% ginseng group reduced the weight of perigonadal and retroperitoneal adipocytes compared with the group fed with HFD only, however, the weight had no significant difference in mesenteric adipocytes among all (Fig. 3B).

3.3. Effects of ginseng supplementation on organ size in HFD-fed C57BL/6 mice

The presence of fatty infiltration in multiple organs like liver, kidneys and spleen may be observed in obesity [19]. Based on these findings, we explored whether organ weights correlate with ginseng in HFD-fed mice. As depicted in Fig. 4, it has been shown that the HFD + 0.5% ginseng group reduced liver weight compared with the HFD group. The color of liver in the HFD group became visibly lighter compared to other group. These findings suggest that HFD leads to fat accumulation in the liver. Nevertheless, no significant difference was found among groups for the weights of kidneys and spleen.

3.4. Effects of ginseng supplementation on serum biochemical parameters in HFD-fed C57BL/6 mice

In this study, we aimed to determine whether ginseng has beneficial effects on HFD-induced obesity. Levels of T-CHO, HDL and LDL in HFD-fed mice were increased compared to those of ND-fed mice. Furthermore, reduced levels of T-CHO, HDL and LDL were found in ginseng supplementation both in HFD-fed and ND-fed. The HFD group had a significant reduction in the HDL/LDL ratio compared with the ND group. The HDL/LDL ratio in the ND + 0.5% ginseng was significantly higher than the ND group. No significant difference was found among groups for the levels of AST, ALT and TG. Data from serum biochemical parameters suggest that ginseng may have beneficial effects on HFD-induced obesity (Table 1).

3.5. Effects of ginseng supplementation on gut microbiota in HFD-fed C57BL/6 mice

Microbial DNA extraction was collected from stool samples and gut microbiota was characterized by the next generation sequencing (NGS) technology. The alteration of gut microbiota in ginseng supplementation was shown in taxonomic composition. The microbial phyla Firmicutes and Bacteroidetes constitute more than 99% of gut bacteria in humans and in mice and Firmicutes/Bacteroidetes ratio. Higher Firmicutes/Bacteroidetes ratio is often observed in overweight, and a decreased ratio is related to weight loss [5,20].

As shown in Fig. 5A, Firmicutes and Bacteroidetes were the most abundant phylum among four groups. At the phylum level, ginseng

