

ORIGINAL ARTICLE

Effects of *Cornus walteri* extract on hepatic lipid-regulating enzyme activities in high fat diet-induced obese rats

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Summary. In this study, we investigated whether the body fat suppressive effects of *Cornus walteri* extract (CE) were due to alterations in lipid-regulating enzyme activities in high fat diet-induced obese rats. Male Sprague-Dawley rats were randomly divided into three groups (n=6): a normal diet group (N-control), a high fat diet group (F-control), and a high fat diet group, treated orally with CE (0.25%) in their drinking water (Treatment). After five weeks, CE supplementation tended to reduce body-weight gain and visceral fat in the diet-induced obese rats. The activity of hepatic glucose-6-phosphate dehydrogenase (G6PD) and malic enzyme (ME), responsible for fatty acid synthesis, tended to be lower in the Treatment group compared to the non-treated groups. This finding indicates that the body fat suppressive effects of CE are most likely due to decreases in hepatic fatty acid synthesis. CE supplementation increased hepatic carnitine palmitoyltransferase (CPT) activity, which suggests that β -oxidation was enhanced. In conclusion, CE supplementation suppressed body fat accumulation by attenuating fatty acid synthesis and enhancing β -oxidation through alteration of lipid-regulating enzyme activities.

Key words: body fat, *Cornus walteri*, glucose-6-phosphate dehydrogenase, malic enzyme, carnitine palmitoyltransferase

List of abbreviations

CE, *Cornus walteri* extract;
CPT, carnitine palmitoyltransferase;
G6PD, glucose-6-phosphate dehydrogenase;
HDL-C, high-density lipoprotein cholesterol;
LDL-C, low density lipoprotein cholesterol;
ME, malic enzyme; TC, total cholesterol;
TGs, triglycerides.

Introduction

Obesity is a medical condition in which excess body fat accumulates to the extent where it may have adverse effects on health, leading to reduced life expectancy and/or increased health problems (1). It is necessary to treat obese individuals with lifestyle interven-

tions and/or treatment. Despite its short-term benefits, pharmacological treatment of obesity is often associated with rebound weight gain after cessation of drug use, side effects from the medication, and a potential for drug abuse (2, 3). Complementary and alternative treatments that have long been used in the Asia are increasingly used worldwide (4). When conventional treatments fail to treat obesity effectively and without adverse events, many people seek unconventional therapeutic methods, including herbal medicine (5,6). Herbal medicine is defined by the use of the medicinal properties of plants, raw or refined products derived from plants, or parts of plants (e.g. leaves, stems, buds, flowers, roots, or tubers) for treatment (7). *Cornus walteri* (*C. walteri*) belongs to the family of Cornaceae, which consists of approximately 55 species. It is a de-

ciduous shrub that grows in the valleys of East Asia, particularly in China and Korea (8, 9). The fruits and leaves of *C. walteri* are used to treat skin inflammation and boils caused by lacquer poison in Chinese folk medicine (9). In Korean folk medicine, the stem of *C. walteri* has been used to treat obesity (8, 10). *C. walteri* contains a variety of cytotoxic constituents such as iridoid glycosides, polyphenols, triterpenoids, lignans, and the benzofuranone derivative, halleridone (11). Previous phytochemical investigations of *C. walteri* led to the isolation of gallic acid and flavonoids (10). It has also been reported that *C. walteri* inhibits nitric oxide (NO) production in lipopolysaccharide (LPS)-activated macrophages, and that it has elastase and tyrosinase inhibitory activities as well as anti-obesity effects (10, 12–14). Anti-obesity studies have concluded that *C. walteri* can lower lipid levels in the blood and liver, and can induce body fat loss (12, 15). However, the mechanisms involved in the anti-obesity effects of *C. walteri* have not been fully investigated. Thus, to understand the anti-obesity effects of *C. walteri* *in vivo*, we investigated whether the body fat suppressing effects of *C. walteri* were due to alterations in lipid-regulating enzyme activities in high fat diet-induced obese rats.

