

Supplementation of Non-Fermented and Fermented Red Ginseng Improves Obese Phenotypes, Lipid and Inflammatory Profiles, and Antioxidant Defense System in High Fat-Fed Rats

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Received February 03, 2021; Revised March 09, 2021; Accepted March 18, 2021

Abstract Obesity is characterized by excessive fat accumulation, chronic inflammation, and enhanced oxidative stress that can present a substantial risk for metabolic complications. The aim of this study was to investigate the protective effects of red ginseng (RG), and its bio-transformed form (fermented RG, FRG) by microorganism on obese phenotype, lipid profile, and inflammation and oxidant profiles in rats fed a high-fat (HF) diet. FRG was prepared by fermentation of RG with *Lactobacillus pentosus* KCCM 12762P. Male Sprague-Dawley rats (n = 8/group; 6 weeks old) were counterbalanced by initial weight into four groups and fed either a low-fat (LF; 10% kcal as fat), high-fat (HF; 45% kcal as fat), or HF diet supplemented with RG or FRG at 1% (w/w) in diet for 6 weeks. General phenotypes (body weight, energy intake, visceral adiposity), lipid and inflammatory profiles, and oxidative states were assessed. RG and FRG supplementation suppressed HF-induced upregulation of proinflammatory cytokine genes, interleukin-6 and tumor necrosis factor alpha, in both white adipose and liver tissues. Similarly, RG and FRG partially reversed HF-induced downregulation of antioxidant enzymes at the gene level. Concomitantly, RG and FRG reduced body weight gain, energy intake, visceral adiposity and the level of serum triglycerides. It is of note that better metabolic profile was observed with FRG than RG for lipid metabolism and inflammation, in the liver in particular. Taken together, we demonstrated that RG and FRG supplementation effectively protected from HF-induced metabolic disturbances by improving obese phenotypes, lipid and inflammatory profiles, and antioxidant defense system.

Keywords: red ginseng, fermented red ginseng, inflammation, oxidative stress, dyslipidemia, diet-induced obesity

Cite This Article: Soyeon Jeong, Misun Park, Minji Kang, Yeong-Ju Park, Minkyong Lee, Cheongbin You, Unsik Hwang, Youngju Song, Hoon Park, Sunhye Lee, and Heejae Suh, "Supplementation of Non-Fermented and Fermented Red Ginseng Improves Obese Phenotypes, Lipid and Inflammatory Profiles, and Antioxidant Defense System in High Fat-Fed Rats." *Journal of Food and Nutrition Research*, vol. 9, no. 3 (2021): 154-162. doi: 10.12691/jfnr-9-3-8.

1. Introduction

Obesity is characterized by abnormal or excessive fat accumulation that can serve as a substantial risk factor for metabolic complications such as dyslipidemia, glucose intolerance, and hypertension [1,2]. An increase in adiposity, especially of visceral fat depots, is associated with macrophage infiltration and release of pro-inflammatory cytokines and consequent production of reactive oxygen species (ROS) in adipose tissue [3].

Accumulating evidence has shown that adipose tissue inflammation itself also acts as an important contributor in the pathogenesis of obesity-related metabolic disturbances

along with inflammation in other metabolically active tissues including the liver [3]. Further, as the depot where ROS is produced following the release of pro-inflammatory adipokines, adipose tissue has been considered an independent mediator of the generation of oxidative stress [4]. With the increase in adipose tissue, the antioxidant ability of the enzymes such as catalase (CAT), GPX (glutathione peroxidase), GSR (glutathione reductase), and SOD (superoxide dismutase) diminishes, consequently favoring metabolic dysfunctions [5].

Due to adverse effects of obesity and related metabolic disorders on human health, a number of studies have sought a wide range of approaches to prevent and/or treat those abnormalities. In particular, since an unbalanced diet has been a primary contributor in the pathogenesis of

obesity and related metabolic dysfunction [6], much attention has been paid to the use of non-invasive dietary approaches such as prebiotics, including natural products, and probiotics in the presence of HF challenge [7,8,9,10].

Korean ginseng (*Panax ginseng* C.A. Meyer) is one of the most commonly consumed medicinal herbs to enhance physiological and metabolic functions in Asian countries [11]. It is to note that its biological and pharmacological abilities can be strengthened with the process of heating, steaming and drying, consequently changing it into red ginseng (RG) [12]. Two major types of ginsenosides, protopanaxadiol and protopanaxatriol, have been reported to mediate RG's pharmacological benefits against obesity-related metabolic disorders via anti-inflammatory and antioxidant mechanism [13]. Moreover, these ginsenosides are bio-transformed by intestinal microbiota (ie fermentation) into their active forms to exert their pharmacological properties [14] to the extent which outweighs those of RG [15,16,17]. Among the metabolites of the ginsenosides, compound K (20S-protopanaxadiol 20-O- β -D-glucopyranoside) as a main metabolite of the protopanaxadiol-type ginsenosides has a variety of metabolic functions with its hepatoprotective, anti-inflammatory, anti-cancer, and even neuroprotective properties [18]. Interestingly, fermentation of RG using probiotics such as *Lactobacillus* or *Bifidobacterium* has shown to increase the proportion of compound K compared to RG [19], enhancing the pharmacological efficacy and metabolic functions of RG [20,21,22].

