Dietary Hesperidin Exerts Hypoglycemic and Hypolipidemic Effects in Streptozotocin-Induced Marginal Type 1 Diabetic Rats

Satoko Akiyama¹, Shin-ichi Katsumata¹, Kazuharu Suzuki¹, Yoshiko Ishimi², Jian Wu²,³ and Mariko Uehara¹,*

¹Department of Nutritional Science, Faculty of Applied Bioscience, Tokyo University of Agriculture, Tokyo 156-8502, Japan
²Food Function and Labeling Program, National Institute of Health and Nutrition, Tokyo 162-8636, Japan
³Division of Healthcare Science, Central Research Laboratory, The Nisshin OilliO Group Ltd, Kanagawa 239-0832, Japan

Received 20 August, 2009; Accepted 10 September, 2009

Summary  Citrus bioflavonoids may offer some protection against the early stage of diabetes mellitus and the development of complications. We investigated the effect of hesperidin on blood glucose levels, hepatic glucose-regulating enzyme activities, serum insulin and adiponectin levels, serum and hepatic lipid levels, and parameters of bone loss in streptozotocin (STZ)-induced marginal type 1 diabetic rats. Weanling male rats were randomly assigned to experimental 3 groups: a control (C) group, a STZ induced marginal type 1 diabetes (S) group, and a diabetes and hesperidin group, and fed their respective diets for 4 weeks. STZ injection increased blood glucose in rats, but the increase was marginal. Serum and hepatic lipids, serum adiponectin and insulin levels were significantly changed by STZ injection. Dietary hesperidin (10 g/kg diet) decreased blood glucose by altering the activity of glucose-regulating enzymes, and normalized the lipids and adiponectin levels, but did not change bone parameters in the marginal type 1 diabetic rats. Hesperidin showed both hypoglycemic and hypolipidemic effects but did not affect bone tissue and bone metabolic makers in STZ-injected marginal diabetic weanling rats without any body weight loss due to STZ injection.

Key Words: hesperidin, streptozotocin, type 1 diabetes, glucose regulating enzymes, lipids

Introduction

Diabetes is the most common serious metabolic disorder, and it is considered to be 1 of the 5 leading causes of death in the world [1]. Hyperglycemia can be initially treated with oral agents and insulin therapy; the latter is occasionally required to achieve target glycemic levels. Moreover, diabetes is associated with increased risk of thrombotic, atherosclerotic and cardiovascular disease [2]. Dyslipidemia is a frequent complication of diabetes and is characterized by low levels of high-density lipoprotein (HDL)-cholesterol, and high levels of low-density lipoprotein (LDL)-cholesterol and triglycerides (TGs). Hyperlipidemia is also a metabolic complication of both clinical and experimental diabetes. In addition, it is well known that diabetes can affect bone remodeling by various mechanisms, including deficient insulin action, increased accumulation of advanced glycation end-products and microangiopathy, although the clinical manifestations of diabetic osteopathy are not fully understood. Type 1 diabetic patients are at high risk for bone loss and bone fracture. One of the mechanisms of diabetes-related bone diseases may be oxidative damage to osteoblasts. Flavonoids are present in several types of vegetables and fruits, and may be associated with potential health benefits [3–4]. Hesperidin, a citrus bioflavonoid, exhibits

*To whom correspondence should be addressed.
Tel: 81-3-5477-2444  Fax: 03-5477-2658
E-mail: mari@nodai.ac.jp
biological and pharmacological properties such as anti-inflammatory, anti-carcinogenic, antioxidative, and lipid-lowering activities [5–7]. Furthermore, we demonstrated that hesperidin not only has cholesterol-lowering effects but also prevents bone loss in ovariectomized (OVX) mice, without exerting any substantial effects on the uterus [7]. Recently, Choi and Kim reported that hesperetin, the aglycone of hesperidin, can act as a biological antioxidant in a cell culture system representative of a diabetic state and protect osteoblasts from oxidative stress-induced toxicity, which may promote bone recovery in diabetic bone diseases [8]. However, the in vivo effects of hesperidin and hesperetin on type 1 diabetic animals remain unknown. In the present study, we investigated the hypoglycemic and hypolipidemic effects of hesperidin, and the effect of hesperidin on bone status in streptozotocin (STZ)-induced type 1 diabetic rats. However, since the body weights of STZ-induced type 1 diabetic rats are decreased [9, 10], the hypolipidemic effects of phytochemicals may not indicate in such a severe type 1 diabetic model. We used marginal type 1 diabetic rats, which the body weight was similar with the control rats in this study.