Materials and methods

Preparation of *C. walteri* extract (CE)

C. walteri used in this study was collected in Korea. *C. walteri* was extracted under optimal water extraction conditions. *C. walteri* was dried, crushed, and homogenized with an Ultra-Turrax® T-50 (Janke & Kunkel IKA-Lab., Staufen, Germany). Next, *C. walteri* (500 g) was refluxed for 5 h at 100°C (7 volumes of water), cooled to room temperature, and filtered with Whatman No. 5 (Whatman International Ltd., Maidstone, UK). After centrifugation at 3,000 × *g* for 30 min at 4°C to remove insoluble materials, the aqueous phase was concentrated with a rotary vacuum evaporator and then lyophilized.

Animals and diets

The experimental protocol was reviewed and approved by the Korea University Animal Care Committee (KUIACUC-20130902-1). Male Sprague-Dawley

rats were obtained at 6 weeks of age from Daehan Biolink (Chungchungbuk-do, Korea). The rats were housed in a room maintained at 24±1°C with 60% atmospheric humidity and a 12 h light/12 h dark cycle. After an adaptation period (7 days), the rats were randomly divided into the following three groups (n=6): a normal diet group (N-control group, positive control), a high fat diet group (F-control group, negative control), and a high fat diet group treated orally with CE (0.25%) in their drinking water (Treatment group). CE 0.25% in the drinking water is equivalent to a dose of 0.25 g/kg body weight (BW) based on daily water consumption. The drinking water was changed every 2 days. The average daily water intakes of the three groups were 29.1 mL (N-control), 30.3 mL (F-control), and 27.9 mL (Treatment) (data not shown). Water intake did not differ between the experimental groups during the experimental period. The composition of the experimental diet was based on the AIN-76 semi-synthetic diet (Table 1). The rats had free access to food and water. After feeding with the experimental diets for 5 weeks, the rats were fasted for 12 h and anatomized under anesthesia with diethyl ether. Blood samples were taken from the inferior vena cava. Serum was separated by centrifugation at 3,000 × *g* for 15 min at 4°C. The liver and visceral fats (epididymal and peri-

Table 1. Composition of the experimental diets

| Ingredient | Experimental diets (g/100 g diet) | |
|-----------------|-----------------------------------|---------------|
| | Normal diet | High fat diet |
| Casein | 19.6 | 26.2 |
| Corn starch | 56.6 | 27.6 |
| Cellulose | 5.0 | 5.0 |
| Sucrose | 9.95 | 9.95 |
| Lard | 0 | 22.4 |
| Soybean oil | 4.0 | 4.0 |
| Mineral mixture | 3.5 | 3.5 |
| Vitamin mixture | 1.0 | 1.0 |
| L-methionine | 0.3 | 0.3 |

Mineral mixture (g/100 g mixture): CaPO₄·2H₂O, 14.6; KH₂PO₄, 25.7; NaH₂PO₄, 9.4; NaCl, 4.7; calcium lactate, 35.1; ferric citrate, 3.2; MgSO₄, 7.2; ZnCO₃, 0.1; MnSO₄·4H₂O, 0.1; CuSO₄·5H₂O, 0.03; KI, 0.01. *Vitamin mixture:* ICN vitamin mixture (No9046549).

renal fat pads) were removed, rinsed with physiological saline, and weighed. All samples were stored at -70°C until the analyses were performed.

Serum lipids

Serum triglycerides (TGs), total cholesterol (TC), and high-density lipoprotein cholesterol (HDL-C) levels were measured with a FUJI DRI-CHEM 3500 (Fuji Photo Film Co., Osaka, Japan). Low density lipoprotein cholesterol (LDL-C) was estimated as described by Friedewald *et al.* (16).

Preparation of hepatic subcellular fractions

Pieces of the liver were homogenized in 6 volumes of a 0.25 M sucrose solution containing 1.0 mM ethylenediaminetetraacetic acid (EDTA) in a 10 mM Tris-HCl buffer (pH 7.4) and then centrifuged at $10,000 \times g$ for 10 min at 4°C to discard any cell debris. The supernatant was subsequently centrifuged at $20,000 \times g$ for 20 min at 4°C to remove the mitochondrial pellet. The resulting mitochondrial pellets were then re-dissolved in 0.8 mL of homogenization buffer. Finally, the supernatant was further ultracentrifuged at $105,000 \times g$ for 60 min at 4°C to obtain the cytosol supernatant. The amounts of protein in the mitochondrial and cytosolic fractions were measured according to the Bradford method (17) using bovine serum albumin as a standard.