Despite mounting evidence on the promising aspects of RG and fermented RG (FRG), there is limited research on the potential benefits of both RG and FRG in obesity and related metabolic disorders and possible differences in their metabolic benefits resulting from the fermentation process using *L. pentosus* KCCM 12762P. Thus, the aim of this study was to investigate the protective effects of RG on obese phenotype, lipid profile, and inflammation and oxidant profiles rats fed a HF diet. We also sought to examine the possible differences in their pharmacological and metabolic effects on the same parameters between non-fermented and fermented RG using *x*. We hypothesized that RG and FRG supplementation would improve the HF-induced inflammatory and oxidative profiles and possibly prevent the development of obesity and related metabolic disturbances with fermentation-derived differences in their pharmacological and metabolic properties between RG and FRG.

2. Materials and Methods

2.1. Materials

Six-year-old RG was purchased from a ginseng market in Geumsan, Korea. Standard ginsenosides (Rb1, Rd, Rg3, Rh2, F2, and CK) were obtained from Dongmyung Scientific, Inc. (Seoul, Korea). Water and acetonitrile were purchased from J. T. Baker (PA, USA), and sodium citrate and sodium carbonate were purchased from Daejung Chemicals Co. (Siheung, Korea). Sumizyme AC and Cellulase KN were obtained from Vision Biochem (Seongnam, Korea), Rapidase C80 MAX and Pyr-flo were obtained from SJD (Seongnam, Korea), Celluclast 1.5L,

Viscozyme L, and Ultimase MFC were obtained from Daejong Enzyme (Seoul). Commercial kits for animal tests were purchased from Elabscience (Houston, Texas, USA). *L. pentosus* KCCM 12762P, which was isolated from kimchi in our laboratory, was used as a starter culture for the production of FRG.

2.2. Preparation of (Fermented) Korean Red Ginseng Powder

The preparation of RG and FRG powder was modified from the method reported in the previous study [23]. For RG extract preparation, 20 g of dried RG root was added to 200 mL of hot water and extracted at 80 °C for 48 hours. This RG root extract was freeze-dried (EYELA freeze dryer, FDU-1200, Tokyo Rikakikai Co.), stored in a refrigerator, and used for experiments. For the production of fermented red ginseng, distilled water was added to the freeze-dried RG extract powder to make 5%, and then 20 mL was taken and used as a substrate for enzyme conversion. Previous study (described above) was subjected to the enzymatic conversion of RG using the enzyme reaction method. In this study, the production of FRG was prepared by fermenting the enzyme-treated RG using *L. pentosus* KCCM 12762P as a starter culture. Briefly, Rapidase C80MAX and Pyr-flo were added to the RG suspension at a concentration of 5% (v/v), respectively, and reacted at 50 °C for 24 hours with shaking at 160 rpm. After boiling for 10 minutes to terminate the reaction, 5% (v/v) Ultimate MFC was added to the solution and reacted at 50 °C for 48 hours with shaking at 160 rpm. The enzyme-treated RG was adjusted to pH 6.5 with 1N sodium citrate and sterilized at 100°C for 10 min. The sterilized RG suspension was inoculated with 1% (v/v) of *L. pentosus* KCCM 12762P culture [1×10^9 colony-forming units (CFU)/mL] to give a final cell concentration of about 1×10^7 CFU/mL, and fermented at 37°C for 72 hours with shaking at 160 rpm. The FRG suspension was lyophilized using an EYELA freeze dryer (FDU-1200, Tokyo Rikakikai Co.) and used for experiments.

2.3. Ginsenoside Analysis of RG and FRG Powders

Freeze-dried RG and FRG extracts were extracted twice with n-butanol saturated with water, and then filtered through a 0.45 μ m membrane (Agilent Technologies, CA, USA). The HPLC analysis was performed by modifying the method reported by Zhou et al. [24]. Analysis was performed at 203 nm using an Agilent HPLC system (1260 series) equipped with a UV detector and a Zorbax SB-C18 column (4.6 \times 150 mm, 5 μ m, Agilent Technologies), and water and acetonitrile were used for the mobile phase.

2.4. Animals and Experimental Design

Animals were maintained and handled in accordance with protocols approved by the Institutional Animal Care and Use Committee (Sun Moon University; SM-2020-01-02). Male Sprague-Dawley rats (n = 8/group; 6 weeks old; Samtako Co., Osan, Korea) were individually housed in a controlled environment at 23 \pm 1°C at 50 \pm 5% relative

humidity under a 12 h light/dark cycle. After acclimation for a week on low-fat (LF) diet, animals were split into four weight-matched groups and fed either a low-fat (LF; 10% kcal as fat), high-fat (HF; 45% kcal as fat), or HF diet supplemented with non-fermented (HF/RG) or fermented red ginseng at 1% (w/w) in diet for 6 weeks (Table S1). Body weight and food intake were measured on a weekly and daily basis, respectively. Food efficiency ratio (FER) was determined as weight gain (g)/energy intake (kcal). After 6 weeks on respective diets, animals were feed-deprived overnight and euthanized by carbon dioxide inhalation. Blood was placed into a sterile Vacutainer plastic tube (BD Vacutainer, Plymouth, UK) and centrifuged at $1000 \times g$ for 10 min at 4°C for serum collection. The liver and visceral fat pads (mesenteric, retroperitoneal and epididymal) were collected and weighed; an adiposity index was determined. Serum and all the tissues were snap-frozen in liquid nitrogen and stored -80°C until analysis.

