Effect of Vaccinium ashei reade Leaves on Lipid Metabolism in Otsuka Long-Evans Tokushima Fatty Rats

Koji NAGAO, Kouki HIGA, Bungo SHIROUCHI, Saori NOMURA, Nao INOUE, Masashi INAFUKU, and Teruyoshi YANAGITA

Department of Applied Biochemistry and Food Science, Saga University, 1 Honjo, Saga 840-8502, Japan

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The effects of blueberry leaf (BBL) on lipid metabolism were studied in obese rats. Feeding of BBL lowered levels of serum lipids and C-reactive protein and alleviated hepatic triglyceride accumulation in the rats. The hypolipidemic effect might be attributable to a reduction of lipogenesis and enhancement of lipolysis in the liver. These results suggest the use of blueberry leaf as a dietary hypolipidemic component.

Key words: Vaccinium ashei reade; blueberry leaf; lipid metabolism; Otsuka Long-Evans Tokushima Fatty rat

Lifestyle-related diseases, such as hyperlipidemia, arteriosclerosis, diabetes mellitus, and hypertension, are widespread and increasingly prevalent diseases in industrialized countries, and they contribute to increases in cardiovascular morbidity and mortality. Accompanied by rapid increases in the numbers of elderly people, this is important not only medically but also socioeconomically. Although the pathogenesis of lifestyle-related diseases is complicated and the precise mechanisms have not yet been elucidated, obesity has emerged as a major cardiovascular risk factor according to epidemiologic studies. Obesity is defined as an increased mass of adipose tissue, and its prevalence and severity are markedly increasing in westernized countries. Otsuka Long-Evans Tokushima Fatty (OLETF) rats develop a syndrome with multiple metabolic and hormonal disorders that shares many features with human obesity. OLETF rats have hyperphagia because they lack receptors for cholecystokinin, and become obese, developing hyperlipidemia, fatty liver, and type-2 diabetes. In this study, we examined effects of blueberry leaf (BBL) on lipid metabolism in obese OLETF rats.

Vaccinium ashei reade (blueberry) belongs to the Ericaceae plant group, and infusions of its leaf are used as a folk medicine treatment for lifestyle-related diseases in Europe. Martineau et al. demonstrated anti-diabetic properties of the Canadian lowbush blueberry in vitro. Recently, we reported that blueberry leaf revealed strong inhibitory effects on angiotensin-converting enzyme activity in vitro, and that feeding of blueberry leaf suppressed the development of essential hypertension in spontaneously hypertensive rats in vivo. The effects of blueberry leaf on lipid metabolism, however, have not been fully evaluated. To determine the physiological function of BBL, we evaluated the effect of BBL feeding on hepatic enzyme activities in relation to lipid metabolism in obese OLETF rats.

Four-week-old male OLETF rats were provided by the Tokusima Research Institute (Otsuka Pharmaceutical, Tokushima, Japan). The rats were housed individually in metal cages in a temperature-controlled room (24°C) under a 12-h light/dark cycle. After a 1-week adaptation period on a powder chow diet (CE-2, Clea Japan, Tokyo), they were assigned to three groups (six rats each), each fed one of three diets: (i) a semi-synthetic diet containing (in weight %) casein, 20; corn oil, 7; cornstarch, 15; vitamin mixture (AIN-76/C212), 1; mineral mixture (AIN-76/C212), 3.5; DL-methionine, 0.3; choline bitartrate, 0.2; cellulose, 5; and sucrose, 45 (control group); (ii) a semi-synthetic diet supplemented with 1% freeze-dried blueberry leaf at the expense of sucrose (low BBL group, LB); or (iii) a semi-synthetic diet supplemented with 5% freeze-dried blueberry leaf at the expense of sucrose (high BBL group, HB). Freeze-dried blueberry leaf was provided by Unkai Shuzo Co., Ltd. (Miyazaki, Japan). It contained (in weight %) carbohydrate, 62.9; protein, 6.2; fat, 5.3; ash, 3.1; moisture, 3.4; and tannin, 18.7. The tannin content was determined according to Folin-Denis method, and tannic acid was used as the standard. The animals received the diets for 4 weeks. All the rats were killed by aortic exsanguination under diethyl ether anesthesia. Perirenal, epididymal, omental, and waist subcutaneous
Table 1. Effect of Blueberry Leaf on Levels of Serum Parameters and Hepatic Lipids in OLETF Rats