Hepatic lipid-regulating enzyme activities

Glucose-6-phosphate dehydrogenase (G6PD; EC1.1.1.49) activity was determined as previously described.¹⁸ Briefly, the reaction solution contained 0.16 M of Tris-HCl buffer (pH 7.6), 30 mM of MgCl_2 , 3.3 mM of glucose-6-phosphate, 1.6 mM of oxidized nicotinamide adenine dinucleotide phosphate (NADP), and 1 U of 6-phosphogluconate dehydrogenase. The reaction was initiated by the addition of 0.3-0.4 mg of a protein source (cytosol) in a final assay volume of 1 mL at 30°C and the absorbance was monitored at 340 nm for 2 min.

Malic enzyme (ME; EC 1.1.1.40) activity was determined as previously described.¹⁹ Briefly, the reaction solution contained 64 mM of triethanolamine hydrochloride (pH 7.4), 1.2 mM of malic acid, 1.2 mM of NADP, and 4 mM of MnCl_2 . The reaction was initi-

ated by the addition of 0.6-0.8 mg of a protein source (cytosol) in a final assay volume of 1 mL at 27°C and the absorbance was monitored at 340 nm for 2 min.

Carnitine palmitoyltransferase (CPT; EC2.3.1.23) activity was determined as previously described.²⁰ Briefly, the reaction solution contained 58 mM of Tris-HCl buffer (pH 8.0), 1.25 mM of EDTA, 1.25 mM of L-carnitine, 0.25 mM of 5,5'-dithiobis-2-nitrobenzoic acid, 37.5 μM of palmitoyl-CoA, and 0.1% Triton-X. The entire solution was equilibrated at 27°C . The reaction was initiated by the addition of 0.2-0.4 mg of a protein source (mitochondria) and the absorbance was monitored for 5 min at 412 nm.

Statistical analysis

All statistical analyses were performed using the Statistical Package for Social Sciences ver. 12.0 (SPSS, Chicago, IL, USA). The differences between groups were statistically evaluated using one-way analysis of variance (ANOVA) and Tukey's multiple range tests. All tests were two-sided with a 5% significance level, and the data are reported as the means \pm standard deviation (SD).

Results

Figure 1 illustrates the rat body-weight gain during the 5 weeks of dietary intervention. High fat feeding caused significant changes in body weights. The body weight gain in the F-group was significantly greater than that of the N-control group ($p < 0.05$). CE supplementation tended to decrease the body weight gain in the diet-induced obese rats. The Treatment group displayed body weight gains that were comparable to those of the F-control group on the 9th, 12th, and 18th day ($p < 0.05$).

Mean body weight gain in the N-control, F-control, and Treatment groups was 5.86 g/day, 7.96 g/day, 7.27 g/day, respectively (data not shown).

The effects of CE supplementation on serum lipids are presented in Table 2. High-fat feeding tended to increase serum levels of TGs, TC, and LDL-C. CE supplementation tended to suppress this increase. However, there was no significant difference in serum lipid levels across all experimental groups.

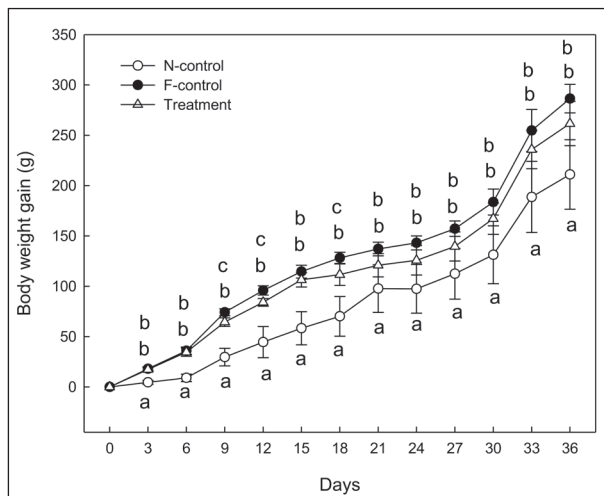


Figure 1. Effects of *Cornus walteri* extract (CE) on body weight gain in diet-induced obese rats. Values are presented as means \pm standard deviations (SD) for 6 rats. Means with different letters are significantly different from each other. Significance was considered at $p < 0.05$ by Tukey's multiple range tests. N-control; normal diet + vehicle water, F-control; high fat diet + vehicle water, Treatment; high fat diet + CE 0.25% water. CE 0.25% in the drinking water is equivalent to a dose of 0.25 g/kg BW based on daily water consumption. Rats were treated with or without CE for 5 weeks.