2.5. RNA Extraction and Quantitative RT-PCR

Total RNA from liver and mesenteric fat samples was extracted using the RNeasy Mini Kit (Qiagen, Hilden, Germany) per the manufacturer's instructions. cDNAs were synthesized from 2 μg of purified RNA samples using TOPscript™ RT DryMIX (dT18 plus; Enzymomix, Daejeon, Korea) following the manufacturer's protocol. Real-time PCR was performed with the CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA) using Topreal™ qPCR 2x PreMIX SYBR Green (Enzymomix) for detection. GAPDH was used as a housekeeping gene. Genes of interest were analyzed according to the $2^{-\Delta\Delta\text{CT}}$ method [25] and compared with control samples. Primer sequences are provided in Table S2

2.6. Biochemical Analysis

The level of serum triglycerides was measured via a colorimetric assay kit (E-BC-K238, Elabscience, Houston, TX, USA) according to the manufacturer's guideline.

2.7. Statistical Analysis

Unless stated otherwise (microbiome analysis), statistical analysis was performed by using Prism software (Prism 8.4.3; GraphPad Software, La Jolla, CA, USA). Two-factor repeated-measures analysis of variance (ANOVA) was used to analyze body weight and one-factor ANOVA was performed to analyze the rest of the parameters. Differences between groups were analyzed by using Fisher's least-significant-difference test. Differences were considered significant if $P < 0.05$. Data are presented as means \pm standard error of the mean.

3. Results

3.1. Ginsenosides of RG and FRG Extract

Red ginseng has various pharmacological effects such as anti-influenza, anti-diabetes, and antioxidant activity, and these beneficial effects are reported to be due to the ginsenoside component of red ginseng [1,2,3,26,27,28]. In recently, it has been reported that de-glycosylated low-molecular ginsenosides, especially compound K (CK), are the main pharmacologically active metabolites found in human bloodstream after oral administration of red ginseng, including anti-inflammatory, anti-allergic, anti-diabetic, anti-aging, and hepatoprotective effects [3,8,9,20,29,30,31].

In this study, in order to increase the amount of CK and health-promoting effects of RG extract, the enzyme-treated RG was fermented by *Lactobacillus pentosus* KK18 (also called *L. pentosus* KCCM 12762P) for the production of FRG. The ginsenoside concentration in RG and FRG are shown in Table 1. The RG contained 20.51 mg/g of Rb1, 10.47 mg/g of Rh1, 8.76 mg/g of Rg2, 7.04 mg/g of Rd, 2.85 mg/g of Rg3, and 2.50 mg/g of F2, but CK was not detected. Fermentation of RG by *L. pentosus* KCCM 12762P following enzyme treatment converted PPD-type major ginsenosides (Rb1 and Rd) into smaller de-glycosylated forms (F2, Rh2, and CK), producing 16.51 mg/g of CK and 6.53 mg/g of Rh2, and increasing the amount of F2 (9.16 mg/g).

3.2. Supplementation of RG And FRG Improves Hyperphagic Phenotypes

After the 3rd week of diet treatment, HF feeding significantly increased body weight compared to the LF group and this increase in body weight was suppressed by RG and/or FRG (week 3: LF vs. HF, $P < 0.05$; week 4: LF vs. HF, $P < 0.01$; week 5: LF vs. HF, $P < 0.01$, LF vs. HF/RG, $P < 0.05$; week 6: LF vs. HF, $P < 0.0001$, LF vs. HF/RG, $P < 0.01$, HF vs. HF/FRG, $P < 0.05$; Figure 1A). Cumulative food intake was significantly increased by HF-feeding compared to the LF group (LF vs. HF, $P < 0.05$), which was not observed in both HF/RG and HF/FRG groups (LF vs. HF/RG or HF/FRG, $P > 0.05$; Figure 1B). HF feeding led to a significant increase in feed efficiency in HF and HF/RG groups (LF vs. HF, $P < 0.01$; LF vs. HF/RG, $P < 0.05$), which was partially suppressed by FRG supplementation (LF vs. FRG, $P > 0.05$; Figure 1C). Visceral adiposity was evaluated from epididymal and retroperitoneal fat depots (Figure 1D). HF feeding significantly increased fat mass compared to the LF group (LF vs. HF, $P < 0.0001$) and this increase was suppressed by both RG and FRG supplementation (HF vs. HF/RG or HF/FRG, $P < 0.05$).

