Note

Alleviation of Weight-Gain in Mice by an Ethanolic Extract from *Rubus coreanus* under Conditions of a High-Fat Diet and Exercise

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The administration of an ethanolic extract (RCE) from *Rubus coreanus* significantly reduced the body weight and epididymal fat tissue of mice under conditions of a high-fat diet (HFD) and exercise. The mice also displayed enhanced muscular carnitine palmitoyltransferase 1 (CPT1) expression and increased superoxide dismutase and glutathione levels. These results suggest that RCE exerted an anti-obesity effect by up-regulating CPT1 and elevating the level of antioxidants.

Key words: *Rubus coreanus*; anti-obesity; weight gain; exercise; antioxidant

*Rubus coreanus* is a type of red raspberry and is one of the Rosaceae family. It grows wild in Korea, Japan, and China. The fruit of *R. coreanus*, known as ‘bokbunja’ in Korea, has been used for a long time as a traditional medicine for treating asthma, allergy, and inflammation.1 It contains various antioxidants, including anthocyanins, epicatechin, ellagic acid, and ferulic acid, as well as high levels of sugar, organic acid, and vitamins. Some studies have suggested the efficacy of the fruit as an anti-rheumatism, anti-oxidant, and anti-cancer agent.2 In our previous study, the ethanolic extract of *R. coreanus* fruit (RCE) was implicated in the relief of fatigue by reducing oxidative stress.3 However, few data have been reported in relation to the inhibitory effect of *R. coreanus* on weight gain under conditions of a high-fat diet (HFD) and exercise.

Excess caloric intake causes weight gain. Overweight leads to obesity-related maladies, including cardiovascular disorders, hyperlipidemia, and hyperglycemia, and also increases the production of reactive oxygen species (ROS) in organ tissues. Weight gain can be alleviated by exercise, although substantial production of ROS occurs during exercise. This combined formation of ROS may restrict efficient weight control due to oxidative stress.4,5 Antioxidants can protect against obesity and exercise-induced oxidative stress, which can reduce the weight gain caused by HFD.6,7 The present study investigates the inhibitory effect of an extract derived from *R. coreanus* fruit on weight gain based on its antioxidative function in regularly exercised mice fed HFD.

*R. coreanus* fruit (1 kg) harvested in Kwangyang city (Jeollanamdo, Korea) was refluxed with 20 L of 80% ethanol at 250 °C for 3 h. The extract (454 g) obtained after filtering was concentrated and lyophilized, and then stored at −20 °C until needed. Five-week-old male balb/c mice (21 ± 1.5 g in body weight (b.w.)) were supplied by Orient Bio (Seongnam, Korea) and housed in cages under automatically controlled conditions of temperature (22 ± 2 °C), humidity (about 60%), and lighting (an alternating 12-h cycle of light and dark). The mice were allowed access to HFD (45% kcal, Research Diet, Seoul, Korea) and water *ad libitum*. Chonnam National University’s Institutional Animal Care and Use Committee approved the protocol for the animal study, and the animals were cared for in accordance with the “Guidelines for Animal Experiments” established by the university.

Prior to the exercise experiment, HFD-fed mice were forced to swim in an adjustable-current water pool (90 × 45 × 45 cm with water filled to a depth of 38 cm) at a flow rate of 8 L/min8 in order to measure the swimming time to exhaustion. The mice were determined to be exhausted when they failed to rise to the surface to breathe within a 7-s period. A period of longer than 7 s frequently resulted in drowning, while a period of less than 5 s reduced the reproducibility of the test.9 The mice were then assigned to two groups (n = 9 per group) with similar mean swimming capacity: the exercised control group (saline) and RCE-administered group (1 g/kg b.w./d). Each group orally received a vehicle or RCE daily via gavage 30 min before swimming without any load for 14 d. The exercise test was conducted by applying 80% of exhaustive swimming time. The body weight and food intake were measured.
daily. After the end of the experiment, the mice were sacrificed under anesthesia to collect serum, tissue, and muscle samples. The samples were stored at \(-70\, ^\circ\text{C}\).

The administration of RCE significantly alleviated weight gain in the HFD-induced obese mice under regular exercise in comparison with the exercised control group. Feeding HFD for 14 d produced an increase in body weight of the exercised mice that did not receive RCE. However, exercise combined with RCE treatment clearly retarded the weight gain (Fig. 1). These results suggest that the treatment with RCE improved the energy expenditure during exercise. We have previously demonstrated that RCE possessed a fatigue-alle viating effect, as judged by enhanced forced swimming capacity.\(^3\)

The weight of fat tissue is an important factor relevant to obesity. Table 1 shows that the HFD-fed mice combined with exercise and the RCE treatment exhibited a 21% decrease in epididymal fat tissue in comparison with the exercised control group. The level of serum triglycerides (TG) is another index of obesity, the level of serum TG being elevated in obese individuals.\(^{10}\) Compared to the mice fed HFD with modest everyday exercise (187.2 \pm 36.5 \text{mg/dL} of serum TG), a relatively lower serum TG level (150.1 \pm 27.8 \text{mg/dL}) was found in the mice with the same conditions as the exercised control group, but differing in the supplementation with RCE (Table 1). These observations are in accordance with those in a previous study.\(^{11}\) The reduction in both the epididymal fat tissue weight and serum triglyceride level confirmed that the administration of RCE alleviated the weight gain from HFD-induced obesity. The weight loss action of plant constituents is generally related to such phenolic compounds as flavonoids and phenolic acids. Even though the bioactive compounds of RCE responsible for the reduced body weight have not been found, the phytochemicals in \textit{Rubus coreanus} including ellagic acid, epicatechin, quercetin, and ferulic acid can be speculated as candidates for anti-obese compounds.\(^{12-15}\)

