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Supplementation of non-fermented and fermented goji berry (*Lycium barbarum*) improves hepatic function and corresponding lipid metabolism via their anti-inflammatory and antioxidant properties in high fat-fed rats

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Abstract

Development of obesity is associated with excessive fat accumulation and oxidative stress along with chronic inflammation. Goji berries (*Lycium barbarum*) are high in polyphenolic compounds and have anti-inflammatory, anti-oxidant, and hypolipidemic properties that may alleviate the pathogenesis of obesity and related metabolic complications. Thus, the aim of this study was to investigate potential metabolic benefits of GB supplementation against high fat (HF) diet-induced obesity and its comorbidities in HF diet-fed rats (male Sprague–Dawley, n = 8/group, 6 weeks old). We also sought to examine the potential metabolic benefits of fermented GB (FGB) with *L. plantarum* CB3 and possible distinctions in the degree and/or mechanism of action compared to GB. GB and FGB supplementation suppressed the gene expression of inflammation indices at the local (adipose tissues) and systemic (liver) levels. In addition, GB and FGB supplementation upregulated the gene expression of antioxidant enzymes compared to the HF and/or even low fat (LF) group with more remarkable antioxidant effects by GB supplementation. Also, GB and FGB supplementation protected from HF-induced damages of the liver and dyslipidemia. In conclusion, we demonstrated that GB and FGB supplementation protected from HF-induced metabolic complications primarily by improving hepatic function and corresponding lipid metabolism via their anti-inflammatory and antioxidant properties. To our knowledge, this is the first in vivo study confirming metabolic benefits of GB in a fermented form. Thus, these findings support the potential application of both GB and FGB to ameliorate obesity-associated metabolic abnormalities.

Keywords: Dyslipidemia, Fermentation, Goji berry (*Lycium barbarum*), Hepatic function, Inflammation, Obesity, Oxidative stress

Introduction

Obesity is primarily characterized by abnormal or excessive fat accumulation and a low-level systemic inflammation [1]. An increase in visceral adiposity serves as a strong predictor of local and systemic inflammation with its association with macrophage infiltration and subsequent secretion of pro-inflammatory cytokines [2]. The pivotal role of pro-inflammatory cytokines such as interleukin-1beta (IL-1β) and

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tumor necrosis factor alpha (TNF α) has been identified in the development of obesity and related metabolic dysregulation [3]. Further, the inflammatory cascade can facilitate the formation of toxic reactive oxygen species and the subsequent generation of oxidative stress, which inhibits the expression of antioxidant enzymes and consequently impairs the antioxidant defense system [3]. Excessive oxidative stress also can impair liver functions, in the regulation of hepatic lipid metabolism in particular [4]. Previous studies have reported that HF-induced hepatic dysfunction demonstrated by the elevated level of alkaline phosphatase (ALP) and aspartate transaminase (AST) enzyme activities in circulation was associated with the upregulation of adipogenesis and lipogenesis transcription factors, resulting in hepatic fat accumulation and dyslipidemia [5, 6].

Extensive attention has been paid to the role of berries in the prevention of obesity and related metabolic disturbances. Goji berries (*Lycium barbarum*) are high in polyphenolic compounds and have anti-inflammatory, anti-oxidant, and hypolipidemic properties that may affect disease pathogenesis [7]. Dietary supplementation with goji berry (GB) reduced biomarkers of oxidative stress and lipid peroxidation as well as inflammatory gene expression in both experimental and clinical studies [8–10]. In addition, Yang et al. reported that GB supplementation ameliorated HF-induced insulin resistance via activation of phosphatidylinositol-3 kinase/protein kinase B/nuclear factor-E2-related factor 2 (PI3K/AKT/Nrf2) pathway [11].

Despite a growing evidence on metabolic benefits of GB, there is limited knowledge on the potential benefits of GB in obesity and related metabolic disorders. Thus, the aim of this study was to investigate metabolic benefits of GB supplementation against HF diet-induced obesity and its comorbidities in rats. We hypothesized that GB supplementation would improve HF-induced obese phenotypes and dysregulation of lipid metabolism in association with anti-inflammatory and anti-oxidant mechanisms. Further, considering that fermentation of bioactive prebiotics with probiotics such as *Lactobacillus* or *Bifidobacterium* has been confirmed to enhance the pharmacological efficacy and metabolic functions of the original non-fermented prebiotics in previous studies [12–14], we also sought to examine the potential metabolic benefits of fermented GB (FGB) with *L. plantarum* CB3 as probiotics and possible distinctions in the degree and/or mechanism of action compared to GB.

Materials and methods

Materials

Commercial kits for animal tests were purchased from Elabscience (Houston, Texas, USA). Goji berries (*Lycium barbarum*) were purchased from local markets in Cheongyang Korea. Pectinex Ultra SP-L was obtained from Vision Biochem (Seongnam, Korea). *Lactobacillus plantarum* CB3, isolated from kimchi in our laboratory, was used as a starter culture for fermentation of goji berries.

