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

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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, storeptozotocin, 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

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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 fed a control diet that was based on the AIN-93G diet and prepared with corn oil instead of soybean oil. After this period, the rats were randomly assigned to 3 experimental groups consisting of 6 rats each: control (C) group, type 1 diabetes (S) group, and type 1 diabetes and hesperidin (SH) group. Rats in the S and SH groups received 2 intraperitoneal (ip) injections of STZ (70 mg/kg body weight; Sigma-Aldrich Co., MO) in a vehicle (0.9% NaCl), and the control rats received ip injections of vehicle alone. Starting at 3 days after the ip injections, the C and S group rats were fed a control diet, and the SH group rats were fed a hesperidin-containing diet (10 g/kg diet) for 4 weeks. We previously have dosed hesperidin containing diet (5 g/kg diet) to OVX mice [*7*]. In the same time, as a pilot study, we indicated that 1% hesperidin (10 g/kg diet) was needed in rats for inhibiting bone loss and due to magnesium-deficiency (unpublished data). 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).

Measurement of serum hesperetin concentration

Serum hesperetin concentration was measured using high-performance liquid chromatography (HPLC) with electrochemical detection (Chrochem 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) 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 adionectin levels, and activities of hepatic glucose regulating enzymes

Group	C	S	SH
Blood glucose (mg/dl)			
Acclimatization period	96.66 ± 8.57	98.00 ± 7.29	95.16 ± 6.66
3 days after the *ip* injections	91.33 ± 6.38^{b}	120.50 ± 7.97^{a}	121.50 ± 5.89^{a}
1 day prior to dissection	89.33 ± 5.11^{b}	131.67 ± 5.02^{a}	94.36 ± 4.77^{b}
Insulin (ng/ml)	1.77 ± 0.58^{b}	0.91 ± 0.47^{b}	4.03 ± 1.22^{a}
G6Pase (μmol/min/mg protein)	518.09 ± 55.45^{b}	774.88 ± 72.28^{a}	288.86 ± 68.02^{c}
GK (μmol/min/mg protein)	322.63 ± 47.52^{a}	191.35 ± 22.93^{b}	370.82 ± 30.06^{a}
Adiponectin (ng/ml)	351.93 ± 71.09^{a}	188.34 ± 23.98^{b}	327.69 ± 42.70^{a}

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

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

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 3. Bone mineral contents (BMC), bone mineral density (BMD) and makers of bone turnover

Group	C	S	SH
Femur BMC (mg)	184.18 ± 3.26	172.16 ± 6.77	176.40 ± 4.57
BMD (mg/cm^2)	100.53 ± 1.19	99.75 ± 2.87	101.01 ± 1.27
Tibia BMC (mg)	139.13 ± 2.74	136.83 ± 4.43	135.53 ± 2.80
BMD (mg/cm^2)	83.00 ± 0.85	83.94 ± 1.53	82.38 ± 0.81
Lumbar BMC (mg)	16.56 ± 0.54	16.63 ± 1.29	16.33 ± 0.93
BMD (mg/cm^2)	48.36 ± 0.96	46.23 ± 2.09	46.71 ± 1.35
Serum Osteocalcin (ng/ml)	63.72 ± 5.94^a	38.05 ± 4.00^b	41.78 ± 3.79^b
Urinary CTx (μg/mmol creatinine)	24.09 ± 3.31	24.94 ± 2.16	22.75 ± 3.14
Urinary DPD (nmol/mmol creatinine)	727.86 ± 82.64	629.17 ± 106.21	583.96 ± 63.02

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

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 gluconeogenetic 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

serum adiponectin levels, in rodents.

