Dual effects of a mixture of grape pomace (Campbell Early) and Omija fruit ethanol extracts on lipid metabolism and the antioxidant defense system in diet-induced obese mice

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BACKGROUND/OBJECTIVES: We investigated the effects of a combination of grape pomace (*Vitis labrusca*, Campbell Early) and Omija fruit (*Schizandra chinensis*, Baillon) ethanol extracts on lipid metabolism and antioxidant defense system in diet-induced obese mice.

MATERIALS/METHODS: Forty male C57BL/6J mice were divided into four groups and fed high-fat diet (control group, CON) or high-fat diet added 0.5% grape pomace extract (GPE), 0.05% Omija fruit extract (OFE) or 0.5% GPE plus 0.05% OFE (GPE+OFE) for 12 weeks.

RESULTS: In contrast to the GPE- or OFE-supplemented groups, the GPE+OFE group showed significantly lower body weight and white adipose tissue weights than the CON group. Moreover, GPE+OFE supplementation significantly decreased plasma total cholesterol and increased the plasma HDL-cholesterol/total-cholesterol ratio (HTR) compared to the control diet. The hepatic triglyceride level was significantly lower in the GPE+OFE and GPE groups by increasing β-oxidation and decreasing lipogenic enzyme compared to the CON group. Furthermore, GPE+OFE supplementation significantly increased antioxidant enzyme activities with a simultaneous decrease in liver H_2O_2 content compared to the control diet.

CONCLUSIONS: Together our results suggest that supplementation with the GPE+OFE mixture may be more effective in improving adiposity, lipid metabolism and oxidative stress in high-fat diet-fed mice than those with GPE and OFE alone.

INTRODUCTION

Obesity is defined as a disease in which excess body fat has accumulated to the extent that it may impair health. In particular, obesity causes or exacerbates diabetes mellitus, dyslipidemia, hypertension, coronary heart disease, and certain forms of cancer [1]. It is also associated with the development of nonalcoholic fatty liver disease [2]. Although some synthetic anti-obesity drugs effectively reduce body weight and the risk of comorbidities, pharmacological treatment strategies have been limited by their undesirable side effects [3]. For this reason, there has recently been a growing interest in nutraceuticals, which have been used since ancient times [4]. A nutraceutical is a food containing health-promoting ingredients or natural components that may provide desirable health benefits and

contribute to the prevention of chronic diseases [5]. It has been suggested that nutraceuticals have the potential to regulate imbalances between energy intake and output and to ameliorate the development of oxidative stress and inflammation in obesity [6].

Grape pomace, which is an industrial waste from wine production and consists of grape seeds, skins, and stems, contains a large number of polyphenolic compounds [7]. The major phenolics found in grape skin include anthocyanin, ellagic acid, myricetin, quercetin, kaempferol, and trans-resveratrol. Gallic acid, catechin, and epicatechin are found in grape seeds, while grape stems contain rutin, quercetin 3-O-glucuronide, trans-resveratrol, and astilbin [8,9]. A previous study has reported that grape pomace has antioxidant and anti-inflammatory effects in diet-induced obese mice [10]. *Schizandra chinensis*,

Baillon is known as Omija in South Korea, which literally means a five-flavor (salty, sweet, sour, pungent and bitter) berry. Its seeds and fruits have been used in traditional medicine for dry cough, asthma, night sweats, nocturnal seminal emissions, and chronic diarrhea [11-13]. A recent article has reported that phenolic phytochemicals, including anthocyans and flavonols, from Omija displayed high antioxidant activity and anti-inflammatory effects in *in vitro* and *in vivo* models [14,15]. Further, the ethanol extract of Omija not only inhibited preadipocyte differentiation and adipogenesis in 3T3-L1 cells, but also reduced body weight gain, adiposity, and plasma triglyceride and total cholesterol levels in high-fat diet-induced obese rats [16].

There are many different grape varieties around the world. The major varieties of grape produced in Korea are the Muscat Bailey A and Campbell Early. Choi *et al.* have reported that the Campbell Early variety has higher levels of cyanidin, peonidin, pelargonidin, gallic acid, catechin, and epicatechin in its skins and seeds, and the Campbell Early seeds exhibit more effective free radical scavenging effects than those of the Muscat Bailey A variety [17]. In a previous study, we demonstrated that Muscat Bailey A grape pomace extract did not significantly affect fat accumulation and hepatic steatosis in high-fat diet-induced obese mice, but, interestingly the combined extracts of Muscat Bailey A grape pomace and Omija fruit improved adiposity, hepatic steatosis, and inflammation [18]. However, there are no reports on the anti-obesity and antioxidant effects of Campbell Early grape pomace combined with Omija in diet-induced obese mice. Accordingly, in the present study, we examined the effects of ethanol extracts of Campbell Early grape pomace and Omija fruit, alone or in combination, on adiposity, lipid metabolism, and the antioxidant defense system in mice fed a high-fat diet.