Preparation of GB and FGB powder

For the preparation of GB extract, 10 g of dried GB was added to 100 mL of 90 °C water and extracted for 6 h. Twenty mL of GB extract was taken and used as a substrate for the production of FGB. Pectinex Ultra SP-L (0.5%, w/v) was added into the GB extract, and the mixture was reacted at 55 °C for 6 h with shaking at 160 rpm and sterilized at 100 °C for 10 min. The sterilized GB suspension was inoculated with 5% (v/v) of *L. plantarum* CB3 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 h with shaking at 160 rpm. The GB and FGB suspensions were lyophilized to 88% solids using an EYELA freeze dryer (FDU-1200, Tokyo Rikakikai Co.) and used for experiments.

Analysis of betaine

Quantitative analysis of betaine was performed using the method proposed by Lee et al. with some modifications [15]. Betaine from GB and FGB samples (lyophilized to 88% solids) was detected using a 1260 Infinity system (Agilent Technologies, Santa Clara, CA, USA) attached with an evaporative light scattering detector (ELSD; Agilent Technologies, Santa Clara, CA, USA) and a Discovery C18 column (4.6 \times 250 mm, 5 μ m, SUPELCO, PA, USA). The mobile phase contained water and acetonitrile and was used in isocratic elution as at a 95:5 ratio (v/v %), respectively. The flow rate of the elution was 0.5 mL/min and the evaporator and the nebulizer temperature for the ELSD were 50 and 70 °C, respectively.

Measurement of total phenolics and antioxidant activity

The total phenolic content of GB and FGB samples (lyophilized to 88% solids) was determined by modifying the method of previous studies [16]. To determine total phenolic compound and antioxidant activity, lyophilized GB and FGB samples were diluted with 80% methanol to a concentration of 5%, respectively. For the calibration curve of phenolic compounds,

gallic acid stock solution (5 mg/mL) was prepared and diluted with water. Five-hundred microliters of each sample extract was mixed with 300 μ l of 1 N Folin-Ciocalteu (Sigma-Aldrich, St. Louis, MO, USA) in a tube. After reacting for 3 min at room temperature, and 3 mL of 2% sodium carbonate was added in the same tube. After reacting at room temperature for 30 min, the absorbance of the mixture was measured at 720 nm in glass cuvettes using a spectrophotometer (Libra S22, Biochrom Ltd., Cambridge, UK). The same procedure was performed for diluted gallic acid (as a standard). A calibration curve for gallic acid was used to determine the total phenolic contents in the samples, and expressed as gallic acid equivalent (GAE).

The antioxidant activities of GB and FGB samples were determined using diphenylpicrylhydrazyl (DPPH) radical scavenging activity and ferric reducing antioxidant power assay (FRAP). DPPH radical scavenging activity was performed with a 100 μ M DPPH solution proposed by Liang et al. and Park et al. [17, 18]. One and half mL of each methanol extract of GB and FGB samples was dispensed into a tube and added to 1.5 mL of 100 μ M DPPH solution. After vortexing and reacting for 30 min in the dark, absorbance was measured at 517 nm using a spectrophotometer (Libra S22, biochrom Co.). 80% of methanol (1.5 mL) and DPPH solution (1.5 mL) were collected as a control sample. All samples were measured in triplicate. DPPH scavenging activity was calculated using the following equation:

$$\text{Antioxidant activity (\%)} = 1 - [(As - Ab)/Ac] \times 100$$

where antioxidant activity-DPPH radical scavenging activity; As, Ab and Ac represent the absorbance of DPPH with specific samples, blank, and DPPH solutions, respectively.

The FRAP analysis is based on reduction of ferric-tripyridyltriazine (Fe^{3+} -TPTZ) complex into ferrous tripyridyltriazine (Fe^{2+} -TPTZ) by interacting with antioxidants in the sample [19]. FRAP assay was performed using the modified Benzie and Strain method [20]. The FRAP reagent was prepared by mixing 300 mM sodium acetate buffer (pH3.6), 10 mM TPTZ solution, and 20 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ solution in a ratio of 10:1:1 (v/v). 50 μ L of each of the methanol extract of GB and FGB was mixed with 1.5 mL of the FRAP reagent and reacted for 5 min at room temperature in the dark. Then, the absorbance of the mixtures was measured at 450 nm. The calibration curve was prepared with ferric sulfate and FRAP results for samples are expressed in μ g/mL. All samples were measured in triplicate.

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-2020-02-01). Male Sprague–Dawley rats (n = 8/group; 6 weeks old; Samtako Co., Osan, Korea) were individually housed in a controlled environment at 23 ± 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/GB) or fermented goji berry at 2% (w/w) in diet for 6 weeks (Additional file 1: Table S1). Selection of the 2% goji berry concentration was based on previously published studies [21–23]. 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 fasted 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 (retroperitoneal and epididymal) were collected and weighed; an adiposity index was determined. Serum and all the tissues were snap-frozen and stored -80 °C until analysis.

RNA extraction and quantitative RT-PCR

Total RNA from liver and epididymal fat tissues was extracted using the RNeasy Mini Kit (Qiagen, Hilden, Germany) per the manufacturer's instructions. cDNAs were synthesized from 2 μ g 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 2 \times 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 [24] and compared with control samples. Primer sequences are provided in Additional file 1: Table S2.

Biochemical analysis

The levels of serum alkaline phosphatase (ALP) and aspartate aminotransferase (AST) were measured via colorimetric assay kits (ALP: E-BC-K091-S and AST: E-BC-K236-M, Elabscience, Houston, TX, USA) according to the manufacturer's guideline.

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 (SEMs).

