SPANDIDOS PUBLICATIONS

Oral administration of phytocomponent *p*-hydroxycinnamic acid has a preventive effect on bone loss in streptozotocin-induced diabetic rats

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Abstract. The phytocomponent *p*-hydroxycinnamic acid (HCA) has been shown to have a stimulatory effect on bone formation and an inhibitory effect on bone resorption in rat femoral tissues *in vitro*. The preventive effect of HCA on bone loss induced in streptozotocin (STZ)-diabetic rats was investigated *in vivo*. Rats received a single subcutaneous administration of STZ (6.0 mg/100 g body weight), and then the animals were orally administered HCA (0.25, 0.5, or 1.0 mg/100 g body weight) once daily for 14 days. STZ administration caused a significant decrease in body weight and a significant increase in serum glucose, triglyceride, and calcium levels, indicating a diabetic state. These alterations were significantly prevented by administration of HCA (0.25, 0.5, or 1.0 mg/100 g). Calcium content in the femoral-diaphyseal and -metaphyseal tissues was significantly decreased in STZ-diabetic rats. This decrease was significantly prevented after administration of HCA (0.25, 0.5, or 1.0 mg/100 g). Alkaline phosphatase activity in the diaphyseal and metaphyseal tissues was significantly decreased in STZ-diabetic rats. The decrease in diaphyseal alkaline phosphatase activity in STZ-diabetic rats was significantly prevented after administration of HCA (0.5 and 1.0 mg/l00 g). The diaphyseal DNA content was also significantly decreased in STZ-diabetic rats. Administration of HCA (0.25, 0.5, or 1.0 mg/100 g) caused a significant increase in DNA content in the diaphyseal and metaphyseal tissues in STZ-diabetic rats. This study demonstrates that the intake of HCA has preventive effects on bone loss in STZ-diabetic rats, and that the intake has partially restorative effects on serum biochemical findings in the diabetic state.

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Introduction

Bone loss with aging induces osteoporosis, which is widely recognized as a major public health problem (1-4). A decrease in bone mass leads to bone fracture; bone loss may be due to decreased bone formation and increased bone resorption. Pharmacologic and nutritional supplements may prevent bone loss with increasing age (5-7). Chemical factors in food and plants may have a useful role in the prevention of osteoporosis with aging.

Cinnamic acid is present in many plants. *p*-hydroxycinnamic acid (HCA) is an intermediate-metabolic substance found in plants and is synthesized from thyrosine. The physiologic role of HCA has not been fully clarified, although the compound has an effect as an anti-oxidant (8,9). More recently, it has been found that among cinnamic acid and other related compounds, HCA has a unique-anabolic effect on bone metabolism (10). HCA has a stimulatory effect on bone formation and an inhibitory effect on bone resorption in rat femoral tissues *in vitro* (10). HCA has been shown to suppress various bone resorption-stimulating factor-induced osteoclast-like cell formations in mouse bone marrow culture *in vitro* (11). Oral administration of HCA has an anabolic effect on bone components in the femoral tissues of rats *in vivo* (12). HCA may have a role in the prevention of osteoporosis with aging. This, however, has not yet been clarified.

This study was undertaken to determine whether the intake of HCA has a preventive effect on bone loss induced in the diabetic state (13). We found that HCA had a preventive effect.

Materials and methods

Chemicals. Streptozotocin (STZ) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Hydroxycinnamic acid (HCA) and other chemicals were of reagent grade from Sigma Chemical Co. and Wako Pure Chemical Industries (Osaka, Japan).

Animals. Male Wistar rats (conventional) weighing 110-120 g (5 weeks old) were obtained from Japan SLC (Hamamatsu, Japan). The animals were fed commercial laboratory chow (solid) containing 1.1% calcium and 1.1% phosphorus and housed at a room temperature of 25˚C with free access to distilled water.

Administration procedure. STZ was dissolved in 50 mM sodium citrate (pH 4.5) solution containing 150 mM NaCl (14). The solution (6.0 mg/0.5 ml/100 g body weight) was subcutaneously administered to rats, and 14 days later the animals were sacrificed by exsanguination. HCA was dissolved in corn oil as a concentration of 0.5, 1.0 or 2.0 mg/ml. HCA (0.25, 0.5, or 1.0 mg/0.5 ml/100 g body weight) was orally administered to rats through a stomach tube once daily for 14 days. HCA was orally administered 3 h after the administration of STZ (6.0 mg/100 g). Rats were sacrificed 24 h after the last administration of HCA, and the blood and femur were removed immediately.