Table 1. Ginsenoside content of red ginseng extract (RG) and fermented red ginseng extract (FRG)

Sample	Ginsenoside (mg/g)									
	Rg2	Rh1	Rc	Rb1	Rd	F2	Rg3	CK	Rh2	
¹⁾ RG	8.76 \pm 0.05	10.47 \pm 0.02	-	20.51 \pm 0.79	7.04 \pm 0.06	2.50 \pm 0.01	2.85 \pm 0.01	-	-	-
²⁾ FRG	2.64 \pm 0.04	5.29 \pm 0.08	1.10 \pm 0.02	-	1.51 \pm 0.02	9.16 \pm 0.09	2.41 \pm 0.05	16.51 \pm 0.17	6.53 \pm 0.08	

RG; freeze dried red ginseng extract

FRG; freeze dried red ginseng extract treated with multiple enzymes and fermented by *L. pentosus* KCCM 12762P

Data are expressed as mean \pm standard deviation.

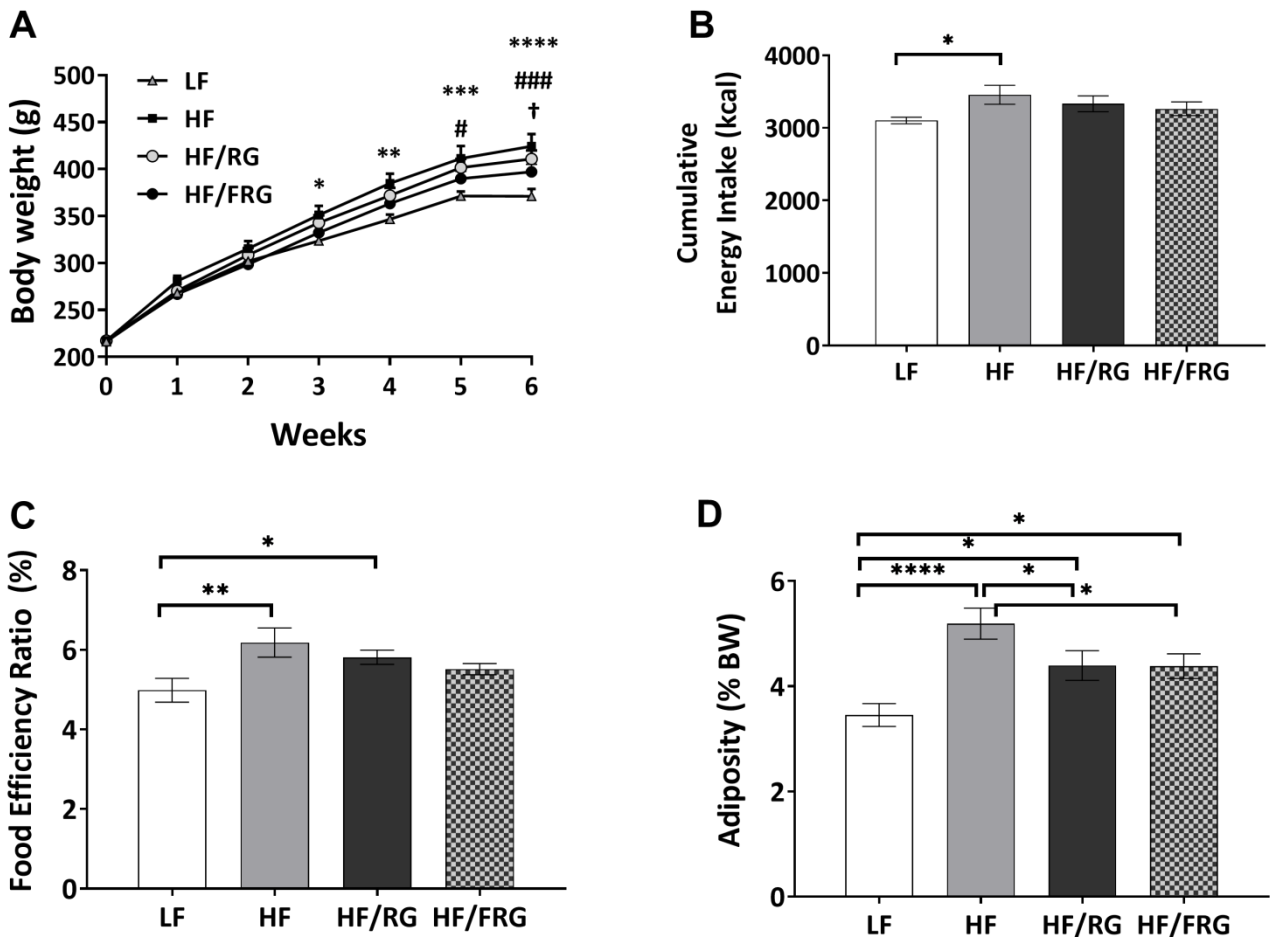


Figure 1. Supplementation of non-fermented and fermented red ginseng improves hyperphagic phenotypes

3.3. Supplementation of RG and FRG Improves Lipid Profiles

Based on reduced fat mass by both RG and FRG supplementation, lipid profile was further examined in the liver and in circulation (Figure 2). As a marker of lipogenesis, the gene expression of SREBP1c was significantly upregulated by HF feeding compared to the LF group, which was normalized to the LF level by RG and FRG supplementation (HF vs. RG, $P = 0.58$; HF vs. FRG, $P < 0.05$; Figure 2A). Similarly, the level of circulating TG was significantly increased by HF feeding compared to the LF-fed rats and this increase was

suppressed by RG and FRG supplementation (HF vs. RG, $P < 0.05$; HF vs. FRG, $P = 0.73$; Figure 2B).