Table 2. Effects of CE on serum lipids in diet-induced obese rats

| Serum lipids | Serum lipids (mg/dL) | | |
|--------------|----------------------|-----------------|-----------------|
| | N-control | F-control | Treatment |
| TGs | 92.0 \pm 11.5 | 97.0 \pm 17.0 | 88.4 \pm 18.2 |
| TC | 67.7 \pm 8.6 | 82.3 \pm 9.9 | 77.0 \pm 12.2 |
| HDL-C | 32.1 \pm 4.4 | 37.0 \pm 5.1 | 36.5 \pm 5.9 |
| LDL-C | 17.1 \pm 6.4 | 25.8 \pm 3.9 | 22.8 \pm 8.1 |

Values are presented as the means \pm standard deviations (SD) for 6 rats. Means with different letters are significantly different from each other. Significance was considered at $p < 0.05$ by Tukey's multiple range tests. N-control; normal diet + vehicle water, F-control; high fat diet + vehicle water, Treatment; high fat diet + CE 0.25% water. CE 0.25% in drinking water is equivalent to a dose of 0.25 g/kg BW based on daily water consumption. Rats were treated with or without CE for 5 weeks. TGs: triglycerides, TC: total cholesterol, HDL-C: high density lipoprotein cholesterol, LDL-C: low density lipoprotein cholesterol.

To examine the effects of CE supplementation on body fat distribution, the weights of the epididymal and perirenal fat tissues relative to body weights were meas-

ured (Fig. 2). CE supplementation tended to effectively reduce visceral fat in the high fat diet-induced obese rats. The Treatment group (epididymal fat: 1.60 g/100 g BW, perirenal fat: 1.52 g/100 g BW) presented smaller fat pads than the F-control group (epididymal fat: 1.79 g/100 g BW, perirenal fat: 1.88 g/100 g BW), but there was no significant difference between the two groups.

To understand the mechanisms involved in the body fat suppressive effects of CE, the activity of hepatic lipid-regulating enzymes was determined (Fig. 3 and 4). High-fat feeding resulted in the down-regu-