MATERIALS AND METHODS

Preparation of grape pomace and Omija fruit extracts

The grapes (*Vitis labrusca*, Campbell Early) and Omija fruit (*Schizandra chinensis*, Baillon) were purchased from Gimcheon and Sangjusi, Gyeonsangbuk-do, Korea and Mungyeong-si, Gyeongsangbuk-do, Korea, respectively. In this study, grape pomace and Omija fruit (Fructus Schisandrae) harvested in 2013 were used. Samples were prepared by adding 1 L of 80% or 50% ethanol to 100 g of dried grape pomace or Omija fruit, respectively, followed by extraction at 80℃ for 2 h and then cooling. The solution was filtered (Whatman paper no. 2), concentrated with a rotary vacuum evaporator, and stored at -70℃. The final weight of the lyophilized powder of grape pomace ethanol extract (GPE) was 17.9 g (17.9%) and of Omija fruit ethanol extract (OFE) was 39.7 g (39.7%). The GPE included 1 mg/g resveratrol, 30 mg/g flavonoids, 64 mg/g polyphenol, 1.35 mg/g catechin, and 17 mg/g gallic acid. The OFE contained 8 mg/g schizandrin, 7 mg/g flavonoids, and 17 mg/g polyphenol.

Animals and diets

Male mice (strain C57BL/6J) were purchased from the Jackson Laboratory (Bar Harbor, ME, USA) at 4 weeks of age. The animals were individually housed with a constant temperature (24℃) and 12 h light/dark cycle, and fed a pelletized commercial nonpurified diet for 1 week after arrival. The mice were then

Table 1. Composition of experimental diets (g of diet, w/w)

Ingredients	Groups[1]			
	CON	GPE	OFE	GPE+OFE
Casein	200	200	200	200
D, L-Methionine	3	3	3	3
Sucrose	369.96	364.96	369.46	364.46
Cellulose	50	50	50	50
AIN-76 mineral mixture	42	42	42	42
AIN-76 vitamin mixture	12	12	12	12
Choline bitartrate	2	2	2	2
Corn Starch	111	111	111	111
Lard	170	170	170	170
Corn oil	30	30	30	30
Cholesterol	10	10	10	10
tert-Butylhydroquinone	0.04	0.04	0.04	0.04
Grape extract		5		5
Omija extract			0.5	0.5
Total	1000	1000	1000	1000

[1] CON: high-fat diet (20% fat, w/w) control; GPE: high-fat diet+grape pomace ethanol extract (0.5%, w/w); OFE: high-fat diet+Omija fruit ethanol extract (0.05%, w/w); GPE+OFE: high-fat diet+grape pomace ethanol extract (0.5%, w/w)+Omija fruit ethanol extract (0.05%, w/w)

randomly divided into 4 groups (n = 10) and fed experimental diets for 12 weeks: high-fat diet (20% fat, w/w) control (CON), high-fat diet with 0.5% (w/w) grape pomace extract (GPE), high-fat diet with 0.05% (w/w) Omija fruit extract (OFE), and high-fat diet with 0.5% (w/w) GPE plus 0.05% (w/w) OFE (GPE+OFE). Based on previous published reports, we chose the dose of experimental materials [18,19]. The composition of each diet is presented in Table 1. The mice had free access to food and distilled water during the experimental period. Their food intake and body weight were measured daily and weekly, respectively. This animal study was approved by the Ethics Committee for animal studies at Kyungpook National University, Republic of Korea (No. 2014-0029).

Preparation of samples

At the end of the experimental period, mice were anaesthetized with diethylether and sacrificed after 12 h of fasting. Blood was taken from the inferior vena cava and then centrifuged at 1000 × g for 15 min at 4℃, and the plasma was separated to analyze plasma biomarkers. After blood collection, the liver and white adipose tissues (WATs), including the epididymal, perirenal, retroperitoneal, mesenteric, subcutaneous, and interscapular WATs, were promptly removed, rinsed, weighed, frozen in liquid nitrogen, and stored at -70℃.