Analytical procedures. Blood samples obtained by cardiac puncture were centrifuged 30 min after collection, and the serum was separated. Serum was frozen at -80˚C until assay. Serum glucose, triglyceride, calcium, and inorganic phosphorus concentrations were determined using an assay kit (Wako Pure Chemical Industries).

The diaphyseal or metaphyseal tissues were dried for 16 h at 110˚C. Calcium was determined using atomic absorption spectrophotometry (15). Calcium content in bone tissues was expressed as milligrams per gram of dry bone.

To assay alkaline phosphatase activity, the diaphyseal or metaphyseal tissues were immersed in 3.0 ml of ice-cold barbital buffer 6.6 mM (pH 7.4), cut into small pieces, and disrupted for 60 sec with an ultrasonic device. The supernatant centrifuged at 600 x g for 5 min was used to measure enzyme activity. Enzyme assay was carried out under optimal conditions. Alkaline phosphatase activity was determined using the method of Walter and Schutt (16). Enzyme activity was expressed as micromole of *p*-nitrophenol liberated per minute per milligram of protein. Protein concentration was determined using the method of Lowry *et al* (17).

To measure bone DNA content, the diaphyseal or metaphyseal tissues were shaken with 4.0 ml of ice-cold 0.1 N NaOH solution for 24 h after homogenization of the bone tissues (18). After alkaline extraction, the samples were centrifuged at 1000 x g for 5 min, and the supernatant was determined using the method of Ceriotti (19) and expressed as the amount of DNA (mg)/g wet weight of bone tissue.

Statistical analysis. Data were expressed as the mean ± SEM. Statistical differences were analyzed using the Student's t-test. A p-value <0.05 was considered to indicate a statistically significant difference. The analysis of variance (ANOVA) multiple comparison test was used to compare the treatment groups.

Results

Effects of administration of HCA on serum biochemical components in STZ-diabetic rats. Rats received a single subcutaneous administration of STZ (6.0 mg/100 g body weight), and the animals were orally administered HCA (0.25, 0.5, or 1.0 mg/100 g body weight) once daily for 14 days. The body weight of the animals was significantly decreased 14 days after administration of STZ, but this reduction was significantly prevented after administration of HCA (0.25, 0.5, or 1.0 mg/100 g) for 14 days (Fig. 1).

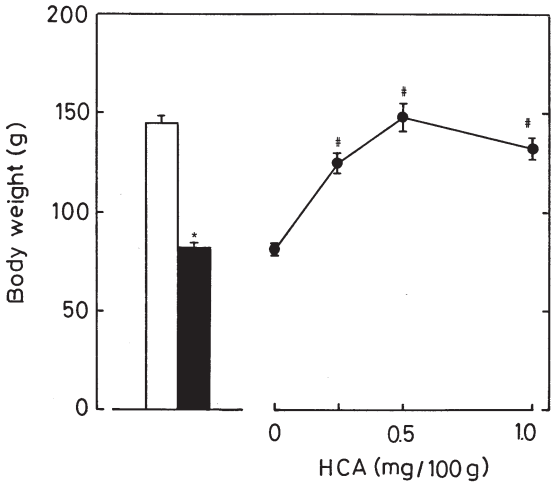

Figure 1. Effects of administration of *p*-hydroxycinnamic acid (HCA) on the change in body weight in STZ-diabetic rats. Rats received a single subcutaneous administration of STZ (6.0 mg/100 g body weight), and 3 h later the animals were orally administered either vehicle (corn oil) or HCA (0.25, 0.5, or 1.0 mg/100 g body weight) once daily for 14 days. Animals were sacrificed 24 h after the last administration. Each value is the mean ± SEM of six rats. *p<0.01 compared with the control (none) value; #p<0.01 comparing the control value with the STZ treatment. White bar, control; black bar, STZ diabetes.