Results

Betaine, total polyphenol contents and antioxidant activities

The concentrations of betaine, total polyphenols and antioxidant activity were measured in GB and FGB samples (lyophilized to 88% solids) (Table 1). After lyophilization to 88% solids, the betaine contents of the GB and FGB samples were measured to be 129.29 mg/mL and 165.51 mg/mL, respectively, indicating that the betaine concentration was increased by about 1.3-fold by fermentation ($p < 0.05$).

Total phenolic contents, DPPH radical scavenging ability, and FRAP assay were significantly increased by fermentation ($p < 0.05$). The total phenolic contents of the GB and FGB were 230.76 mg/mL and 280.53 mg/mL, respectively, indicating that the total phenolic contents of the GB sample were significantly increased by about 1.2-fold that of initial during fermentation. The FRAP assay in the GB and FGB were 273.03 μ g/mL and 479.56 μ g/mL, respectively. This means that the FRAP assay of GB was increased dramatically to about 1.8-fold after fermentation. On the other hand, the DPPH radical scavenging ability of the GB was slightly increased by about 3.6% after fermentation.

Supplementation of GB and FGB improves hyperphagic phenotypes

After six weeks on respective diets, all HF fed groups showed significantly (HF, HF/FGB) or partially (HF/GB) higher body weight than the control LF group (LF vs. HF or HF/FGB, $p < 0.05$; LF vs. HF/GB, $p = 0.057$; Fig. 1A).

On the other hand, little difference was observed in cumulative food intake and food efficiency among groups (Fig. 1B, C). Visceral adiposity was evaluated from epididymal and retroperitoneal fat depots (Fig. 1D). HF feeding significantly increased fat mass compared to the LF group (LF vs. HF, $p < 0.0001$) and little difference was found among HF-fed.

Supplementation of GB and FGB attenuates HF-induced inflammation

In adipose tissues, both GB and FGB supplementation significantly down-regulated the gene expression of macrophage chemoattractant protein 1 (MCP-1), as a marker of macrophage infiltration, and pro-inflammatory cytokines, IL-6 and TNF α , compared to the HF group (MCP-1: HF vs. GB or FGB, $p < 0.01$; IL-1 β : HF vs. GB or FGB, $p < 0.05$; TNF α : HF vs. GB or FGB, $p < 0.05$; Fig. 2A–C).

At the systemic level, HF feeding significantly upregulated the gene expression of IL-1 β in the liver compared to the LF group, which was suppressed by GB (significantly) and FGB (partially) supplementation (LF vs. HF, $p < 0.01$; HF vs. GB, $p < 0.05$; HF vs. FGB, $p = 0.075$; Fig. 2D). Further, the gene expression of IL-10 as an anti-inflammatory cytokine was upregulated by GB and FGB supplementation by 98% and 97% compared to the HF group although this did not reach statistical significance (HF vs. GB, $p = 0.075$; HF vs. FGB, $p = 0.076$; Fig. 2E).

Supplementation of GB and FGB exerts antioxidant effects

To investigate the antioxidant properties of GB and FGB supplementation against the HF challenge, the gene expression of antioxidant enzymes was examined. While HF feeding showed little effect on the gene expression of antioxidant enzymes, both GB and FGB supplementation significantly upregulated their gene expression compared to the HF and/or even LF group with more remarkable antioxidant effects by GB supplementation (CAT: LF or HF vs. HF/GB, $p < 0.001$; HF/GB vs. HF/FGB, $p < 0.05$; GPX: LF or HF vs. HF/GB, $p < 0.0001$; HF vs. HF/FGB, $p < 0.01$; HF/GB vs. HF/FGB, $p < 0.01$; GSR: LF or HF vs. HF/GB, $p < 0.01$; SOD1: LF or HF vs. HF/GB,