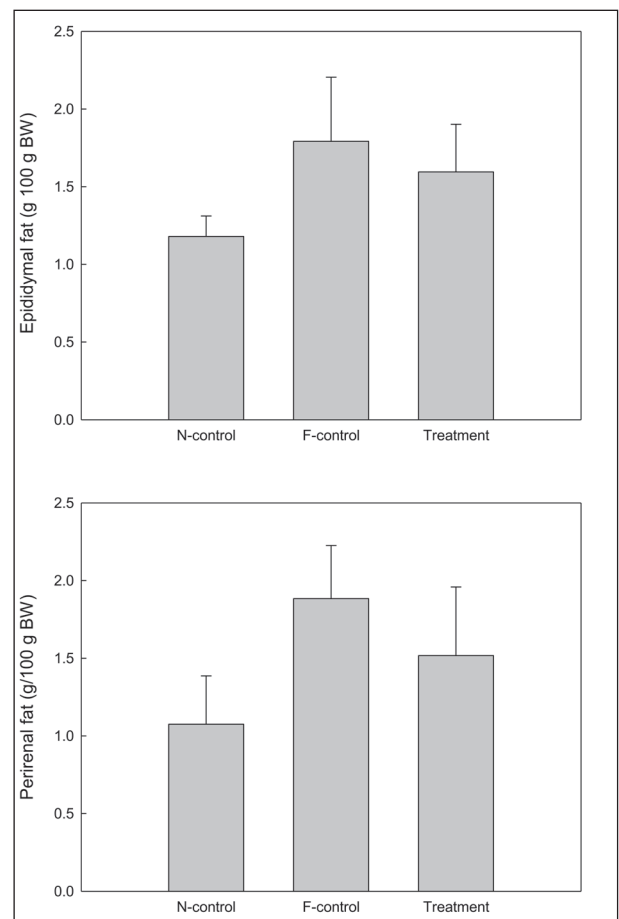


Figure 2. Effects of *Cornus walteri* extract (CE) on fat pads in diet-induced obese rats. Values are presented as means \pm standard deviations (SD) for 6 rats. Means with different letters are significantly different from each other. Significance was considered at $p < 0.05$ by Tukey's multiple range tests. N-control; normal diet + vehicle water, F-control; high fat diet + vehicle water, Treatment; high fat diet + CE 0.25% water. CE 0.25% in drinking water is equivalent to a dose of 0.25 g/kg BW based on daily water consumption. Rats were treated with or without CE for 5 weeks.

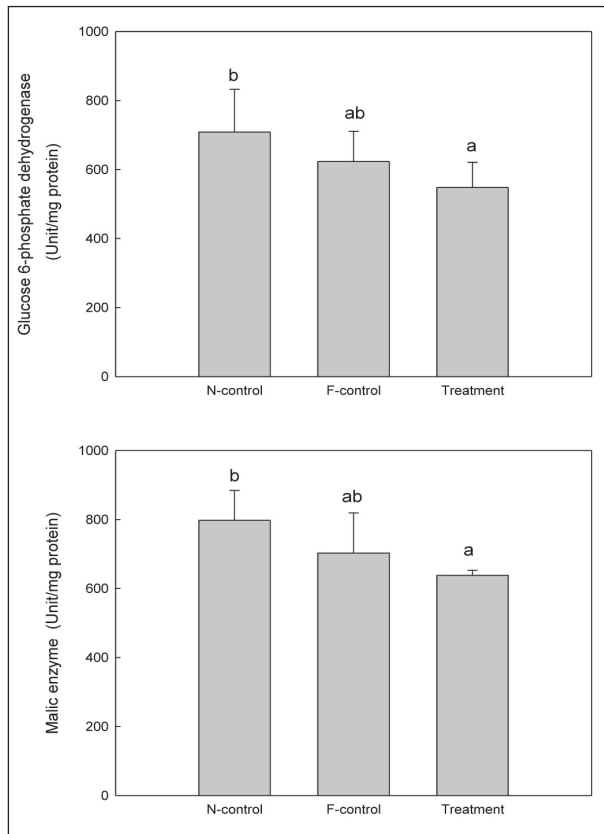


Figure 3. Effects of *Cornus walteri* extract (CE) on hepatic lipogenesis enzymes in diet-induced obese rats. Values are presented as means \pm standard deviations (SD) for 6 rats. Means with different letters are significantly different from each other. Significance was considered at $p < 0.05$ by Tukey's multiple range tests. N-control; normal diet + vehicle water, F-control; high fat diet + vehicle water, Treatment; high fat diet + CE 0.25% water. CE 0.25% in drinking water is equivalent to a dose of 0.25 g/kg BW based on daily water consumption. Rats were treated with or without CE for 5 weeks.

lation of G6PD and ME activities, which provide reduced nicotinamide adenine dinucleotide phosphate (NADPH) required for fatty acid synthesis. CE supplementation enhanced the fat-induced suppression of G6PD and ME activities; G6PD activity was significantly inhibited in the Treatment group compared to the N-control group (N-control group: 708.9 Unit/mg proteins vs. Treatment group: 548.0 Unit/mg protein, $p < 0.05$). Rats treated orally with CE also exhibited a significantly decreased ME activity compared to normal rats (N-control group: 797.9 Unit/mg proteins vs. Treatment group: 638.0 Unit/mg proteins, $p < 0.05$).