The enzyme source fraction from the liver was prepared and analyzed according to Hulcher and Oleson with slight modifications in order to measure the hepatic lipid-regulating and antioxidant enzyme activities [20]. A 20% (w/v) homogenate was prepared in a buffer containing 0.1 mol triethanolamine, 0.02 mol ethylenediaminetetraacetic acid (EDTA) and 2 mmol dithiothreitol (pH 7.0) and then centrifuged at 600 x g for 10 min to remove any cell debris. The supernatant was centrifuged at 10,000 x g and again at 12,000 x g for 20 min at 4℃ to remove the mitochondrial pellet. Subsequently, the supernatant was

ultracentrifuged twice at 100,000 x g for 60 min at 4℃ to obtain the cytosolic supernatant. The mitochondrial and microsomal pellets were then dissolved in 800 μL of homogenization buffer and the protein content was determined using the Thermo Scientific Pierce BCA Protein Assay Kit.

Plasma and hepatic lipids

Plasma total cholesterol, triglyceride, and high-density lipoprotein (HDL)-cholesterol concentrations were determined using commercially available kits (Asan, Seoul, Republic of Korea). The hepatic lipids were extracted using the procedure developed by Folch *et al.* [21]. The dried lipid residues were dissolved in 1 mL of ethanol for the cholesterol and triglyceride assays. Triton X-100 and a sodium cholate solution in distilled water were added to 200 μL of the dissolved lipid solution to produce final concentrations of 5 g/L and 3 mmol/L, respectively. The hepatic cholesterol and triglyceride were analyzed using the same enzymatic kit used in the plasma analysis. Levels of plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined using enzymatic kits (Asan, Seoul, Republic of Korea).

Hepatic lipid-regulating enzyme activity

Malic enzyme (ME) activity was measured according to the method of Ochoa by monitoring the production of NADPH at 340 nm, where activity was represented by the formation of nicotinamide adenine dinucleotide phosphate (NADPH) nmol/min/mg protein [22]. Glucose-6-phosphate dehydrogenase (G6PD) activity was assayed using spectrophotometric methods according to the procedures described by Pitkanen *et al.* where the activity was expressed as the reduced NADPH nmol/min/mg protein [23]. Fatty acid synthase (FAS) activity was determined using a spectrophotometric assay according to the method by Carl *et al.* [24]; one unit of FAS activity represented the oxidation of 1 nmol of NADPH per minute at 30℃. Phosphatidate phosphohydrolase (PAP) activity was determined using the method of Walton and Possmayer [25]. The fatty acid β-oxidation was determined using the method of Lazarow by monitoring the reduction of NAD to NADH at 340 nm, where the activity was expressed as the reduced NAD nmol/min/mg protein [26].

Hepatic antioxidant enzyme activity and hydrogen peroxide content

The superoxide dismutase (SOD) activity was estimated according to the method of Marklund, which is based on the color change due to the auto-oxidation of pyrogallol [27]. The catalase (CAT) activity was measured using Aebi's method, in which the decrease of hydrogen peroxide was monitored spectrophotometrically at 240 nm for 5 min using a spectrophotometer [28]. The glutathion peroxidase (GSH-Px) activity was measured using a spectrophotometric assay, as described previously by Paglia and Valentine with a slight modification [29]. The reaction mixture included 30 mmol glutathione, 6 mmol NADPH, and hydrogen peroxide (H_2O_2) in a 0.1 mol Tris-HCl (pH 7.2) buffer. The reaction was initiated by adding the enzyme source and the absorbance was measured at 340 nm for 5 min. The glutathione reductase (GR) activity was measured by monitoring the oxidation of NADPH at 340 nm

[30]. The H_2O_2 levels in the liver were measured using Wolff's method [31]. FOX 1 (ferrous oxidation) reagent was prepared from 0.25 mol H_2SO_4, 1 mol sorbitol, 2.5 mmol ammonium iron (II), and 1 mmol xylenol orange, and H_2O_2 levels were determined at 560 nm absorbance.

Statistical analysis

Data were expressed as the mean ± standard error of the mean. Significant differences among the groups were determined using one-way analysis of variance (ANOVA) in SPSS (version 11.0, SPSS Inc., Chicago, IL, USA). Duncan's multiple-range test was performed if differences were identified between the groups at $P < 0.05$.