In this study, hesperidin also showed hypolipidemic effects in STZ-induced diabetic rats. The serum and hepatic TG, TC and serum LDL + VLDL cholesterol concentrations were significantly increased by STZ injection, but hesperidin suppressed the increase in these levels. In contrast, HDL-cholesterol concentration and HDL/TC ratio were decreased by STZ-injection, but were normalized by hesperidin administration. By using weanling rats that were maintained for 28 days under conditions that were almost identical to those in our study, Myake *et al.* [*5*] reported that hesperidin exerts protective effects against oxidative stress but not against the effects of increased serum glucose and lipid levels. It is well known that 3-hydroxy-3-methyl- glutaryl-CoA (HMG-CoA) reductase inhibitors are very effective in lowering plasma cholesterol in most animal species, including humans, and these inhibitors are now widely used as hypocholesterolemic drugs. Bok *et al.* demonstrated that in rats, naringin and hesperidin significantly lowered the plasma and hepatic levels of cholesterol and TG by inhibiting HMG-CoA reductase and acyl CoA:cholesterol acyltransferase (ACAT) [*21*]. Furthermore, hesperidin enhances expression of the gene encoding the LDL receptor [*22*]. These are some possible mechanisms underlying the hypolipidemic effects of hesperidin; we, however, did not measure the activities of HMG-CoA reductase and ACAT or the expression of the gene encoding the LDL receptor. We demonstrated increasing adiponectin, which can reduce lipid accumulation, by hesperidin administration.

Previous researchers have reported that STZ-injected rats exhibit decreased bone formation and increased bone resorption [*23*]. In this study, however, the femoral BMD and BMC, and the levels of bone resorption markers such as DPD and CTx 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. As mentioned above, we made a marginal model of type 1 diabetes. Bone loss was not occurred in this model. Further, we used weanling rats (aged 4 weeks) in this study, while other researchers have used adult rats (aged 10–16 weeks). This is another reason why we did not observe bone loss in the STZ-injected rats in the present study. During the growing period in rats, STZ does not seem to affect bone tissue to a great extent. Consequently, only marginal bone loss was induced, and hesperidin did not affect any bone parameters in this study. It has been reported [*24*] that the flavonol quercetin inhibits bone loss due to STZ-induced diabetes by using rats aged 16 weeks.

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.

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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

[1] Gispen, W.H. and Biessels, G.J.: Cognition and synaptic plasticity in diabetes mellitus. *Trends. Neurosci.*, **23**, 542–549, 2000.

[2] Beppu, H., Shimpo, K., Chihara, T., Kaneko, T., Tamai, I., Yamaji, S., Ozaki, S., Kuzuya, H., and Sonoda, S.: Anti-diabetic effects of dietary administration of Aloe arborescens Miller components in multiple low-dose streptozotocin-induced diabetes in mice: Investigation on hypoglycemic action and systemic absorption dynamics of aloe components. *J. Ethnopharmacol.*, **103**, 468–477, 2006.

[3] Sharma, B., Balomajumder, C., and Roy, P.: Hypoglycemic and hypolipidemic effects of flavonoid rich extract from Eugenia jambolana seeds on streptozotocin induced diabetic rats. *Food Chem. Toxicol.*, **46**, 2376–2383, 2008.

[4] Cao, H., Hininger-Favier, I., Kelly, M.A., Benaraba, R., Dawson, H.D., Coves, S., Roussel, A.M., and Anderson, R.A.: Green tea polyphenol extract regulates the expression of genes involved in glucose uptake and insulin signaling in rats fed a high fructose diet. *J. Agric. Food Chem.*, **55**, 6372–6378, 2007.

[5] Miyake, Y., Yamamoto, K., Tsujihara, N., and Osawa, T.: Protective effects of lemon flavonoids on oxidative stress in diabetic rats. *Lipids*, **33**, 689–695, 1998.

[6] Middleton, E. and Kandaswami, C.: Potential health-promoting properties of citrus flavonoids. *Food Techoni.*, **48**, 115–119, 1994.

[7] Chiba, H., Uehara, M., Wu, J., Wang, X., Masuyama, R., Suzuki, K., Kanazawa, K., and Ishimi, Y.: Hperidin, a Citrus flavonoid, inhibits bone loss and decreases serum and hepatic lipids in ovariectomized mice. *J. Nutr.*, **133**, 1892–1897, 2003.