Materials and Methods

Experimental Design and Sample Treatments

Eighteen 3-week-old male Wistar rats (Clea Japan, Tokyo, Japan) were individually housed in stainless-steel rat cages at 22°C and maintained under a 12-h light/12-h dark cycle. The Animal Use Committee of the Tokyo University of Agriculture approved the study, and the animals were maintained in accordance with the guidelines of the university for the care and use of laboratory animals. For a 3-day acclimatization period, all rats were sacrificed, and blood and liver samples were collected. Measurements of bone mineral contents and bone mineral density were measured with commercial kits (Rat Insulin ELISA Kit and Mouse/Rat High Molecular Adiponectin ELISA Kit, Shibayagi, Gunma, Japan); cholesterol C test, triglyceride E test and HDL cholesterol test (Wako Pure Chemicals); Osteocalcin rat ELISA system (Amersham Pharmacia Biotech, Buckinghamshire, UK); Metra DPD (Quidel Co., San Diego, CA); RatLaps ELISA (Norbic Bioscience Diagnostics A/S, Herlev, Denmark); and Creatinine-test Wako (Wako Pure Chemicals, Osaka, Japan).

Measurement of serum hesperetin concentration

Serum hesperetin concentration was measured using high-performance liquid chromatography (HPLC) with electrochemical detection (Chirochrome 2; ESA, Chelmsford, MA) and UV detection (SPD-10A; Shimazu, Tokyo, Japan), as described, previously [7].

Measurements of hepatic enzymes

Glucokinase (GK) and Glucose-6-phosphatase (G6Pase) activities in liver microsomes was determined as described by Davidson and Arion [12], and according to the method of Bickerstaff and Burchell [13], respectively.

Measurements of bone mineral contents and bone mineral density

Bone samples from each rat were used for analysis of bone mineral content (BMC) and bone mineral density (BMD). Moreover, Horcajada et al. suggested that the effective dose of hesperetin on bone and lipids in rats was 0.5% in the diet [11]. This dosage of hesperetin can be calculated around 1% as hesperidin. Thus, the dosage of 1% (10 g/kg diet) hesperidin in the diet was also employed for this study. All rats were given free access to distilled water. After 4 weeks of feeding, all rats were sacrificed, and blood and liver samples were collected for analysis. The blood samples were centrifuged, and the supernatants were used as serum samples. Urine was collected during the 24 h prior to dissection. Serum and urine samples were stored at –80°C until analysis. The liver was perfused with cold 0.9% NaCl solution and then removed. The femur and tibia were removed, cleansed of all soft tissues and stored in 70% ethanol solution at 4°C until analysis. The lumbar vertebrae were removed, cleansed of all soft tissues and frozen at –80°C until analysis.

Measurements of blood glucose, serum insulin and adiponectin, serum and hepatic lipids, and serum and urinary bone metabolic markers

Blood glucose concentration was measured during the acclimatization period, at 3 days after the ip injections, and 1 day prior to dissection. Samples were collected from the tail vein after the animals had been fasted for 12 h, and glucose concentrations were measured with a blood glucose meter (Medisafe-mini GR-102; Terumo Co., Tokyo, Japan). Serum insulin, serum adiponectin, serum and hepatic lipids, serum and urinary bone metabolic markers and urinary creatinine levels were measured with commercial kits (Rat Insulin ELISA Kit and Mouse/Rat High Molecular Adiponectin ELISA Kit, Shibayagi, Gunma, Japan); cholesterol C test, triglyceride E test and HDL cholesterol test (Wako Pure Chemicals); Osteocalcin rat ELISA system (Amersham Pharmacia Biotech, Buckinghamshire, UK); Metra DPD (Quidel Co., San Diego, CA); RatLaps ELISA (Norbic Bioscience Diagnostics A/S, Herlev, Denmark); and Creatinine-test Wako (Wako Pure Chemicals, Osaka, Japan).
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(BMD) by dual-energy X-ray absorptiometry (DXA; model DCS- 600R; Aloka, Tokyo, Japan). BMD was calculated on the basis of the BMC of the measured area.