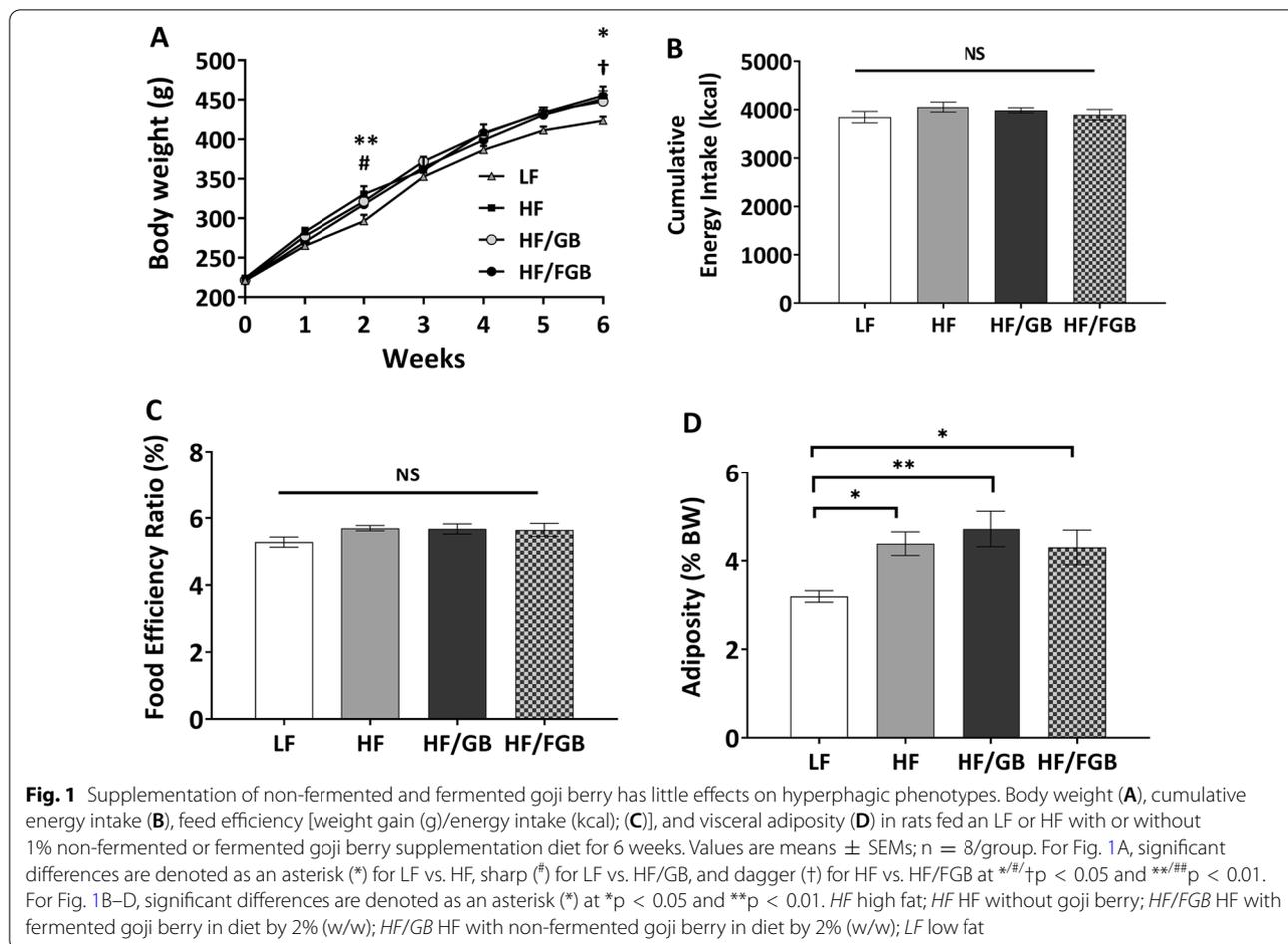
Table 1 Betaine, total phenol contents and antioxidant activities of GB and FGB

Sample	Betaine (mg/mL)	Total polyphenol (mg/mL)	DPPH (%)	FRAP (μ g/mL)
GB	129.29 \pm 0.49 ^b	230.76 \pm 0.59 ^b	64.93 \pm 0.57 ^b	273.03 \pm 6.40 ^b
FGB	165.51 \pm 1.37 ^a	280.52 \pm 1.01 ^a	68.51 \pm 0.68 ^a	479.56 \pm 6.61 ^a

Data are expressed as mean \pm SD

Different superscript letters in the same column mean significantly different at $p < 0.05$ by Duncan's multiple range test

GB Freeze-dried goji berry sample (88% solids); FGB Freeze-dried goji berry samples (88% solids) pretreated with Pectinex Ultra SP-L and fermented with *L. plantarum* CB3



p < 0.0001; LF vs. HF/FGB, p < 0.001; HF vs. HF/FGB, p < 0.01; Fig. 3).

Supplementation of GB and FGB improves hepatic functions

Hepatic functions were examined by the level of ALP and AST in circulation as markers of liver damage (Fig. 4). HF feeding had little effect on the level of serum ALP but the level was significantly decreased by 2% GB supplementation compared to the HF group (Fig. 4A). The serum ALP level in the HF/GB group was even lower than the LF group by 27%. Similarly, the serum AST level was significantly reduced by both GB and FGB supplementation compared to the HF group (HF vs. GB, p < 0.001; HF vs. FGB, p < 0.05; Fig. 4B).

Supplementation of GB and FGB improves lipid profiles

In addition to visceral adiposity, lipid profile was further examined in the liver and in circulation (Fig. 5). As a marker of lipogenesis, the gene expression of hepatic peroxisome proliferator-activated receptor gamma

(PPAR γ) was significantly upregulated by HF feeding compared to the LF group, which was normalized to the LF level by both GB and FGB supplementation (LF vs. HF, p < 0.0001; HF vs. GB or FGB, p < 0.0001; Fig. 5). Similarly, the gene expression of hepatic sterol regulator element-binding protein 1c (SREBP1c), as a marker of adipogenesis, was partially upregulated by HF feeding compared to the LF group and this was significantly suppressed by both GB and FGB supplementation (LF vs. HF, p = 0.059; HF vs. GB, p < 0.01; HF vs. FGB, p < 0.001; Fig. 5B). The similar effect of GB and FGB supplementation on lipid profile was also confirmed at the systemic level; the serum TG level was significantly reduced by both GB and RGB supplementation compared to the HF group (HF vs. GB or FGB, p < 0.01; Fig. 5C).