Exercise is useful for burning body fat, because fat is typically used as the primary energy source during exercise.\(^{16}\) However, the utilization of fat by \(\beta\)-oxidation results in substantial ROS generation that in turn may decrease the efficiency of fat utilization.\(^{17}\) An excess caloric intake increases the incidence of obesity. The obese demonstrate a higher level of oxygen consumption than the non-obese, eventually resulting in elevated oxidative stress due to the increased formation of superoxide anion radicals.\(^{18}\) The antioxidative effects of RCE are shown in Table 1. After sacrificing the mice, the muscular tissue was collected and homogenated in a 50 \text{mm} phosphate buffer. The suspension was centrifuged at 13,000 \times g for 15 min at 4 \text{C}, and the supernatant was used for subsequent measurements. The activity of superoxide dismutase (SOD) was determined as described by McCord and Fridovich.\(^{19}\) The level of reduced glutathione (GSH) was assayed by the method of Akerboom and Sies.\(^{20}\) The activity of SOD, an antioxidant enzyme, and the level of GSH, a key intracellular non-enzymatic antioxidant, in the muscles of the RCE-administered mice were significantly enhanced in comparison with the exercised control group. The elevation of the intracellular antioxidative defense system by RCE has been reported in a mouse model,\(^3\) the present results being consistent with the previously reported data. Excess ROS generation by oxidative stress may inactivate an enzymatic antioxidant like SOD. The present treatment with RCE significantly protected the mice from the inactivation of enzymatic antioxidants. GSH is an important non-enzymatic antioxidant in the body and is involved in various enzymatic processes that reduce ROS.\(^{21}\) The present results indicate that RCE could be helpful in protecting GSH from oxidative stress. The administration of RCE, which possesses antioxidative potential, could stabilize the intracellular antioxidative defense system in HFD-fed mice, leading to the inhibition of weight gain by more efficient burning of fat. Carnitine palmitoyltransferase 1 (CPT1), which is present in the outer membrane of mitochondria, plays an important role in the rate-limiting step for \(\beta\)-oxidation. The fatty acid uptake by mitochondria is controlled by CPT1, which converts fatty acids to acylated fatty acids.

### Table 1. Changes in Fat Tissue Weights, Serum Triglyceride Levels, and Muscular Antioxidative Activities Due to the Administration of the Ethanolic Extract from \textit{Rubus coreanus} to High-Fat Diet Fed Mice in Combination with Exercise\(^1\)

<table>
<thead>
<tr>
<th>Group(^2)</th>
<th>Epididymal fat (g/100 g b.w.)</th>
<th>Perirenal fat (g/100 g b.w.)</th>
<th>TG(^3) (mg/dL)</th>
<th>SOD(^4) (mU/mg of protein)</th>
<th>GSH(^5) (mmole/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exercised control</td>
<td>2.79 \pm 0.23(^a)</td>
<td>0.83 \pm 0.75</td>
<td>187.2 \pm 36.5(^a)</td>
<td>181.0 \pm 15.3(^a)</td>
<td>5.06 \pm 1.93(^a)</td>
</tr>
<tr>
<td>RCE-administered(^6)</td>
<td>2.19 \pm 0.23(^b)</td>
<td>0.75 \pm 0.25</td>
<td>150.1 \pm 27.8(^b)</td>
<td>219.6 \pm 10.4(^b)</td>
<td>9.65 \pm 2.10(^b)</td>
</tr>
</tbody>
</table>

\(^1\)Data are expressed by the mean \pm SE for 9 mice.

\(^2\)The mice were fed the high-fat diet and given the vehicle (control) or RCE (1 g/kg of body weight) before exercise.

\(^3\)TG, triglycerides; SOD, superoxide dismutase; GSH, reduced glutathione.

\(^4\)RCE, 80% ethanol extract of \textit{R. coreanus}.

\(^5\)Values with different letters in a column are significantly different by Student’s \(t\)-test \((p < 0.05)\).
before their transport into mitochondria. Figure 2 shows an increased expression of muscular CPT1 in HFD-fed mice combined with exercise and the RCE treatment when compared with HFD-fed mice subjected only to exercise. The elevated level of CPT1 is an indication of increased acylated fatty acids in mitochondria, which is essential for \( \beta \)-oxidation. The present findings therefore suggest that RCE contributed to the alleviation of weight gain by promoting \( \beta \)-oxidation.

In summary, an excess caloric intake leads to weight gain, which can be ameliorated by exercise. The main fuel during exercise is \( \beta \)-oxidation, which produces ROS. Oxidative stress can render fat oxidation inefficient. The administration of RCE to HFD-fed mice combined with exercise accelerated the energy expenditure and protected against oxidative stress. This result suggests that the alleviation of weight gain by RCE might have been due, in part, to its protective effect against oxidative stress. To the best of our knowledge, the present study is the first to investigate the inhibitory effects of \( R. coreanus \) on weight gain in combination with exercise. The results indicate that obesity can be efficiently avoided by an agent with the dual action of promoting \( \beta \)-oxidation and antioxidative activity. Further research is currently underway to isolate and identify the structure of the compound in \( R. coreanus \) responsible for the weight-loss activity and to further elucidate the mechanism for its weight-loss action.

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References