Statistics

The results were expressed as the mean ± SEM for each group of 6 rats. After one-way analysis of variance (ANOVA), Fisher’s protected least significant difference (PLSD) test was used to determine significant differences. Differences were considered significant at \( p < 0.05 \).

Results

Body weight and food intake

The initial and final body weights (54 g and 242–247 g, respectively), and mean food intake during the feeding period (16 g/day) did not differ among the 3 groups. Average of hesperidin intake was 160 mg/day.

Blood glucose and serum insulin concentrations

Three days after the ip injections, blood glucose was significantly higher in the S and SH groups than in the C group, although the increase of blood glucose by STZ injection was marginal. However, the final blood glucose concentration was significantly decreased in the SH group compared with that in the S group (Table 1). The serum insulin concentration was the highest in the SH group (Table 1).

Serum hesperetin concentration

Serum hesperetin detected in the SH (12.3 ± 3.3 μmol/l), but not in other two groups.

Hepatic GK and G6Pase activities and serum adiponectin

The activity of G6Pase was increased and that of GK was decreased in STZ-injected rats. In the STZ rats that were fed the hesperidin diet, the activities of both enzymes had normalized (Table 1). Serum Adiponectin concentration was decreased by STZ-injection, but normalized by hesperidin administration (Table 1).

Serum and hepatic lipids

The serum TG, total cholesterol (TC) and LDL + VLDL cholesterol concentrations were significantly increased by STZ injection, but hesperidin suppressed the increase in these levels (Table 2). Serum HDL-cholesterol concentration and HDL/TC ratio were lower in the S group than in the C and SH groups. The hepatic TG and TC concentrations

Table 1. Blood glucose, serum insulin and adiponectin levels, and activities of hepatic glucose regulating enzymes

<table>
<thead>
<tr>
<th>Group</th>
<th>Blood glucose (mg/dl)</th>
<th>Insulin (ng/ml)</th>
<th>G6Pase (μmol/min/mg protein)</th>
<th>GK (μmol/min/mg protein)</th>
<th>Adiponectin (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acclimatization period</td>
<td>96.66 ± 8.57</td>
<td>1.77 ± 0.58b</td>
<td>518.09 ± 55.45b</td>
<td>351.93 ± 71.09b</td>
</tr>
<tr>
<td></td>
<td>3 days after the ip injections</td>
<td>91.33 ± 6.38b</td>
<td>89.33 ± 5.11b</td>
<td>774.88 ± 72.28b</td>
<td>188.34 ± 23.98b</td>
</tr>
<tr>
<td></td>
<td>1 day prior to dissection</td>
<td>89.33 ± 5.11b</td>
<td>131.67 ± 5.02a</td>
<td>191.35 ± 22.93b</td>
<td>327.69 ± 42.70b</td>
</tr>
<tr>
<td>C</td>
<td>98.00 ± 7.29</td>
<td>0.91 ± 0.47b</td>
<td>774.88 ± 72.28b</td>
<td>191.35 ± 22.93b</td>
<td>327.69 ± 42.70b</td>
</tr>
<tr>
<td>S</td>
<td>120.50 ± 7.97a</td>
<td>4.03 ± 1.22a</td>
<td>288.86 ± 68.02c</td>
<td>370.82 ± 30.06a</td>
<td></td>
</tr>
<tr>
<td>SH</td>
<td>121.50 ± 5.89a</td>
<td>94.36 ± 4.77b</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

G6Pase, Glucose-6-phosphatase; GK, Glucokinase. Values are means ± SEM, \( n = 6 \). C, Control group; S, STZ injected group; SH, STZ + hesperidin group. Means without a common letter differ, \( p < 0.05 \).