RESULTS

Effect on body weight, body weight gain, food intake, and food efficiency ratio (FER)

The time courses for changes in body weights are shown in Fig. 1. Compared with the control, neither GPE nor OFE alone significantly altered body weight in high-fat diet-fed mice. The GPE and OFE alone groups also showed no difference in body weight gain and FER (Table 2). However, the body weight of
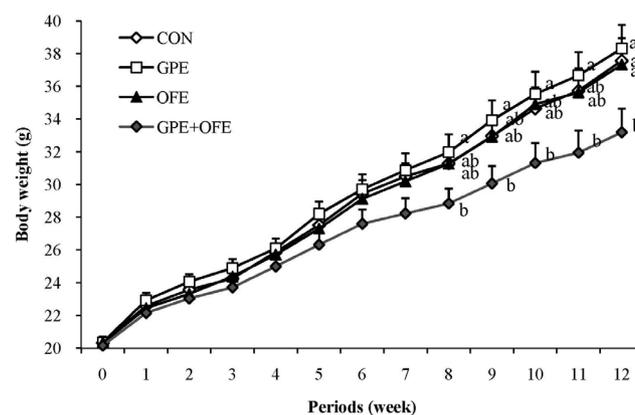

Fig. 1. Effects of supplementation with grape pomace and Omija fruit ethanol extracts for 12 weeks on changes in body weight in C57BL/6J mice fed a high-fat diet. Data are mean ± SE. Superscripts not sharing a common letter are significantly different among groups at $P < 0.05$. CON: high-fat diet (20% fat, w/w) control; GPE: high-fat diet+grape pomace ethanol extract (0.5%, w/w); OFE: high-fat diet+Omija fruit ethanol extract (0.05%, w/w); GPE+OFE: high-fat diet+grape pomace ethanol extract (0.5%, w/w)+Omija fruit ethanol extract (0.05%, w/w).

Table 2. Effects of supplementation with grape pomace and Omija fruit ethanol extracts on body weight gain, food intake, and food efficiency ratio in C57BL/6J mice fed a high-fat diet

Groups[1]	CON	GPE	OFE	GPE+OFE
Body weight gain (g)	17.25 ± 1.27[a]	17.95 ± 1.26[a]	17.00 ± 0.98[a]	13.03 ± 1.29[b]
Food intake (g/day)	3.25 ± 0.07[ab]	3.45 ± 0.07[a]	3.09 ± 0.08[b]	3.39 ± 0.11[a]
FER[2]	0.06 ± 0.004[a]	0.06 ± 0.004[a]	0.07 ± 0.004[a]	0.05 ± 0.006[b]

Data are mean ± SE.
Superscripts not sharing a common letter within the same row are significantly different among groups at $P < 0.05$.
[1] CON: high-fat diet (20% fat, w/w) control; GPE: high-fat diet+grape pomace ethanol extract (0.5%, w/w); OFE: high-fat diet+Omija fruit ethanol extract (0.05%, w/w); GPE+OFE: high-fat diet+grape pomace ethanol extract (0.5%, w/w)+Omija fruit ethanol extract (0.05%, w/w)
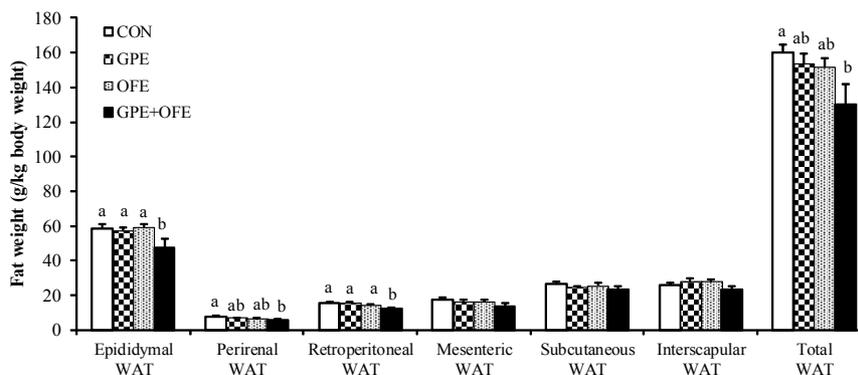[2] FER: food efficiency ratio

Fig. 2. Effects of supplementation with grape pomace and Omija fruit ethanol extracts on white adipose tissue (WAT) weights in C57BL/6J mice fed a high-fat diet. Data are mean ± SE. Superscripts not sharing a common letter are significantly different among groups at $P < 0.05$. CON: high-fat diet (20% fat, w/w) control; GPE: high-fat diet+grape pomace ethanol extract (0.5%, w/w); OFE: high-fat diet+Omija fruit ethanol extract (0.05%, w/w); GPE+OFE: high-fat diet+grape pomace ethanol extract (0.5%, w/w)+Omija fruit ethanol extract (0.05%, w/w).