<table>
<thead>
<tr>
<th>Serum parametersa,b</th>
<th>Control</th>
<th>LB</th>
<th>HB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>149 ± 12</td>
<td>143 ± 17</td>
<td>107 ± 15</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>151 ± 6a</td>
<td>139 ± 6a</td>
<td>117 ± 2b</td>
</tr>
<tr>
<td>Phospholipids (mg/dl)</td>
<td>216 ± 6b</td>
<td>195 ± 6b</td>
<td>181 ± 7b</td>
</tr>
<tr>
<td>NEFA (mEq/l)</td>
<td>0.670 ± 0.043</td>
<td>0.703 ± 0.032</td>
<td>0.632 ± 0.024</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>168 ± 5</td>
<td>165 ± 6</td>
<td>162 ± 5</td>
</tr>
<tr>
<td>Insulin (ng/ml)</td>
<td>2.80 ± 0.40</td>
<td>3.35 ± 0.85</td>
<td>4.13 ± 0.40</td>
</tr>
<tr>
<td>Adiponectin (µg/ml)</td>
<td>7.16 ± 0.49</td>
<td>7.35 ± 0.52</td>
<td>7.43 ± 0.49</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>1.90 ± 0.20</td>
<td>2.03 ± 0.37</td>
<td>1.91 ± 0.21</td>
</tr>
<tr>
<td>C-reactive protein (µg/ml)</td>
<td>922 ± 19a</td>
<td>833 ± 29b</td>
<td>808 ± 19b</td>
</tr>
<tr>
<td>Hepatic lipids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglyceride (mg/g Liver)</td>
<td>24.8 ± 1.3a</td>
<td>19.5 ± 1.3b</td>
<td>13.4 ± 1.5c</td>
</tr>
<tr>
<td>Cholesterol (mg/g Liver)</td>
<td>3.41 ± 0.18</td>
<td>3.20 ± 0.14</td>
<td>2.89 ± 0.18</td>
</tr>
<tr>
<td>Phospholipids (mg/g Liver)</td>
<td>40.8 ± 1.3</td>
<td>39.9 ± 1.1</td>
<td>38.8 ± 1.2</td>
</tr>
</tbody>
</table>

Rats were fed a control, 1% BBL (LB), or 5% BBL (HB) diet for 4 weeks. Values are given as means ± S.E. (n = 6). Data were analyzed by one-way analysis of variance, and all differences were inspected by Tukey-Kramer (Super ANOVA, Abacus Concepts, Berkeley, CA). Different superscript letters show significant differences at P < 0.05.

aSerum levels of triglyceride, cholesterol, phospholipids, NEFA, and glucose were measured using commercial enzyme assay kits (Wako Pure Chemicals, Tokyo).
bSerum levels of insulin, adiponectin, leptin, and C-reactive protein were measured using commercial rat enzyme-linked immunosorbent assay kits (Rat Insulin ELISA kit, Shibayagi, Gunma, Japan; Otsuka Rat Adiponectin ELISA kit, Otsuka Pharmaceutical, Tokyo; Rat Leptin ELISA kit, Yanaihara Institute, Fujinomiya, Japan; and Rat C-Reactive Protein ELISA kit from Alpha Diagnostic International, San Antonio, TX, respectively).

white adipose tissues (WATs) and the liver were also excised for analysis. All aspects of the experiment were conducted according to the guidelines provided by the ethical committee on experimental animal care of Saga University.

After the 4-week feeding period, the amount of food intake (control, 617 ± 12; LB, 616 ± 24; HB, 619 ± 2 g), final body weight (control, 324 ± 6; LB, 321 ± 12; HB, 318 ± 3 g) and total WAT weights (control, 9.28 ± 0.19; LB, 9.53 ± 0.17; HB, 8.79 ± 0.35 g/100 g body weight) were not significantly altered by dietary BBL in OLETF rats. The effects of dietary BBL on serum parameters are shown in Table 1. After the 4-week feeding period, 9-week-old OLETF rats showed mild hyperlipidemia. The serum triacylglycerol level was 28% lower, but not significantly, in the HB rats than in the rats fed the control diet. Serum cholesterol and phospholipids levels were lowered dose-dependently by BBL feeding in the OLETF rats. Serum glucose, non-esterified fatty acids (NEFA), insulin, adiponectin, and leptin levels were not significantly altered by dietary BBL.