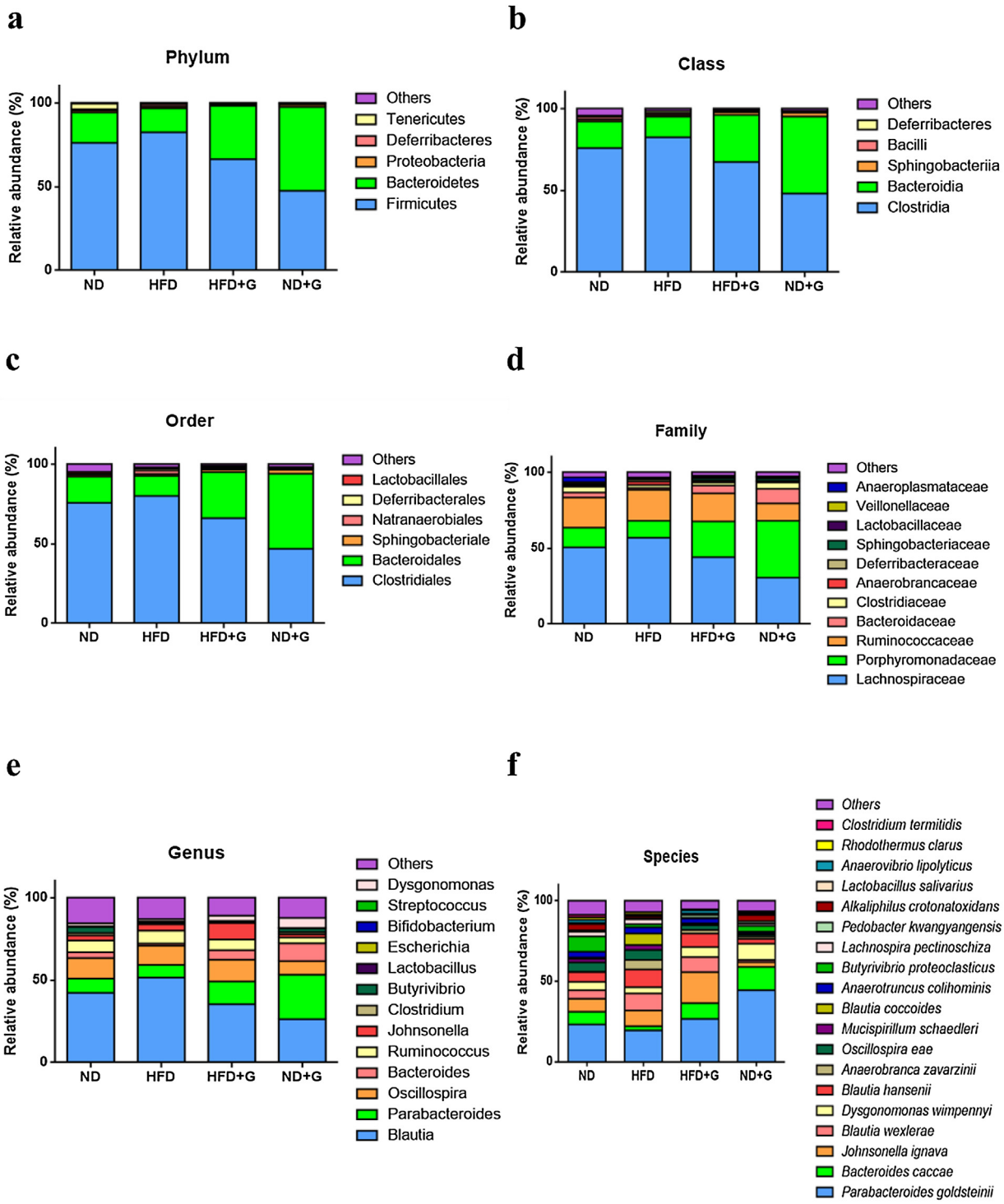


Fig. 5. Effects of ginseng supplementation at taxonomic levels by the relative abundances of phylum (a), class (b), order (c), family (d), genus (e) and species (f).

supplementation decreased Firmicutes abundance and increased Bacteroidetes abundance. In contrast, Firmicutes/Bacteroidetes ratio in ginseng supplementation was reduced both in HFD-fed and ND-fed mice. At class level, Bacteroidia while phylum Bacteroidetes abundance was increased and Clostridia abundance while phylum Firmicutes was reduced in the HFD + 0.5% ginseng group compared with the HFD group (Fig. 5B).

Order-level microbiota assignments was showed that the relative abundance of Bacteroidales and Sphingobacteriales while phylum Bacteroidetes were increased and the relative abundance of Clostridiales while phylum Firmicutes was reduced in the HFD + 0.5% ginseng group compared with the HFD group (Fig. 5C). At the family level, Bacteroidaceae and Porphyromonadaceae abundance while phylum Bacteroidetes were increased and Ruminococcaceae while phylum Firmicutes was reduced in the HFD + 0.5% ginseng group compared with the HFD group (Fig. 5D).

Grouping gut bacterial by genus, *Parabacteroides* while phylum Bacteroidetes was increased and *Blautia* while phylum Firmicutes was reduced in the HFD + 0.5% ginseng group than in the HFD group

(Fig. 5E). As shown in Fig. 5F, *Parabacteroides goldsteinii* while phylum Bacteroidetes was the most abundant bacteria among the four groups. The HFD + 0.5% ginseng group had higher abundance of *P. goldsteinii* and *Bacteroides caccae* while phylum Bacteroidetes than in the HFD group. Otherwise, the ND + 0.5% ginseng group had reduced the levels of *Johnsonella ignava* while phylum Firmicutes and increased the levels of *P. goldsteinii* and *B. caccae* while phylum Bacteroidetes as compared to the ND group at the species level. Recent research indicated that *P. goldsteinii* reduced obesity and was associated with increased adipose tissue thermogenesis [21]. Some texts showed a positive correlation between Bacteroidetes and weight loss in a rodent model [22,23]. Abundance analysis at all taxonomic levels showed that gut microbiota composition in ginseng supplementation has changed and more abundant (Fig. 5A–F). These observations indicate that ginseng supplementation might has a greater effect on gut microbiota composition.

4. Conclusion

In this study, the dietary administration of ginseng suppressed HFD-induced obesity, associated with a decrease in adipose tissue weight, plasma levels of T-CHO and LDL, Ginseng supplementation altered gut microbiota by decreasing Firmicutes while increasing Bacteroidetes. These results show that ginseng could modulate the energy storage and promote healthier by changing the gut microbiota. Future research is needed to elucidate the roles of ginseng on lipid metabolism related gene expression, lipogenic enzyme activities and comprehensive analysis of microbial communities.

Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledgment

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