Table 3. Effects of supplementation with grape pomace and Omija fruit ethanol extracts on plasma lipid profiles in C57BL/6J mice fed a high-fat diet

Groups[1]	CON	GPE	OFE	GPE+OFE
Plasma				
Triglyceride (mmol/L)	1.53 ± 0.02	1.57 ± 0.11	1.81 ± 0.06	1.53 ± 0.09
Total-C[2] (mmol/L)	5.60 ± 0.26^a	5.47 ± 0.19^{ab}	5.48 ± 0.16^{ab}	4.86 ± 0.26^b
HDL-C[3] (mmol/L)	1.14 ± 0.08	1.20 ± 0.04	1.12 ± 0.04	1.16 ± 0.06
HTR[4] (%)	20.36 ± 0.91^b	21.94 ± 0.61^a	20.44 ± 0.57^{ab}	23.87 ± 0.51^a
Liver				
Triglyceride (mmol/g liver)	7.12 ± 0.34^a	6.22 ± 0.28^b	7.11 ± 0.19^a	6.13 ± 0.29^b
Cholesterol (mmol/g liver)	3.02 ± 0.41	2.55 ± 0.32	3.06 ± 0.17	2.79 ± 0.32

Data are mean ± SE.
Superscripts not sharing a common letter within the same row are significantly different among groups at $P < 0.05$.
[1] CON: high-fat diet (20% fat, w/w) control; GPE: high-fat diet+grape pomace ethanol extract (0.5%, w/w); OFE: high-fat diet+Omija fruit ethanol extract (0.05%, w/w); GPE+OFE: high-fat diet+grape pomace ethanol extract (0.5%, w/w)+Omija fruit ethanol extract (0.05%, w/w)
[2] Total-C: total cholesterol
[3] HDL-C: high-density lipoprotein cholesterol
[4] HTR: HDL-C/Total-C ratio ([HDL-C]/[Total-C]×100)

Table 4. Effects of supplementation with grape pomace and Omija fruit ethanol extracts on hepatic lipid-regulating enzyme activities in C57BL/6J mice fed a high-fat diet

Groups[1]	FAS[2]	ME[3]	G6PD[4]	PAP[5]	β-oxidation[6]
	nmol/min/mg protein				
CON	11.31 ± 0.29^a	119.01 ± 7.43^a	22.80 ± 1.90^a	36.52 ± 0.58^a	33.07 ± 1.51^b
GPE	9.91 ± 0.60^b	92.37 ± 5.47^b	17.07 ± 1.86^b	32.32 ± 1.40^b	47.24 ± 3.53^a
OFE	11.21 ± 0.54^{ab}	99.22 ± 6.95^{ab}	18.69 ± 1.66^{ab}	33.49 ± 1.27^{ab}	40.40 ± 2.31^{ab}
GPE+OFE	10.87 ± 0.23^{ab}	95.77 ± 7.78^b	21.73 ± 0.96^{ab}	32.76 ± 1.21^b	42.53 ± 1.82^a

Data are mean ± SE.
Superscripts not sharing a common letter within the same column are significantly different among groups at $P < 0.05$.
[1] CON: high-fat diet (20% fat, w/w) control; GPE: high-fat diet+grape pomace ethanol extract (0.5%, w/w); OFE: high-fat diet+Omija fruit ethanol extract (0.05%, w/w); GPE+OFE: high-fat diet+grape pomace ethanol extract (0.5%, w/w)+Omija fruit ethanol extract (0.05%, w/w)
[2] FAS: fatty acid synthase
[3] ME: malic enzyme
[4] G6PD: glucose-6-phosphate dehydrogenase
[5] PAP: phosphatidate phosphohydrolase
[6] Fatty acid β-oxidation

the GPE+OFE group tended to be lower than that of the CON group between 8 and 11 weeks of the experimental period, and at 12 weeks, but final body weight was significantly lower in the GPE+OFE group than in the CON group (Fig. 1). Moreover, the GPE+OFE group showed significantly lower body weight gain than the CON group (Table 2). FER was significantly lower in the GPE+OFE group than in the control group, although food intake was not different between these 2 groups. Diet supplementation with OFE led to slightly lower food intake than that with GPE or GPE+OFE.