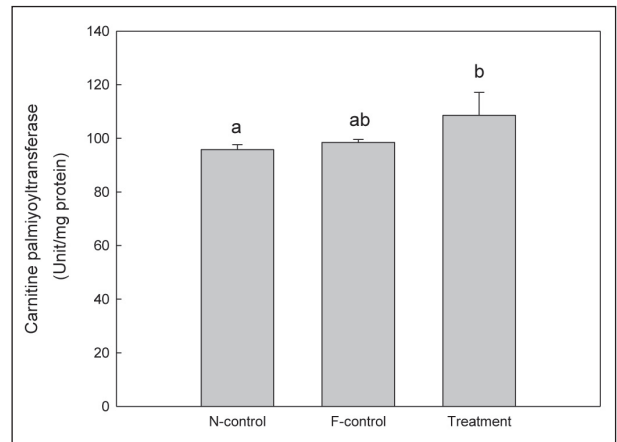


Figure 4. Effects of *Cornus walteri* extract (CE) on hepatic lipolysis enzymes in diet-induced obese rats. Values are presented as means \pm standard deviations (SD) for 6 rats. Means with different letters are significantly different from each other. Significance was considered at $p < 0.05$ by Tukey's multiple range tests. N-control; normal diet + vehicle water, F-control; high fat diet + vehicle water, Treatment; high fat diet + CE 0.25% water. CE 0.25% in drinking water is equivalent to a dose of 0.25 g/kg BW based on daily water consumption. Rats were treated with or without CE for 5 weeks.

The addition of CE to the high fat diet increased CPT activity compared to the normal group (N-control group: 95.8 Unit/mg proteins vs. Treatment group: 108.6 Unit/mg proteins, $p < 0.05$). Thus, it seems likely that CE enhanced fatty acid oxidation in the fatty infiltration condition induced by the high fat diet.

Discussion

This study assessed whether the effects of CE on body fat and serum lipids were linked to modulation of hepatic lipid-regulating enzyme activities. The results demonstrated that CE supplementation tended to decrease body fat in obese rats, which indicates that CE affects the hepatic lipid metabolism. Moreover, CE supplementation tended to decrease serum TGs, TC, and LDL-C levels. Dobrian *et al.* (21) reported that the decrease of body weight is associated with diminished serum lipids, and that a particularly strong positive correlation exists between body fat mass and serum TGs. Therefore, the influence of CE on energy balance may be related to an improvement in serum lipid profiles.

Body fat is increased when the number and/or size of adipocytes rise due to increased proliferation and differentiation. Differentiated adipocytes store fatty acids in the form of TGs in the cytoplasm (22). This overall process of lipid biosynthesis, or the sequence of reactions involved in the formation of lipids, is known as lipogenesis. In contrast to lipid degradation, which occurs in the mitochondria, lipogenesis occurs in the cytoplasm. Lipolysis is defined as the metabolic breakdown of TGs into free fatty acids within cells. Free fatty acids are then broken-down for energy production via β -oxidation (22,23). Lipid homeostasis is maintained by a fine-tuning between lipogenesis and lipolysis, which are regulated by the cooperative action of various enzymes in metabolic organs, such as the liver (24, 25).

This study assessed the activity of the lipid-regulating enzymes G6PD, ME, and CPT, in the liver of rats treated orally with CE. The hepatic G6PD and ME activities tended to be lower in rats that were fed a high-fat diet compared to rats that were fed a normal diet. Decreased G6PD and ME activities may suggest that there is an adaptive reaction to excess dietary fat. Fatty acid synthesis and storage have important roles in metabolic homeostasis (25, 26). In this study, G6PD and ME activities responsible for fatty acid synthesis tended to be lower in rats treated with CE than in rats that were not treated with CE. The results of this study indicate that the body fat suppressive effects of CE are likely due to a decrease in hepatic fatty acid synthesis.

Because CPT is a rate-limiting enzyme of mitochondrial β -oxidation, we examined its activity. CE supplementation increased hepatic CPT activity, which suggests an enhancement of β -oxidation. This enhancement may contribute to the reduction of hepatic TG depots and steatosis because of the reduced flux of fatty acids that are used for TG synthesis (27, 28). This mechanism is proposed to be one of the mechanisms that are associated with the hypolipidemic effects of CE.

In conclusion, these results suggest that CE delays the development of high fat diet-induced obesity by modulating hepatic lipid metabolism. CE supplementation suppressed the accumulation of body fat by attenuating fatty acid synthesis and by enhancing β -oxidation through modulation of the activity of li-

pid-regulating enzymes. Additional investigations are warranted to determine the chemical identity of the bioactive functional constituents of CE.

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