Discussion

In this present study, we investigated the beneficial effects of GB supplementation on obesity and related metabolic disorders in HF diet-fed rats. We also sought to examine the beneficial effects of fermented GB with *L. Plantarum*

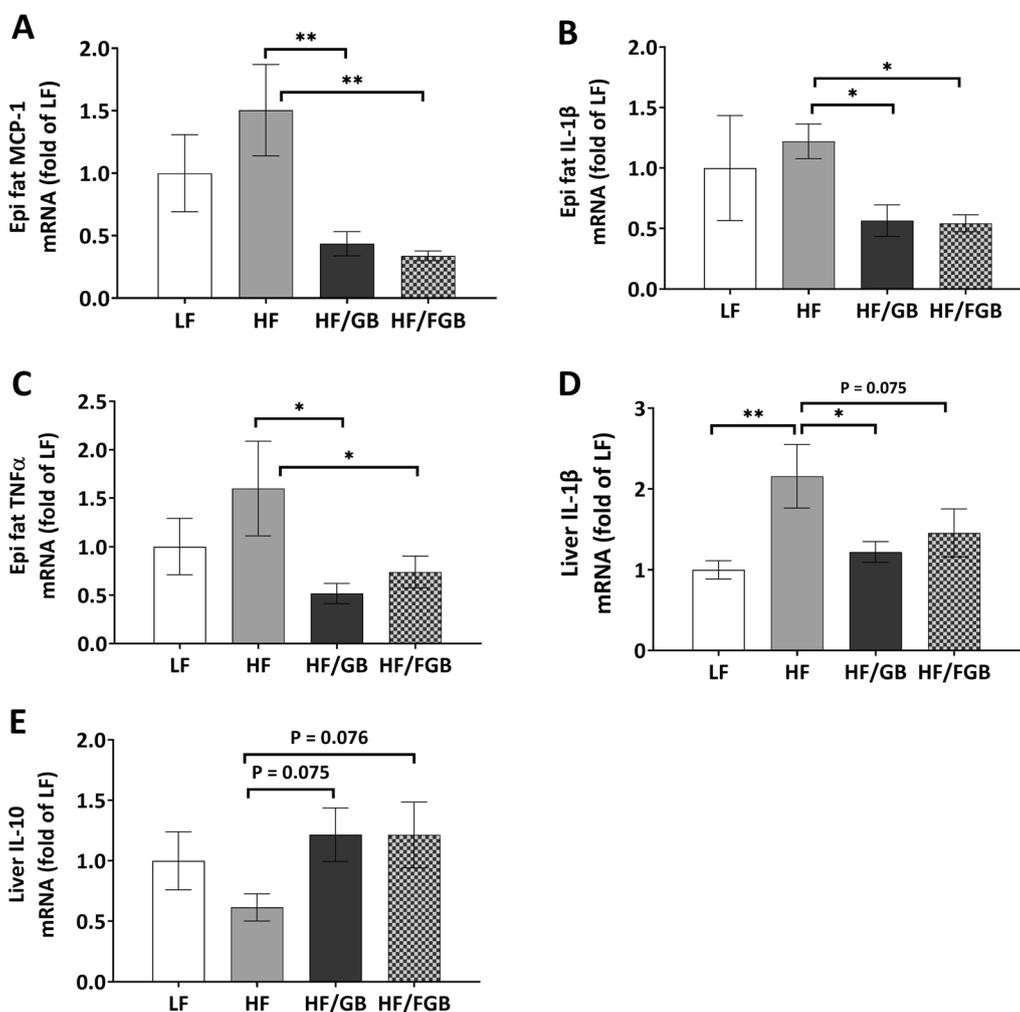


Fig. 2 Supplementation of non-fermented and fermented goji berry attenuates HF-induced inflammation. Gene expression of IL-6 and TNFα in epididymal fat (A–C) and liver (D, E) tissues of rats fed an LF or HF with or without 2% non-fermented or fermented goji berry supplementation diet for 6 weeks. Values are means ± SEMs; n = 8/group. Significant differences are denoted as an asterisk (*) at *p < 0.05 and **p < 0.01. HF high fat; HF HF without goji berry; HF/FGB HF with fermented goji berry in diet by 2% (w/w); HF/GB HF with non-fermented goji berry in diet by 2% (w/w); IL interleukin; LF low fat; MCP-1 macrophage chemoattractant protein 1; TNFα tumor necrosis factor alpha

CB3, elucidating the possible metabolic benefits resulting from the fermentation process. Our hypothesis was GB and FGB supplementation as a part of HF diet would improve the HF-induced disturbances in lipid, inflammatory, and oxidative profiles along with hepatic functions, possibly preventing the development of obesity and related metabolic abnormalities, and that these effects would be enhanced by fermentation.

As major bioactive components of GB, concentrations of betaine and total polyphenolic and their corresponding antioxidant activities were measured in GB and FGB samples. Betaine has similar functions to choline, folic acid, vitamin B₁₂, and the amino acid methionine known as SAmE (S-adenosylmethionine). In the human body,

it functions as a methyl donor and has the effects of improving liver function, cell replication, detoxification, and kidney protection [25, 26].