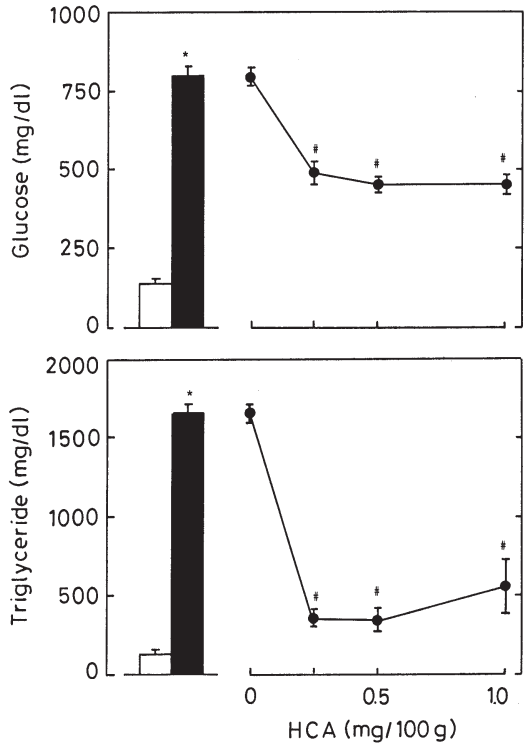

Figure 2. Effects of administration of *p*-hydroxycinnamic acid (HCA) on the change in glucose and triglyceride concentrations in STZ-diabetic rats. The administration procedure is described in the legend of Fig. 1. Each value is the mean ± SEM of six rats. *p<0.01 compared with the control (none) value; #p<0.01 comparing the control value with the STZ treatment. White bars, control; black bars, STZ diabetes.

The serum glucose and triglyceride levels were markedly elevated in STZ-administered rats, indicating that the administration induced a diabetic state. These increases were

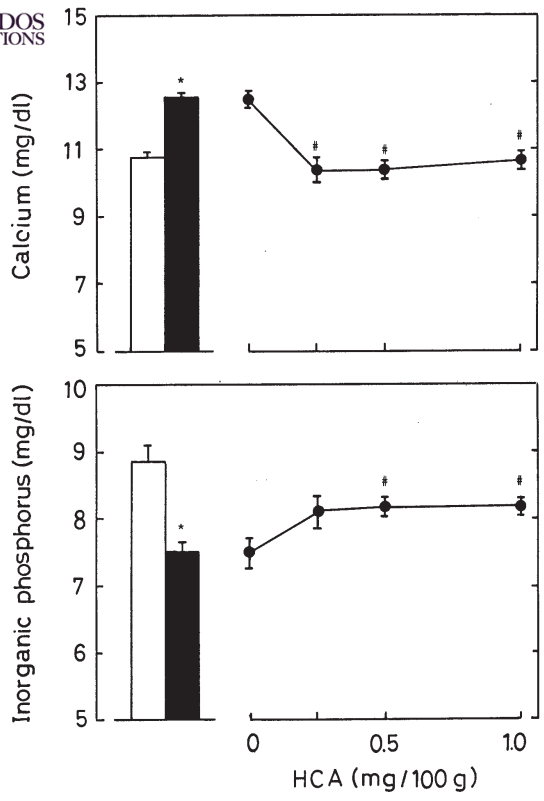

Figure 3. Effects of administration of *p*-hydroxycinnamic acid (HCA) on the change in calcium and inorganic phosphorus concentrations in STZ-diabetic rats. The administration procedure is described in the legend of Fig. 1. Each value is the mean ± SEM of six rats. *p<0.01 compared with the control (none) value; #p<0.01 comparing the control value with the STZ treatment. White bars, control; black bars, STZ diabetes.

significantly prevented after administration of HCA (0.25, 0.5, or 1.0 mg/100 g) for 14 days (Fig. 2).

The serum calcium levels were significantly increased after STZ administration (Fig. 3), and the increase was significantly prevented after administration of HCA (0.25, 0.5 or 1.0 mg/100 g) for 14 days. Serum inorganic phosphorus levels were also significantly decreased 14 days after STZ administration (Fig. 3), and this decrease was significantly prevented after administration of HCA (0.5 or 1.0 mg/100 g) for 14 days.

Effects of administration of HCA on bone components in STZ-diabetic rats. The calcium content in the femoral-diaphyseal and -metaphyseal tissues of rats was significantly decreased 14 days after the administration of STZ (6.0 mg/100 g) (Fig. 4), and the decrease was significantly prevented after administration of HCA (0.25, 0.5, or 1.0 mg/100 g) for 14 days.

The alkaline phosphatase activity in femoral tissues was also significantly decreased in STZ-diabetic rats (Fig. 5), and the decrease in the diaphyseal tissues was significantly prevented after administration of HCA (0.5 or 1.0 mg/100 g) for 14 days. The dose of HCA (0.25, 0.5, or 1.0 mg/100 g) for 14 days did not have a significant preventive effect on the decrease in metaphyseal alkaline phosphatase activity in STZ-diabetic rats.