Effect on white adipose tissue weights

The weights of the WATs are shown in Fig. 2. Supplementation with GPE or OFE alone only tended to reduce the perirenal WAT and total WAT weights from those seen in the CON group. However, in the GPE+OFE group the weights of epididymal, perirenal, retroperitoneal and total WATs were significantly lower than those in the CON group. The mesenteric, subcutaneous, and interscapular WAT weights were not significantly different among groups.
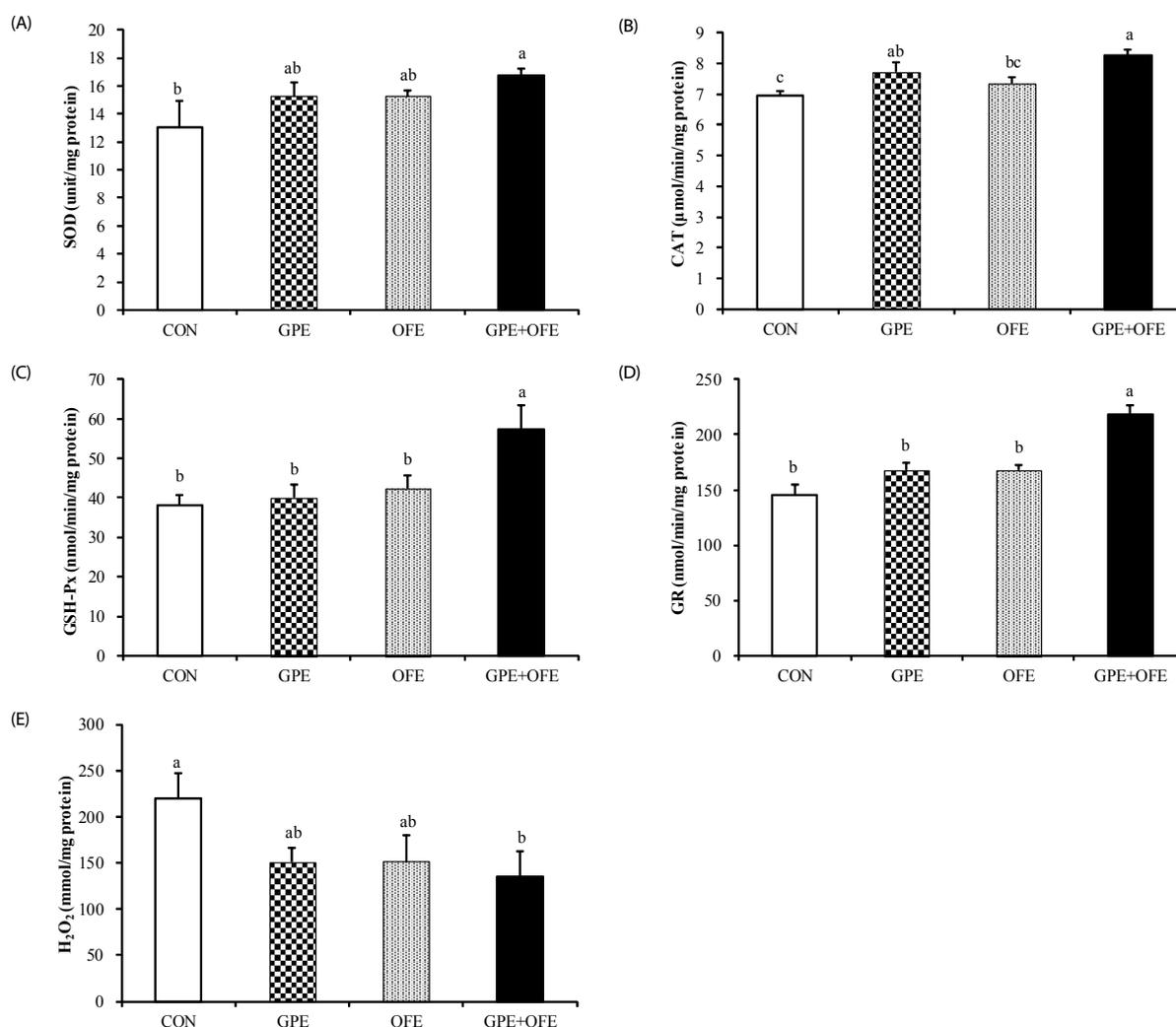
Fig. 3. Effects of supplementation with grape pomace and Omija fruit ethanol extracts on hepatic antioxidant enzyme activities and hydrogen peroxide content in C57BL/6J mice fed a high-fat diet. Data are mean ± SE. Superscripts not sharing a common letter are significantly different among groups at *P* < 0.05. CON: high-fat diet (20% fat, w/w) control; GPE: high-fat diet+grape pomace ethanol extract (0.5%, w/w); OFE: high-fat diet+Omija fruit ethanol extract (0.05%, w/w); GPE+OFE: high-fat diet+grape pomace ethanol extract (0.5%, w/w)+Omija fruit ethanol extract (0.05%, w/w). (A) SOD: superoxide dismutase, (B) CAT: catalase, (C) GSH-Px: glutathion peroxidase, (D) GR: glutathione reductase, (E) H_2O_2: hydrogen peroxide