In this study, we observed that betaine concentration of goji berry was significantly increased by enzyme (Pectinex Ultra SP-L) treatment and *L. plantarum* CB3 fermentation, confirming the previous finding that the betaine concentration increases after fermenting goji berries with various lactic acid bacteria [27]. Total phenolic contents, DPPH radical scavenging ability, and FRAP assay were significantly increased after fermentation with *L. plantarum* CB3 inoculation, which corresponds to the finding of the previous studies [28]. This seems to be because the enzyme and carboxylic acid produced by *L.*

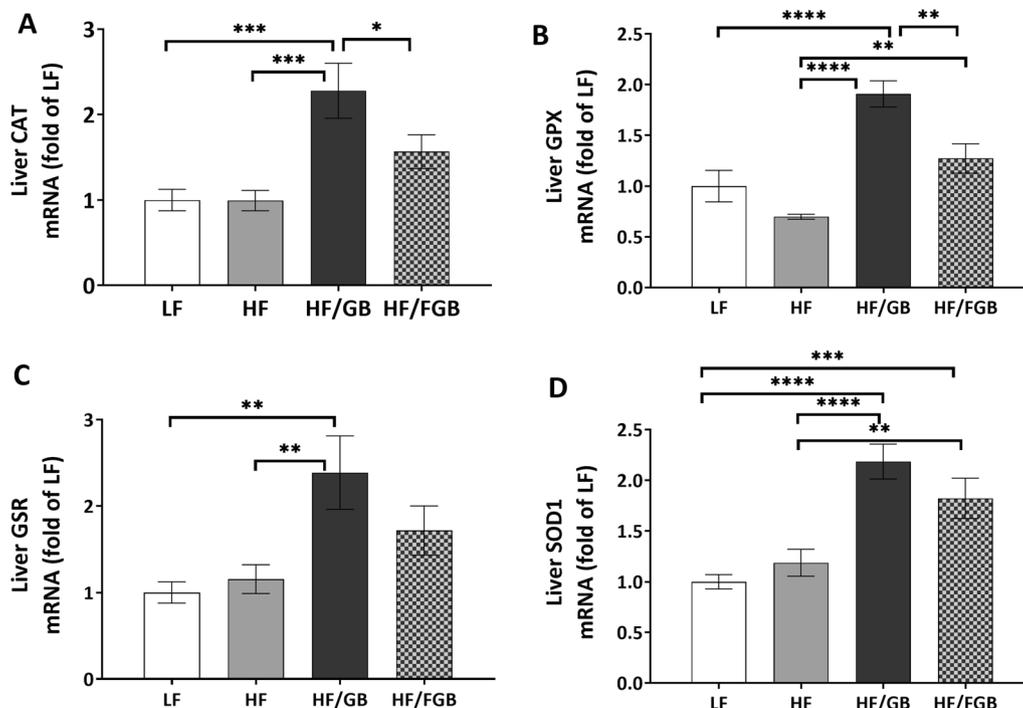


Fig. 3 Supplementation of non-fermented and fermented goji berry exerts antioxidant effects. Gene expression of antioxidant enzymes, CAT (A), GPX (B), GSR (C), and SOD1 (D), in the liver of rats fed an LF or HF with or without 2% non-fermented or fermented goji berry supplementation diet for 6 weeks. Values are means ± SEMs; n = 8/group. Significant differences are denoted as an asterisk (*) at *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001. CAT catalase; GPX glutathione peroxidases; GSR glutathione reductase; HF high fat; HF HF without goji berry; HF/FGB HF with fermented goji berry in diet by 2% (w/w); HF/GB HF with non-fermented goji berry in diet by 2% (w/w); LF low fat; SOD1 superoxide dismutase 1

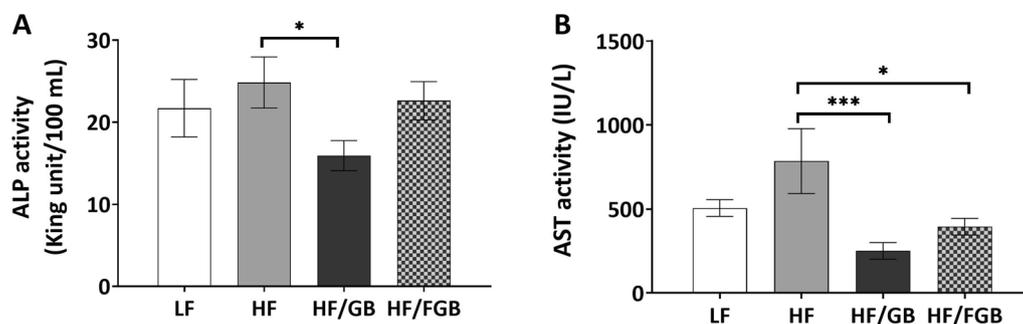
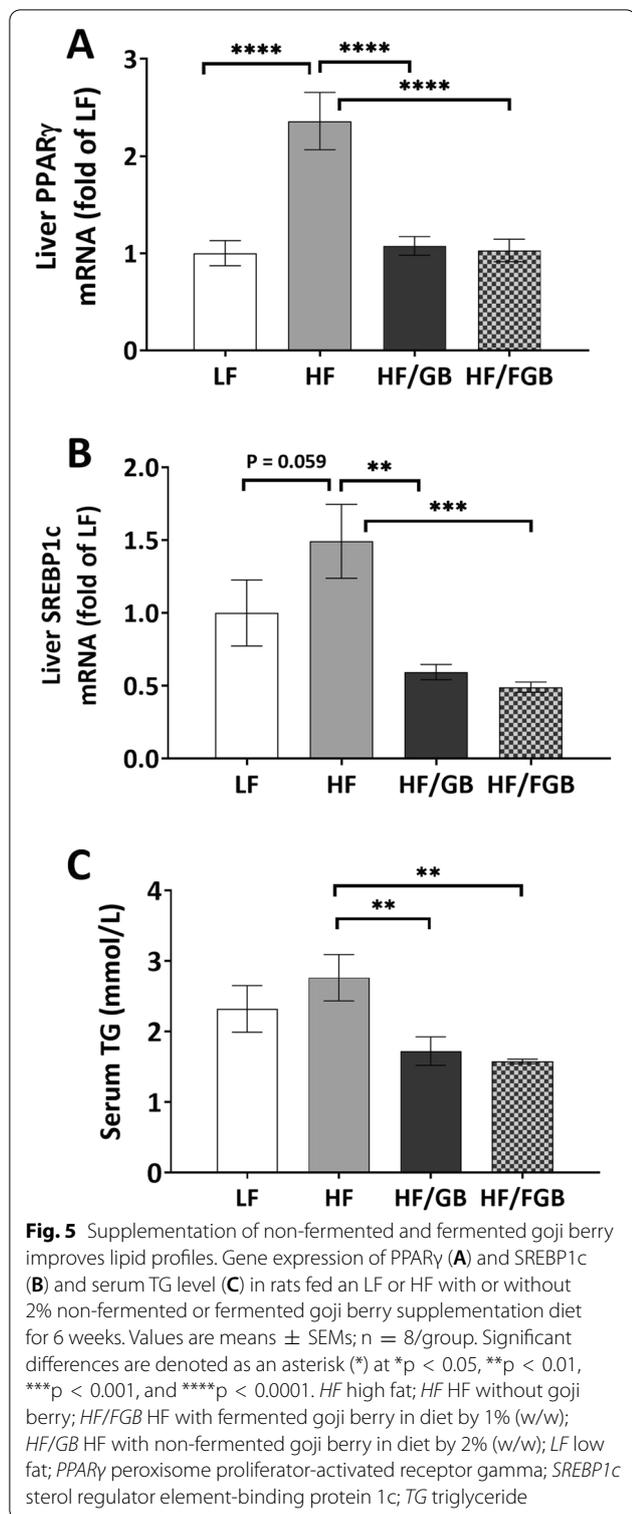