The DNA content in the femoral-diaphyseal tissues was significantly decreased in STZ-diabetic rats (Fig. 6), and the decrease was significantly prevented after administration of HCA (0.25, 0.5, or 1.0 mg/100 g) for 14 days. The metaphyseal DNA content was not significantly changed in STZ-diabetic rats. Administration of HCA (0.25, 0.5, or 1.0 mg/100 g) for 14 days caused a significant increase in metaphyseal DNA content in STZ-diabetic rats.

Discussion

The effects of cinnamic acid and other related phytocomponent compounds on bone metabolism have not yet been clarified. Recently, we found that *p*-hydroxycinnamic acid (HCA) stimulated bone formation and inhibited bone resorption *in vitro* (10), and that this compound had an inhibitory effect on various bone-resorbing factor-induced osteoclast-like cell formation in mouse bone marrow culture *in vitro* (11). Moreover, oral administration of HCA had an anabolic effect on bone components in the femoral tissues of

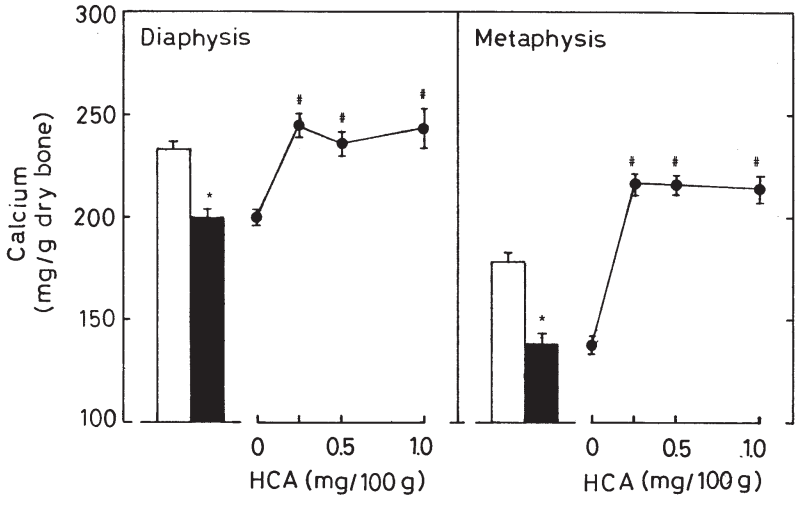

Figure 4. Effects of administration of *p*-hydroxycinnamic acid (HCA) on the change in calcium content in the femoral-diaphyseal and -metaphyseal tissues of STZ-diabetic rats. The administration procedure is described in the legend of Fig. 1. Each value is the mean ± SEM of six rats. *p<0.01 comparing with the control (none) value; #p<0.01 comparing the control value with the STZ treatment. White bars, control; black bars, STZ diabetes.

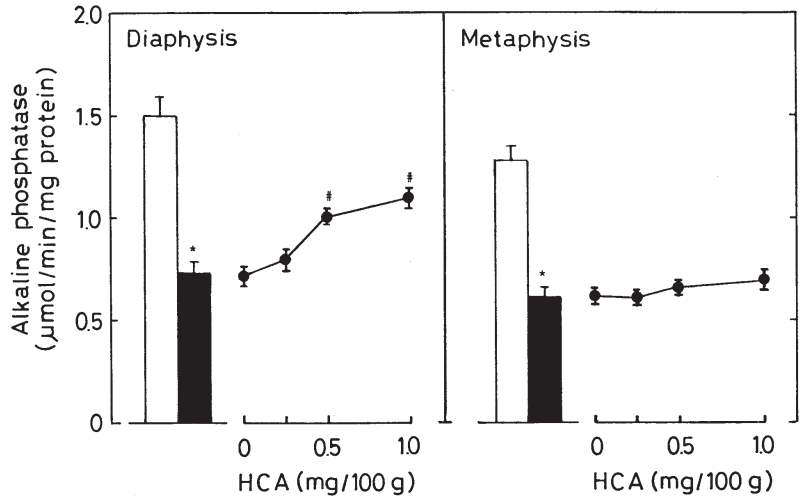

Figure 5. Effects of administration of *p*-hydroxycinnamic acid (HCA) on the change in alkaline phosphatase activity in the femoral-diaphyseal and -metaphyseal tissues of STZ-diabetic rats. The administration procedure is described in the legend of Fig. 1. Each value is the mean ± SEM of six rats. *p<0.01 comparing with the control (none) value; #p<0.01 comparing the control value with the STZ treatment. White bars, control; black bars, STZ diabetes.