Effect on plasma and hepatic lipid levels

As shown in Table 3, there was no significant difference in plasma triglyceride concentrations among groups. However, plasma total cholesterol concentration was significantly lower in the GPE+OFE group than in the CON group. Although plasma HDL-cholesterol levels were not significantly different among groups, the HDL-C/Total-C ratio (HTR) was significantly higher in the GPE alone and GPE+OFE groups than in the CON group. Hepatic triglyceride levels were also significantly lower in the GPE alone and GPE+OFE groups than in the CON group, but there were no significant differences in hepatic total cholesterol levels among the groups.

Effect on lipid-regulating enzyme activities in the liver

The activities of lipogenic enzymes and β-oxidation in the liver are shown Table 4. The GPE group showed significantly lower activities of hepatic lipogenic enzymes, including ME,

G6PD, FAS and PAP, than the CON group. The hepatic ME and PAP activities were significantly lower in the GPE+OFE group than in the CON group. In contrast to the lipogenic enzyme activities, fatty acid β-oxidation was significantly higher in the GPE alone and GPE+OFE groups than in the CON group. The OFE group exhibited no significant differences in lipid-regulating enzyme activities.

Effect on antioxidant enzyme activities and hydrogen peroxide content in the liver

When examining the antioxidant enzyme activities, hepatic SOD, CAT, GSH-Px, and GR activities were significantly higher in the GPE+OFE group than in the CON group (Fig. 3). Compared to the control group, the group with GPE supplementation also showed significantly increased hepatic CAT activity and tended to have increased hepatic SOD activity. The hepatic SOD and CAT activities observed in the OFE group tended to be higher

Table 5. Effects of supplementation with grape pomace and Omija fruit ethanol extracts on plasma aminotransferase activities in C57BL/6J mice fed a high-fat diet

Groups[1]	AST[2]	ALT[3]
	IU/L	
CON	43.07 ± 7.19	30.88 ± 7.76
GPE	42.83 ± 6.55	27.21 ± 7.36
OFE	43.21 ± 5.92	27.20 ± 6.75
GPE+OFE	40.33 ± 3.75	26.35 ± 8.63

Data are mean ± SE.
[1] CON: high-fat diet (20% fat, w/w) control; GPE: high-fat diet+grape pomace ethanol extract (0.5%, w/w); OFE: high-fat diet+Omija fruit ethanol extract (0.05%, w/w); GPE+OFE: high-fat diet+grape pomace ethanol extract (0.5%, w/w)+Omija fruit ethanol extract (0.05%, w/w)
[2] AST: aspartate aminotransferase
[3] ALT: alanine aminotransferase

than those of the CON group, but these differences were not statistically significant. The hepatic mitochondrial hydrogen peroxide content was the lowest in the GPE+OFE group.

Effect on plasma aminotransferase activities

The AST and ALT activities in plasma are shown in Table 5. There were no significant differences in plasma AST and ALT activities among groups.

DISCUSSION

Our study was carried out to clarify whether a plant mixture containing grape pomace and Omija fruit ethanol extracts synergistically affects lipid and antioxidant metabolism in diet-induced obese mice. Compared with the control, supplementation with GPE+OFE significantly decreased body weight, body weight gain, body fat accumulation, and FER in mice fed a high-fat diet, although supplementation with GPE or OFE alone did not influence these parameters. Similarly, previous studies reported no significant effects of GPE on body weight gain and fat mass in high-fat diet-induced obese mice or fructose-fed obese rats [18,32,33], but GPE+OFE supplementation significantly lowered body weight gain in high-fat diet-fed mice [18]. On the other hand, in contrast to our results, Park et al. demonstrated that supplementation with OFE (50 mg/kg body weight and 200 mg/kg body weight) for 6 weeks decreased body weight in a dose-dependent manner in high-fat diet-fed rats, which may be due to higher doses of OFE and different animal models [16].