Fig. 4 Supplementation of non-fermented and fermented goji berry protects hepatic functions; levels of serum ALP (A) and AST (B) in rats fed an LF or HF with or without 2% non-fermented or fermented goji berry supplementation diet for 6 weeks. Values are means ± SEMs; n = 8/group. Significant differences are denoted as an asterisk (*) at *p < 0.05 and ****p < 0.0001. HF high fat; HF HF without goji berry; HF/FGB HF with fermented goji berry in diet by 2% (w/w); HF/GB HF with non-fermented goji berry in diet by 2% (w/w). ALP alkaline phosphatase; AST aspartate transaminase; HF/GB HF with non-fermented goji berry in diet by 2% (w/w); LF low fat

plantarum destroy the cellular structure of GB and cause the release of phenolic substances in the fermentation process [29].

In vivo, GB and FGB supplementation suppressed the gene expression of inflammation indices at the local (MCP-1, IL-1β, TNFα in the adipose tissues) and

systemic (IL-1β in the liver) levels. In addition, GB and FGB supplementation upregulated the gene expression of antioxidant enzymes, catalase (CAT), glutathione peroxidases (GPX), glutathione reductase (GSR), superoxide dismutase (SOD1) compared to the HF and/or even LF group with more remarkable antioxidant effects by GB



supplementation. Also, GB and FGB supplementation protected from HF-induced damages of the liver and dyslipidemia. Taken together, these data show that both

GB and FGB supplementation improved HF-induced metabolic disturbances and these improvements were associated with their anti-inflammatory and anti-oxidant properties.

It is widely demonstrated that consumption of a HF diet can lead to increases in body weight, energy intake, and visceral fat mass [30]. In this present study, HF feeding for six weeks significantly increased the body weight. While little difference was observed in overall energy intake and feed efficiency between LF and HF fed groups, an increase in adiposity was still observed in HF-fed rats compared to the LF group, confirming the previous finding that total and visceral body fat weights increases proportionally to the level of fat in the diet even when fed isocalorically [31]. It was unexpected that GB and FGB supplementation did not prevent HF-diet induced increases in body weight gain and adiposity. Thus, to better understand the limited effects of supplementation on these obesity phenotypes, we further investigated the related parameters at the molecular level.

The pathogenesis of obesity involves infiltration of macrophages into expanding adipose tissue and the activated macrophages can contribute to local (adipose tissue) and systemic (liver) inflammation, which is primarily promoted by the release of pro-inflammatory cytokines [2]. In particular, studies have demonstrated that HF diet-induced obese animals developed chronic inflammation in adipose and hepatic tissues [32–34]. In this present study, the HF group showed the highest expression of MCP-1 gene, which was remarkably down-regulated by GB and FGB supplementation. Correspondingly, GB and FGB supplementation also exerted anti-inflammatory effects against other pro-inflammatory cytokines, IL-1 β and TNF α and in favor of IL-10 as an anti-inflammatory cytokine at the local and systemic levels, which corresponds to the finding of the previous study [35].