3.4. Supplementation of RG and FRG attenuates HF-induced inflammation.

In adipose tissues, HF feeding significantly increased the gene expression of the pro-inflammatory cytokine, interleukin (IL)-6 and $TNF\alpha$, compared to the LF group (LF vs. HF, $P < 0.05$; Figures 3A, B) and this was significantly reduced by RG supplementation and/or FRG supplementation (IL-6: HF vs. HF/RG, $P < 0.05$; HF vs. HF/FRG, $P = 0.66$; $TNF\alpha$: HF vs. HF/RG or HF/FRG, $P < 0.05$).

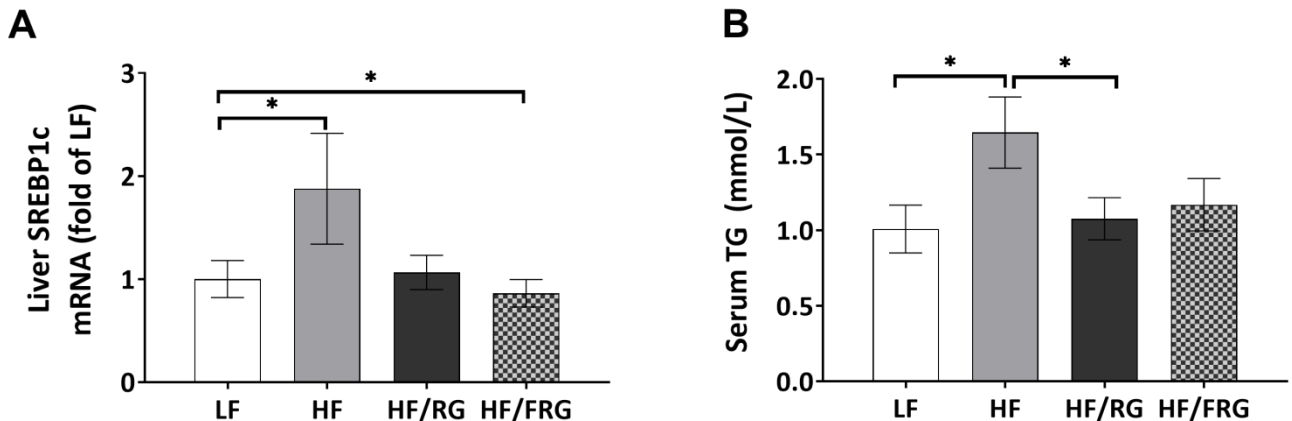


Figure 2. Supplementation of non-fermented and fermented red ginseng improves lipid profiles