Table 2. Serum and hepatic lipids

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum TG (mg/dl)</th>
<th>Serum TC (mg/dl)</th>
<th>Serum HDL-C (mg/dl)</th>
<th>Serum LDL + VLDL-C (mg/dl)</th>
<th>HDL-C/TC</th>
<th>Liver TG (mg/g)</th>
<th>Liver TC (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>99.53 ± 6.55b</td>
<td>65.76 ± 2.60b</td>
<td>46.57 ± 1.20a</td>
<td>19.19 ± 2.41b</td>
<td>0.71 ± 0.02a</td>
<td>24.28 ± 1.24b</td>
<td>2.56 ± 0.06b</td>
</tr>
<tr>
<td>S</td>
<td>136.41 ± 9.42e</td>
<td>76.05 ± 4.29e</td>
<td>36.08 ± 2.43b</td>
<td>39.97 ± 4.89e</td>
<td>0.48 ± 0.04b</td>
<td>27.38 ± 2.25e</td>
<td>3.12 ± 0.10e</td>
</tr>
<tr>
<td>SH</td>
<td>110.89 ± 6.02b</td>
<td>65.59 ± 4.25b</td>
<td>47.97 ± 1.95a</td>
<td>17.62 ± 3.01b</td>
<td>0.73 ± 0.03a</td>
<td>23.40 ± 2.25b</td>
<td>2.04 ± 0.10b</td>
</tr>
</tbody>
</table>

HDL-C/TC

Liver TG (mg/g)

Liver TC (mg/g)

Values are means ± SEM, \( n = 6 \). C, Control group; S, STZ injected group; SH, STZ + hesperidin group. Means without a common letter differ, \( p < 0.05 \).

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were also significantly increased in the S group, but in the SH group, hesperidin suppressed this increase (Table 2).

**BMC and BMD in femur, tibia and limbar, and bone metabolic markers**

The femoral BMD and BMC, and the levels of deoxypyridinoline (DPD) and C-telopeptide of type 1 collagen (CTX), which are markers of bone resorption, were not altered by STZ injection. Only osteocalcin, a marker of bone formation, was decreased in the STZ-injected groups, but there was no significant difference in the osteocalcin levels between the S and SH group rats (Table 3).

**Discussion**

Although the dose of STZ to the rats was high (70 mg/kg BW), blood glucose level was not particularly increased in this study. It seems that STZ-injection was failed. Therefore, this dose of STZ was slightly but significantly increased blood glucose, and changed enzymes related with glucose metabolism without any change in body weight and food intake. In other words, in marginal status of diabetes, the rats were fed the hesperidin diet in this study. Regarding normal rats, there were no effects of hesperidin administration on serum and hepatic lipids in weanling Sprague-Dawley male rats, and on serum glucose in aging Wistar male rats in our pilot studies (data is not shown), while plasma total cholesterol and triglyceride concentrations showed lower values in hesperidin-treating sham operated (SH) female Wistar rats than in the SH control rats (6 and 9 month-old ) from the study of Horcajada et al. [11]. Many studies have shown that some flavonoids and other phytochemicals decrease blood glucose levels and oxidative stress, and increase serum insulin concentrations [3–5, 14–16]. One of the reasons flavonoids ameliorate diabetes might be alterations in the activity of key enzymes involved in glucose metabolism. It is reported that hesperidin and naringin increase the mRNA level of GK, a key enzyme of glucose catabolism, and decrease the level of G6Pase, a gluconeogenic enzyme, in type 2 diabetic mouse liver [17]. In this study, the activities of both GK and G6Pase were also changed in STZ-injected rats. In contrast, in the STZ rats that were fed the hesperidin diet, the activities of both enzymes had normalized, and the insulin levels had increased; this has been previously reported in type 2 diabetic mice, but not in type 1 diabetic rodents. Adiponectin, a metabolically active protein secreted by adipocytes, plays an important role in the regulation of glucose and lipid metabolism, and modifies insulin sensitivity and energy balance [18, 19]. In this study, the serum insulin level was increased, and the blood glucose level was decreased on hesperidin administration to the STZ-injected rats. Hesperidin might inhibit the damage of pancreatic islet by the antioxidative efficacy, and the treatment with hesperidin might increase insulin and consequently decrease glucose levels and alter glycogen contents in the diabetic tissues due to improving glycolytic and gluconeogenic enzymes like as rutin [16]. However, hesperidin administration showed beyond the control level of insulin. We analyzed serum hesperetin value and the mean concentration of the SH group was 12.3 μmol/L, while it was not detectable in the control and S groups. It was higher in the SH rats than that (3.57 μmol/L) in rats from the study of Horcajada et al. [11], although the dosage of hesperetin (as aglycone) was similar in the both studies, while strain, age, sex and feeding term were different. Further, the serum adiponectin concentration was significantly decreased in the STZ-injected rats, but the value was normalized by hesperidin administration. Liu et al. reported that naringenin and hesperetin up-regulated the transcription of adiponectin and induced the expression of peroxisome proliferator-activated receptor (PPAR) γ-controlled luciferase in vitro but not in vivo [20]. We observed the in vivo hypoglycemic and hypolipidemic effects of hesperidin, which are mediated by increasing