Adipose tissue is not simply an energy storage organ but it also regulates lipid metabolism locally as well as at distant sites such as the liver, muscle, and brain [34]. Obesity is associated with an increased risk of dyslipidemia and nonalcoholic fatty liver disease [35,36]. In the present study, we found that compared with the control, supplementation with GPE+OFE led to significant reductions in the levels of plasma total cholesterol and hepatic triglyceride and a marked increase in the plasma HTR, a good predictor of cardiovascular risk [37]. Compared with the control, GPE supplementation also significantly increased the plasma HTR and markedly decreased hepatic triglyceride content, whereas OFE supplementation did not alter plasma and hepatic lipid profiles. Consistent with the decreased hepatic triglyceride content, GPE with or without OFE, significantly

decreased the activities of hepatic fatty acid synthesis-related enzymes (FAS, G6PD, and/or ME) from those seen in the CON group. FAS catalyzes the reductive synthesis of long-chain fatty acids, and its inhibition reduced food intake and body weight in mice [38]. Cellular NADPH, which is required for the biosynthesis of fatty acids and cholesterol, can be produced by enzymatic reaction of ME and G6PD. A previous study observed that the enzyme activity and expression levels of G6PD were significantly elevated in obese mice models [39]. In our previous study in which Muscat Bailey A was used instead of the Campbell Early grape variety, the mRNA expression of hepatic lipogenic genes (FAS, acetyl-CoA carboxylase, or ME) and their transcription factor (PPAR γ) was significantly down-regulated by a mixture of GPE and OFE extracts [18]. In the present study, we also found that the activity of PAP, a central enzyme in the synthesis of triglycerides, was significantly lower in the GPE and GPE+OFE groups than in the CON group, and hepatic fatty acid β-oxidation was higher in the GPE and GPE+OFE groups than in the CON group, which supports the finding of lower hepatic triglyceride content.

A study by Furukawa et al. reported fat accumulation correlated with oxidative stress in obese mice [40]. Oxidative stress contributes to tissue injury by reactive oxygen species (ROS) attack, and tissues are protected from ROS damage by antioxidant enzymes [41]. Among the antioxidant enzymes, SOD catalyzes the dismutation of the superoxide anion to hydrogen peroxide, and CAT and GSH-Px can break down hydrogen peroxide. GSH-Px also oxidizes glutathione to glutathione disulfide (GSSG). The reduction of GSSG to GSH can be catalyzed by GR again. In the present study, hepatic SOD, CAT, GSH-Px, and GR activities were significantly higher in the GPE+OFE group than in the CON group, whereas hepatic H_2O_2 content was lower. It is well known that GPE contains substantial amounts of antioxidant phenolic compounds, in particular anthocyanins, catechin, epicatechin, and several phenolic acids [10]. In rats fed a high-fat diet, grape skin supplementation was effective in protecting against oxidative damage caused by lipid peroxidation [42]. Phenolic compounds from Omija dose-dependently promote antioxidant activities such as electron donating ability, SOD-like ability, hydroxyl radical scavenging ability, and nitrite scavenging ability in vitro [43]. In the present study, the beneficial effects on hepatic antioxidant capacity as well as adiposity were greater with the GPE+OFE mixture than with GPE or OFE alone. This suggests a synergistic effect of the bioactive phytochemical compounds in grape pomace and Omija. Anthocyanins from grape and Omija improved obesity induced by high-fat feeding [44]. Resveratrol present in grapes is also effective for protecting against diet-induced obesity in vivo [45,46]. Thus, phytochemical combinations have greater additive and synergistic effects on health benefits than single preparations [5]. It is supposed that some flavonoids and polyphenols in GPE+OFE may have synergistic effects via their interaction, although we did not determine it in the present study. This hypothesis is supported by a previous our study in which the effects of GPE+OFE were compared with those of resveratrol and schizandrin mixture (RS), which is a mixture of the major index components of GPE+OFE [47]. GPE+OFE have more protective effect on hyperglycemia, adiposity and hepatic

steatosis in type 2 diabetic mice than RS [47]. Another possibility is that other unknown active components present in extracts of GPE+OFE also play important in their biological effects. To confirm the safety of the experimental supplements in the liver, AST and ALT were measured in plasma, because increased AST and ALT activities have been observed in liver injury [48]. The ethanol extracts used did not exhibit hepatic toxicity.

In conclusion, supplementation with a GPE+OFE mixture, in which the Campbell Early grape variety was used, improves adiposity, lipid metabolism, and antioxidant capacity in high-fat diet-fed mice by suppressing hepatic lipogenic enzyme activities and simultaneously increasing hepatic antioxidant enzyme activities. The anti-adiposity and antioxidant effects of GPE+OFE supplementation appeared to be more potent than that of GPE and OFE alone. These results suggest that GPE+OFE is more beneficial for the prevention of obesity than GPE or OFE alone.

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