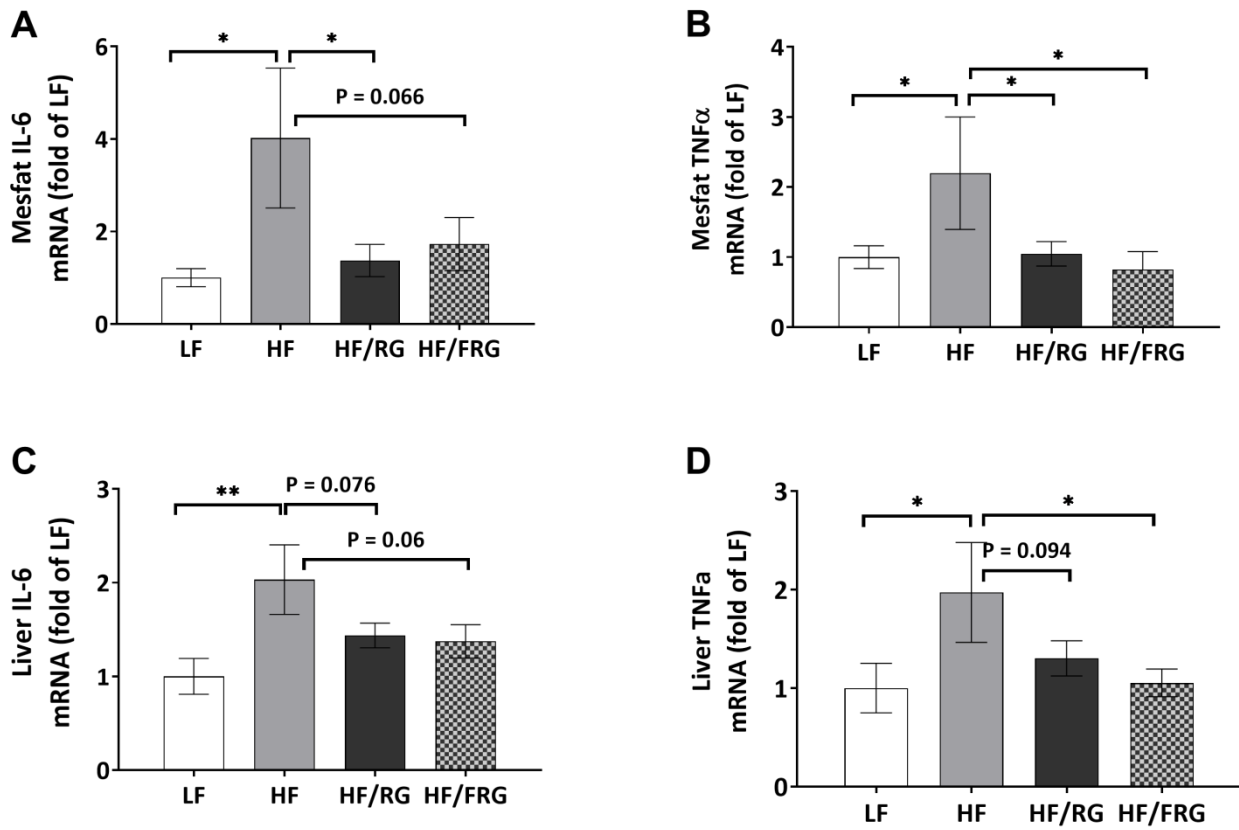


Figure 3. Supplementation of non-fermented and fermented red ginseng attenuates HF-induced inflammation

Furthermore, HF feeding also significantly upregulated the gene expression of these two pro-inflammatory cytokines in the liver compared to the LF group (IL-6, TNF α : LF vs. HF, $P < 0.05$; **Figures 3C, D**). However, the HF-induced upregulation of IL-6 was attenuated by RG and FGR supplementation by 29% and

46% although this did not reach statistical significance (HF vs. RG, $P = 0.76$; HF vs. FRG, $P = 0.6$). Similarly, the upregulated TNF α gene expression by HF feeding was suppressed by FRG and RG by 71% and 34% with or without significance (HF vs. FRG, $P < 0.05$; HF vs. RG, $P 0.94$).

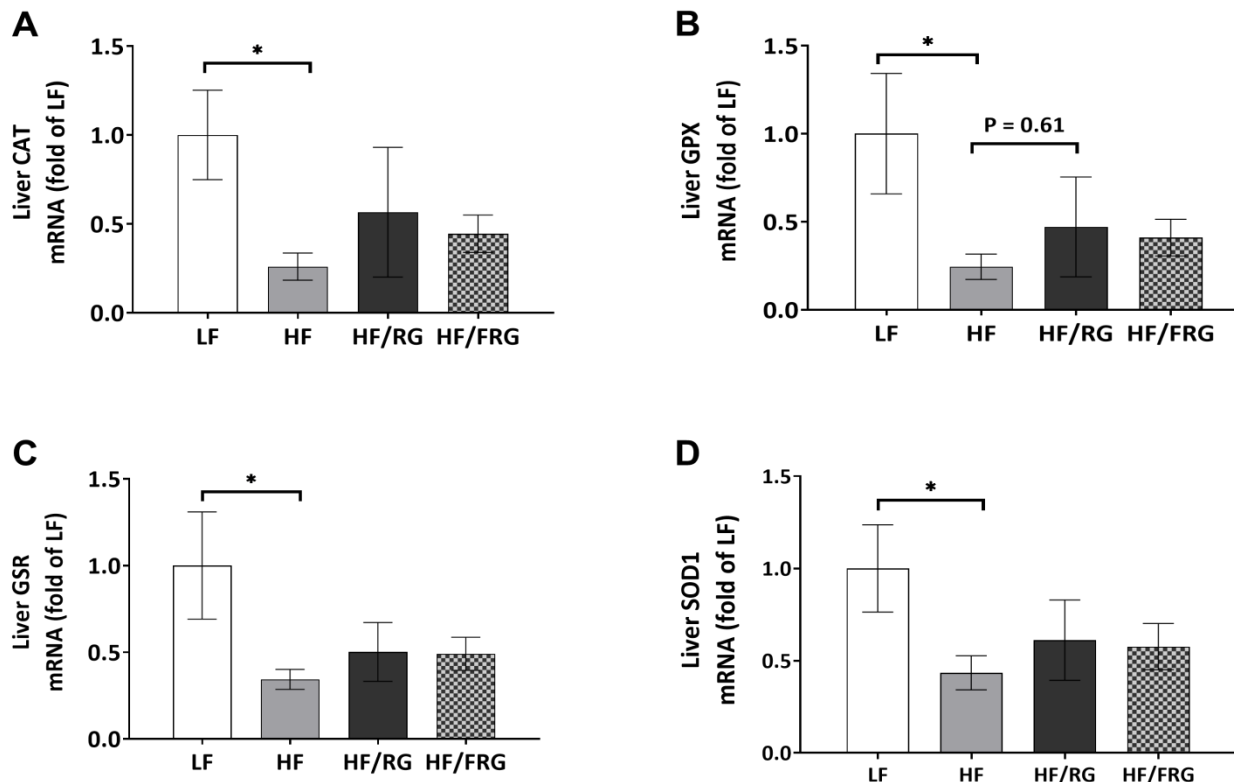


Figure 4. Supplementation of non-fermented and fermented red ginseng exerts antioxidant effects

3.5. Supplementation of RG and FRG Exerts Antioxidant Effects

To characterize the antioxidant properties of RG and FRG supplementation against the HF challenge, the gene expression of antioxidant enzymes was examined. It was observed that HF feeding significantly downregulated the gene expression of CAT, GPX, GSR, and SOD1, which was partially reversed by RG and FRG supplementation (LF vs. RG or FRG, $P > 0.05$; Figure 4). In specific, the downregulated gene expression by HF was reversed by RG and FRG by 118% and 33% for CAT, by 93% and 35% for GPX, by 46% and 29% for GSR, and by 41% and 23% for SOD1.