The inflammatory cascade facilitates the formation of toxic reactive oxygen species and the subsequent production of oxidative stress, which inhibits the expression of antioxidant enzymes and consequently impairs the antioxidant defense system [3]. Here, we observed that GB and/or FGB supplementation remarkably upregulated the gene expression of antioxidant enzymes in the liver including CAT, GPX, GSR, and SOD1 compared both the LF and HF groups, which is consistent to the previous findings of antioxidant effects of GB and FGB in vitro and in vivo [10, 36, 37]

Excessive oxidative stress also can cause tissue damage to the liver [4]. It is known that ALP and AST enzymes are reliable markers of hepatic function and increased levels of circulating ALP and AST indicate hepatic damages [38, 39]. Here, the highest levels of serum ALP and AST were observed in the HF group and their activities

were significantly inhibited by GB and/or FGB supplementation. It is important to note that the elevation in these hepatic enzymes have been associated with abnormal hepatic lipid metabolism [5]. Further, studies have previously shown that HF feeding caused ectopic fat deposition, which was closely associated with the upregulated expression of hepatic PPAR γ as a transcription factor for adipogenesis [32]. Considered as the master regulator of adipogenesis [40], its overexpression has been known to be sufficient for inducement of hepatic fat deposition [41] in concert with SREBP1c as a transcription factor of lipogenesis and also a mediator of PPAR γ expression [42]. In this study, both GB and FGB significantly suppressed HF-induced upregulation of PPAR γ and SREBP1c gene expression in the liver. This was also confirmed at the systemic level. As a well-known indicator of obesity, dyslipidemia is primarily characterized by the elevation of triglycerides in circulation [6]. In this present study, the serum TG level was increased in the HF group by 19% compared to the LF group and this elevation was significantly suppressed by both GB and FGB supplementation. The beneficial effects of GB supplementation also has been reported in previous studies via inhibiting the expression of hepatic lipogenesis factors and/or decreasing the level of triglyceride in circulation, which confirms the findings of this study [43, 44].

Taken together, it can be confirmed that GB and FGB supplementation improved HF-induced metabolic dysregulation in adipose and liver tissues at the molecular level primarily by enhancing anti-inflammatory and antioxidant responses.

There are some considerations to this study that deserve more attention. First, we employed a HF regime for six weeks to induce diet-induced obesity and related metabolic disturbances. This protocol was sufficient to induce obese phenotypes (increases in body weight and visceral fat mass), which was further supported by HF-induced dysregulation of hepatic metabolism (IL-1 β , PPAR γ , and SREBP1c). However, we failed to find any remarkable differences between the LF and HF groups for the other metabolic parameters at the molecular level including those for inflammation, antioxidant actions, and hepatic functions. Thus, a study with longer duration than six weeks would better enable the establishment of other measures for the development of HF-induced obesity. In the meanwhile, despite little difference the LF and HF groups in those metabolic parameters, both GB and FGB supplementation demonstrated metabolic benefits compared to the HF group, even compared to the LF group for the regulation of the antioxidant defense system. Notably, these beneficial effects were more outstanding in the HF/GB group. Previous studies have reported different degree and/or mechanism for

metabolic effects between non-fermented and fermented prebiotics resulting from fermentation with probiotics [45–47]. In these studies, metabolic benefits were more enhanced or different mechanism of action was resulted by fermentation compared to the original prebiotics. In this regard, it is possible that different pathways might have been involved in metabolic benefits observed in the HF/FGB group. Thus, further investigation into multiple possible metabolic pathways would identify the potential mechanism of action by FGB for its metabolic benefits. Further, since the gut microbiota has been identified the primary target by prebiotics and probiotics for their metabolic benefits, metagenomic and metabolomic analyses would provide a better understanding of distinct mechanism of action on metabolic profiles between GB and FGB.

In conclusion, we demonstrated that GB and FGB supplementation protected from HF-induced metabolic complications primarily by improving hepatic function and corresponding lipid metabolism via their anti-inflammatory and antioxidant properties. To our knowledge, this is the first in vivo study confirming metabolic benefits of GB in a fermented form. Thus, these findings support the potential application of both GB and FGB to ameliorate obesity-associated metabolic abnormalities.

Abbreviations

ALP: Alkaline phosphatase; AST: Aspartate transaminase; ANOVA: Analysis of variance; CAT: Catalase; GPX: Glutathione peroxidases; GSR: Glutathione reductase; HF: High fat; HF/FGB: HF with fermented goji berry in diet by 2% (w/w); HF/GB: HF with non-fermented goji berry in diet by 2% (w/w); IL: Interleukin; LF: Low fat; PPAR γ : Peroxisome proliferator-activated receptor gamma; ROS: Reactive oxygen species; SOD1: Superoxide dismutase; SREBP1c: Sterol regulator element-binding protein 1c; TG: Triglyceride; TNF α : Tumor necrosis factor alpha; MCP-1: Macrophage chemoattractant protein.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13765-021-00642-1>.

Additional file 1: Table S1. Composition of experimental diets. **Table S2.** Primer sequences used for RT-PCR.

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Authors' contributions

Conceptualization: HS (Hee-jae Suh), HP, SL. Animal experiment: SL, SJ, YP, CY, UH. Sample preparation: SJ, YP, HS (Hyunji Seo), CY, UH. Sample analysis: SL, SJ. Data analysis: SL. Writing, review, and editing: HS (Hee-jae Suh), HP, SL. Funding acquisition: HS (Hee-jae Suh). All authors have read and approved the final manuscript.

Declarations

Competing interests

The authors declare that there is no competing interests.

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