<table>
<thead>
<tr>
<th>Group</th>
<th>C</th>
<th>S</th>
<th>SH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Femur BMC (mg)</td>
<td>184.18 ± 3.26</td>
<td>172.16 ± 6.77</td>
<td>176.40 ± 4.57</td>
</tr>
<tr>
<td>BMD (mg/cm²)</td>
<td>100.53 ± 1.19</td>
<td>99.75 ± 2.87</td>
<td>101.01 ± 1.27</td>
</tr>
<tr>
<td>Tibia BMC (mg)</td>
<td>139.13 ± 2.74</td>
<td>136.83 ± 4.43</td>
<td>135.53 ± 2.80</td>
</tr>
<tr>
<td>BMD (mg/cm²)</td>
<td>83.00 ± 0.85</td>
<td>83.94 ± 1.53</td>
<td>82.38 ± 0.81</td>
</tr>
<tr>
<td>Lumbar BMC (mg)</td>
<td>16.56 ± 0.54</td>
<td>16.63 ± 1.29</td>
<td>16.33 ± 0.93</td>
</tr>
<tr>
<td>BMD (mg/cm²)</td>
<td>48.36 ± 0.96</td>
<td>46.23 ± 2.09</td>
<td>46.71 ± 1.35</td>
</tr>
<tr>
<td>Serum Osteocalcin (ng/ml)</td>
<td>63.72 ± 5.94</td>
<td>38.05 ± 4.00</td>
<td>41.78 ± 3.79</td>
</tr>
<tr>
<td>Urinary CTx (μg/mmol creatinine)</td>
<td>24.09 ± 3.31</td>
<td>24.94 ± 2.16</td>
<td>22.75 ± 3.14</td>
</tr>
<tr>
<td>Urinary DPD (nmol/mmol creatinine)</td>
<td>727.86 ± 82.64</td>
<td>629.17 ± 106.21</td>
<td>583.96 ± 63.02</td>
</tr>
</tbody>
</table>

Values are means ± SEM, n = 6. C, Control group; S, STZ injected group; SH, STZ + hesperidin group. Means without a common letter differ, p<0.05.
In conclusion, we demonstrated that hesperidin normalizes blood glucose by altering the activity of glucose-regulating enzymes, and lowering serum and liver lipid levels in STZ-induced marginal type 1 diabetic rats without any body weight loss due to STZ injection. Thus, hesperidin showed both hypoglycemic and hypolipidemic effects but did not affect bone status in marginal type 1 diabetic weanling rats.

Acknowledgments

We thank Toyo Sugar Refining (Tokyo, Japan) for providing hesperidin.

Abbreviations

ACAT, acyl CoA:cholesterol acyltransferase; BMC, bone mineral contents; BMD, bone mineral density; CTx, C-telopeptide of type 1 collagen; DPD, deoxypyridinoline; GKase, glucokinase; G6Pase, glucose-6-phosphatase; HDL, high density lipoprotein; HMG-CoA, 3-hydroxy-3-methyl-glutaryl-CoA; LDL, low density lipoprotein; PPAR, peroxisome proliferator-activated receptor; STZ, streptozotocin; TC, total cholesterol; TG, triglyceride; VLDL, very low density lipoprotein.

References