4. Discussion

Here we investigated the effect of RG supplementation on obese phenotype, lipid and inflammatory profiles, and oxidative states in rats fed a HF diet. We also sought to examine the possible pharmacological and metabolic differences by fermentation of RG with *L. pentosus*. Our hypothesis was that RG and FRG supplementation would improve the HF-induced inflammatory and oxidative profiles, possibly preventing the development of obesity and related metabolic disturbances with different degree of pharmacological and metabolic effects between RG and FRG.

In the present study, it was shown that RG and FRG supplementation suppressed HF-induced upregulation of proinflammatory cytokine genes, IL-6 and TNF α , in both white adipose and liver tissues. On the other hand, RG and FRG supplementation partially reversed HF-induced downregulation of antioxidant enzymes, CAT, GPX, GSR, SOD1, at the gene level. Concomitantly, RG and FRG supplementation reduced body weight gain, energy intake, visceral adiposity and improved lipid profiles. Taken together, these data show that both RG and FRG supplementation led to improvements in obese phenotypes and metabolic profiles in HF-fed rats and these improvements were associated with its anti-inflammatory and anti-oxidant properties.

In rodents, consumption of a HF diet (45% fat by kcal) for more than five weeks results in significant increases in body weight, energy intake, and visceral fat mass. In this present study, HF feeding for six weeks resulted in significant increases in body weight, cumulative energy intake, FER, and visceral adiposity compared to the LF group, confirming the development of obese phenotypes by HF feeding. However, these obese phenotypes were partially prevented by both RG and FRG supplementation as previously found in preclinical studies with various types of ginseng [32,33]. In particular, visceral fat mass, a well-known characteristic of obesity, was markedly reduced by RG and FRG supplementation compared to the HF group. It is to note that the increased visceral fat mass has been associated with liver-specific expression of transcriptionally active SREBP1c as a lipogenesis factor [34]. In this study, RG and FRG reversed HF-induced upregulation of hepatic SREBP1c gene expression to the LF level, which corresponds to the finding of the previous study examining the anti-obesity effect of Korean white ginseng in HF-fed obese mice [35]. These data confirm

that RG and FRG supplementation protected from the development of obese phenotypes and related metabolic profiles.

Along with excessive visceral fat accumulation, dyslipidemia contributes to the progression of obesity and related metabolic disturbances. Of note, the hallmark of dyslipidemia is hypertriglyceridemia [36]. In this present study, HF feeding resulted in a significant increase in serum TG and this increase was prevented by RG and FRG supplementation. It has been also reported in previous studies that non-ferment or fermented ginseng improves lipid metabolism [17,37]. Thus, the present findings suggest that RG and FRG supplementation improved HF-induced lipid dysregulation.

An increasing number of studies have confirmed that inflammatory response and oxidative stress serve as a pivotal mechanism involved in the development and progression of obesity and metabolic disorders. In an obese state by HF feeding, macrophages are recruited to the expanding adipose tissue and both activated macrophages and enlarged adipocytes promote the production of pro-inflammatory factors, leading to increased inflammation in adipose and liver tissues, which is primarily triggered by the release of inflammatory cytokines, such as interleukin (IL)-6 and tumor necrosis factor alpha (TNF α) [38]. Further, consequent formation of toxic reactive oxygen species and subsequent generation of oxidative stress can impair the antioxidant defense system especially by downregulating antioxidant enzymes such CAT, GPX, GSR, and SODs [5].

In this present study, RG and FRG supplementation suppressed HF-induced upregulation of pro-inflammatory cytokine expression (IL-6, TNF α) in both visceral adipose and liver tissues, accompanied by attenuated oxidative stress through enhanced antioxidant enzyme defense (CAT, GPX, GSR, and SOD1) in HF-fed groups. Taken together, it can be inferred from these results that RG and FRG protected from HF-induced metabolic dysregulation in adipose and liver tissues primarily by promoting anti-inflammatory and antioxidant responses.