Atherosclerosis and metabolic syndrome are considered to be multifactorial diseases driven by inflammatory reactions. C-reactive protein (CRP) is an inflammatory cytokine and its serum concentration reflects the inflammatory condition of animals and humans. In the present study, levels of CRP in serum were significantly lowered by BBL feeding in OLETF rats, and the results suggest that BBL has anti-inflammatory properties. Given that obesity enhances CRP synthesis and elevated CRP levels have been suggested as risk factors of cardiovascular diseases and type-2 diabetes in animals and humans, we assumed that the decrease in serum CRP levels in BBL-fed OLETF rats contributed to suppression of the development of obesity-induced metabolic abnormalities in OLETF rats.

Next we investigated the effect of dietary BBL on the distribution of lipids to the liver. Liver lipids were extracted and purified by the method of Folch et al., and concentrations of triacylglycerol, cholesterol, and phospholipids were measured as previously described. As shown in Table 1, hepatic triacylglycerol accumulation was markedly alleviated by BBL dose-dependently. Hepatic cholesterol and phospholipids concentrations in the liver tended to decrease, but not significantly, by the feeding of BBL diets (Table 1). These results suggest that the BBL diet can prevent the development of obesity-related diseases in obese OLETF rats.

To further investigate the regulation of hepatic lipid metabolism, we analyzed the effect of dietary BBL on the activities of enzymes related to fatty acid synthesis and fatty acid beta-oxidation. Measurements of the activities of fatty acid synthase (FAS), glucose 6-phosphate dehydrogenase (G6PDH), malic enzyme, peroxisomal beta-oxidation, and carnitine palmitoyltransferase (CPT) were carried out as described previously. As shown in Fig. 1, the activity of FAS, a key enzyme in fatty acid synthesis, was significantly suppressed by BBL diets dose-dependently in the OLETF rats. The activities of G6PDH and malic enzyme, which provide the NADPH required for fatty acid synthesis, were not significantly altered by dietary BBL (data not shown). Although the activity of peroxisomal beta-oxidation was not different among the groups (data not shown), the BBL diets significantly enhanced the activity of CPT, a key enzyme in mitochondrial fatty acid beta-oxidation, as compared with the control diet in OLETF rats (Fig. 1). These results suggest that the hypolipidemic effect of BBL can be attributed to a reduction of FAS activity and...
enhancement of CPT activity in the liver. Although the active components are yet to be identified, the BBL used in this study contained 18.7% tannin. Li et al. reported that isoflavones, flavones, bilavonoids, hydrolysable tannin-related derivatives, and triterpenoids from plants showed FAS inhibitory activity in vitro.27 We have reported that feeding of tea catechins, rich in (→)-epigallocatechin gallate and (→)-epicatechin gallate, resulted in visceral fat deposition through a reduction of hepatic FAS activity in Sprague-Dawley rats.28 In addition, Murase et al. reported that green tea extract, which contains high levels of polyphenolic compounds, increased fatty acid beta-oxidation in the liver and muscles of mice.29,30 Li et al. also showed that green tea leaf extract enhanced hepatic expression of lipolytic transcriptional factor PPAR-alpha and improved lipid homeostasis in a fructose-fed insulin-resistant hamster model.31 In addition, previous reports have indicated that BBL contains a variety of phenolic compounds, such as chlorogenic acid, quercetin, caffeic acid, and procyanidins, and has oxygen radical absorbance capacity and antileukaemic activity.32–34 Therefore, it is likely that tannin, contained in BBL, partly contributes to preventing the development of obesity-induced diseases in OLETF rats. Further investigation is necessary to characterize the active components from BBL.

In conclusion, our results indicate that blueberry leaf reveals a hypolipidemic effect in obese OLETF rats and suggests that blueberry leaf can be useful as a dietary hypolipidemic component. Although we evaluated the physiological function of BBL during the onset of obesity-induced lipid-abnormalities in young OLETF rats in this study, evaluating the anti-diabetic properties of BBL using adult OLETF rats is of great interest for the future.

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References