[8] Choi, E.M. and Kim, Y.H.: Hesperetin attenuates the highly reducing sugar-triggered inhibition of osteoblast differentiation. *Cell Biol. Toxicol.*, **24**, 225–231, 2008.
[9] Cameron-Smith, D., Habito, R., Barnett, M., and Collier, G.R.: Dietary guar gum improves insulin sensitivity in streptozotocin-induced diabetic rats. *J. Nutr.*, **127**, 359–364, 1997.
[10] Ueno, Y., Kizaki, M., Nakagiri, R., Kamiya, T., Sumi, H., and Osawa, T.: Dietary glutathione protects rats from diabetic nephropathy and neuropathy. *J. Nutr.*, **132**, 897–900, 2002.
[11] Horcajada, M.N., Habauzit, V., Trzeciakiewicz, A., Morand, C., Gil-Izquierdo, A., Mardon, J., Lebecque, P., Davicco, M.J., Chee, W.S., Coxam, V., and Offord, E.: Hesperidin inhibits ovariectomized-induced osteopenia and shows differential effects on bone mass and strength in young and adult intact rats. *J. Appl. Physiol.*, **104**, 648–654, 2008.
[12] Davidson, A.L. and Arion, W.J.: Factors underlying significant underestimations of glucokinase activity in crude liver extracts: physiological implications of higher cellular activity. *Arch. Biochem. Biophys.*, **15**, 156–167, 1987.
[13] Bickerstaff, G.F. and Burchell, B.: Studies on the purification of glucose 6-phosphatase from rabbit liver microsomal fraction [proceedings]. *Biochem. Soc. Trans.*, **8**, 389–390, 1980.
[14] Azuma, K., Minami, Y., Ippoushi, K., and Terao, J.: Lowering effects of onion intake on oxidative stress biomarkers in streptozotocin-induced diabetic rats. *J. Clin. Biochem. Nutr.*, **40**, 131–140, 2007.
[15] Jalal, R., Majid, S., Bagheri, S.M., Moghimi, A., and Rasuli, M.B.: Hypoglycemic effect of aqueous shallot and garlic extracts in rats with fructose-induced insulin resistance. *J. Clin. Biochem. Nutri.*, **41**, 218–223, 2007.
[16] Stanley Mainzen Prince, P. and Kamalakkanna, N.: Rutin improves glucose homeostasis in streptozotocin diabetic tissues by altering glycolytic and gluconeogenic enzymes. *J. Biochem. Mol. Toxicol.*, **20**, 96–102, 2006.
[17] Jung, U.J., Lee, M.K., Park, Y.B., Kang, M.A., and Choi, M.S.: Effect of citrus flavonoids on lipid metabolism and glucose-regulating enzyme mRNA levels in type-2 diabetic mice. *Int. J. Biochem. Cell Biol.*, **38**, 1134–1145, 2006.
[18] Guerre-Millo, M.: Adipose tissue and adipokines: for better or worse. *Diabete. Metab.*, **30**, 13–19, 2004.
[19] Kadowaki, T. and Yamauchi, T.: Adiponectin and adiponectin receptors. *Endocr. Rev.*, **26**, 439–451, 2005.
[20] Liu, L., Shan, S., Zhang, K., Ning, Z.Q., Lu, X.P., and Cheng, Y.Y.: Naringenin and hesperetin, two flavonoids derived from Citrus aurantium up-regulate transcription of adiponectin. *Phytother. Res.*, **22**, 1400–1403, 2008.
[21] Bok, S.H., Lee, S.H., Park, Y.B., Bae, K.H., Son, K.H., Jeong, T.S., and Choi, M.S.: Plasma and hepatic cholesterol and hepatic activities of 3-hydroxy-3-methyl-glutaryl-CoA reductase and acyl CoA: cholesterol transferase are lower in rats fed citrus peel extract or a mixture of citrus bioflavonoids. *J. Nutr.*, **129**, 1182–1185, 1999.
[22] Wilcox, L.J., Borradaile, N.M., Dreu, L.E., and Huff, M.W.: Secretion of hepatocyte apoB is inhibited by the flavonoids, naringenin and hesperetin, via reduced activity and expression of ACAT2 and MTP. *J. Lipid Res.*, **42**, 725–734, 2001.
[23] Tein, M.S., Breen, S.A., Loveday, B.E., Devlin, H., Balment, R. J., Boyd, R.D., Sibley, C.P., and Garland, H.O.: Bone mineral density and composition in rat pregnancy: effect of streptozotocin-induced diabetes mellitus and insulin replacement. *Exp. Physiol.*, **83**, 165–174, 1998.
[24] Kanter, M., Altan, M.F., Donmez, S., Ocakci, A., and Kartal, M.E.: The effects of quercetin on bone minerals, biomechanical behavior, and structure in streptozotocin-induced diabetic rats. *Cell Biochem. Funct.*, **25**, 747–752, 2007.