There are some limitations to this study that deserve consideration. First, while both RG and FRG supplementation demonstrated metabolic benefits on the same parameters against an HF challenge as hypothesized, we failed to find any remarkable differences in degree of their respective pharmacological and metabolic benefits. Previous studies have reported differences in bioactivity and subsequent metabolic effects by fermentation compared to the original non-fermented material of interest, including the differences between RG and FRG [15,16,17,39,40,41]. Thus, further studies at the molecular and/or even cellular level would enable to identify the potential differences between RG and FRG in their pharmacological and metabolic effects. Also, since the gut microbiota has been identified as a potential driver in the development of obesity and it is also the underlying mechanism through which prebiotics and probiotics exert their beneficial effects on metabolic profiles [8,9,10], metagenomic analysis of the gut microbiota of the animals would confirm the fundamental triggering factor for possible pharmacological and metabolic differences between RG and FRG. Lastly, metabolomics would demonstrate metabolite changes derived from fermentation process

and/or gut microbiota and possibly provide a better understanding of the pharmacological and metabolic differences between RG and FRG.

5. Conclusion

We demonstrated that RG and FRG supplementation effectively protected from HF-induced metabolic disturbances by improving obese phenotypes, lipid and inflammatory profiles, and antioxidant defense system. Thus, these findings support the use of RG and FRG for modulating the hyperphagic response to HF diets and improving obesity-related metabolic disorders.

Acknowledgements

We acknowledge the financial support from the Ministry of Trade, Industry and Energy in 2019 [grant number P0010311].

Statement of Competing Interests

The authors declare that there is no conflict of interest.

List of Abbreviations

ANOVA, analysis of variance; CAT, catalase; GPX, glutathione peroxidases; GSR, glutathione reductase; HF, high fat; HF/FRG, HF with fermented red ginseng in diet by 1% (w/w); HF/RG, HF with non-fermented red ginseng in diet by 1% (w/w); IL, interleukin; LF, low fat; ROS, reactive oxygen species; SOD1, superoxide dismutase; SREBP1c, sterol regulator element-binding protein 1c; TG, triglyceride; TNF α , tumor necrosis factor alpha.

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Supplemental Data

Table S1. Composition of experimental diets

%	LF		HF		HF/RG		HF/FRG	
	g	kcal	g	kcal	g	kcal	g	kcal
Protein	19.3	20.1	23.8	20.1	23.8	20.1	23.8	20.1
Carbohydrate	67.1	69.8	29.3	24.8	29.3	24.8	29.3	24.8
Fat	4.3	10.0	23.8	45.2	23.8	45.2	23.8	45.2
Total		100.0		90.1		90.1		90.1
kcal/g	3.8		4.7		4.7		4.7	
Sucrose	6.9	7.2	20.7	17.65	20.7	17.54	20.7	17.54
Ingredient	g	kcal	g	kcal	g	kcal	g	kcal
Casein	200	800	200	800	200	800	200	800
L-Cystine	3	12	3	12	3	12	3	12
Corn Starch	506.2	2025	72.8	291	72.8	291	72.8	291
Sucrose	72.8	291	176.8	707	168.3	673	168.3	673
Maltodextrose	125	500	100	400	100	400	100	400
Cellulose	50	0	50	0	50	0	50	0
Soybean Oil	25	225	25	225	25	225	25	225
Lard	20	180	177.5	1598	177.5	1598	177.5	1598
AIN-93G Mineral Mix	35	0.0	35	0.0	35	0.0	35	0.0
AIN-93 Vitamin Mix	10	0.00	10	0.00	10	0.00	10	0.00
Choline Bitartrate	2.5	0.0	2.5	0.0	2.5	0.0	2.5	0.0
tert-Butylhydroquinone	0.014	0.000	0.014	0.000	0.014	0.000	0.014	0.000
Red ginseng powder	0	0	0	0	8.5	34	0	0
Fermented red ginseng	0	0	0	0	0	0	8.5	34
Total	1049.51	4033	852.61	4033	852.61	4033	852.61	4033

HF/FRG, HF with fermented red ginseng in diet by 1% (w/w); HF/RG, HF with non-fermented red ginseng in diet by 1% (w/w); LF, low fat.

Table S2. Primer sequences used for RT-PCR

Gene	Accession no.	Forward primers (5' to 3')	Reverse primers (5' to 3')
CAT	NM_012520.2	AAC TCC CAG AAG CCT AAG A	CTC CAT CCA GCG ATG ATT AC
GAPDH	NM_017008.4	GAG CAT CTC CCT CAC AAT TC	GGG TGC AGC GAA CTT TAT
GPX	NM_030826.4	GAG AAG TGC GAG GTG AAT G	CTG GAC CTA CCA GGA ACT T
GSR	NM_053906.2	CCA CGA GGA AGA CGA AAT G	ATG AAT GGC GAC CGT ATT G
IL-6	NM_012589.2	TGT TGT GGG TGG TAT CCT	CCT TCT TGG GAC TGA TGT TG
SOD1	NM_001276707.1	CGA CTA CAT CCG CTT CTT AC	GTC AGC GTT TCT ACC ACT T
SREBP1c	NM_017050.1	GTG GTG TCA GGA CAG ATT AC	CTC CAA CAT GCC TCT CTT C

CAT, catalase; GAPDH, glyceraldehyde 3- phosphate dehydrogenase; GPX, glutathione peroxidases; GSR, glutathione reductase; IL-6, interleukin 6; SOD1, superoxide dismutase 1; SREBP1, sterol regulator element-binding protein 1; TNA α , tumor necrosis factor alpha.



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