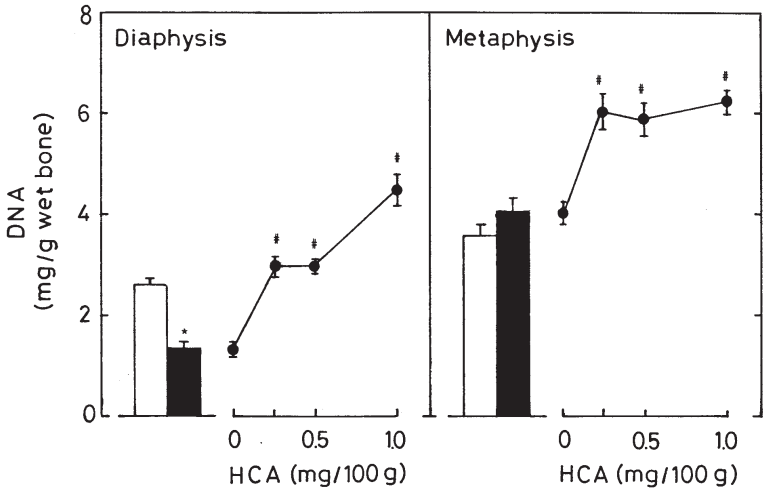

Figure 6. Effects of administration of *p*-hydroxycinnamic acid (HCA) on the change in DNA content in the femoral-diaphyseal and -metaphyseal tissues of STZ-diabetic rats. The administration procedure is described in the legend of Fig. 1. Each value is the mean ± SEM of six rats. *p<0.01 comparing with the control (none) value; #p<0.01 comparing the control value with the STZ treatment. White bars, control; black bars, STZ diabetes.

rats *in vivo* (12). This study was undertaken to determine whether HCA has a preventive effect on bone loss in STZ-diabetic rats.

Oral administration of HCA to STZ-diabetic rats was found to have a preventive effect on the decrease in body weight and the increase in serum glucose and triglyceride levels induced in the diabetic state. This is a novel finding. Intake of HCA has a partially restorative effect on serum biochemical findings involving diabetes *in vivo*. HCA may have a restorative role in the prevention of diabetic states.

The serum calcium level was found to increase in STZ-diabetic rats. Intestinal calcium absorption has been shown to be impaired in the diabetic state (20-22). The increase in serum calcium concentration in STZ-diabetic rats may result from the release of calcium from bone tissues; the femoral calcium content was found to decrease markedly in STZ-diabetic rats. Oral administration of HCA to these rats had a significant preventive effect on hypercalcemia and bone calcium loss in the diabetic state. Intake of HCA may thus have an inhibitory effect on bone resorption in these rats.

Alkaline phosphatase activity in the femoral tissues was found to decrease in STZ-diabetic rats. This enzyme participates in osteoblastic mineralization (23). The femoral alkaline phosphatase activity was significantly decreased in these rats, suggesting that osteoblastic bone mineralization is impaired in the diabetic state. The decrease in femoral-diaphyseal alkaline phosphatase activity in STZ-diabetic rats was significantly prevented after administration of HCA. Presumably, intake of HCA has a stimulatory effect on osteoblastic bone formation in this state.

HCA has been shown to increase DNA content in rat femoral tissues *in vitro* (10) and *in vivo* (12). Oral administration of HCA caused a significant increase in DNA content in the femoral-diaphyseal and -metaphyseal tissues of STZ-diabetic rats. DNA content in bone tissues is an index of the number of existing bone cells (24). It is speculated that

ulates bone cells including osteoblastic cells in the al and metaphyseal tissues of STZ-diabetic rats *in vivo*. This may be partly attributed to the increase in bone calcium content in STZ-diabetic rats.

Oral administration of HCA was found to have a preventive effect on bone loss in the STZ-diabetic state. HCA may have a role in the prevention of osteoporosis. Additional study is warranted using the ovariectomized rat, an animal model of osteoporosis, to further elucidate the present results.

In conclusion, it has been shown that the intake of HCA has a preventive effect on diabetic-induced bone loss, and also has a partially preventive effect on the increase in serum glucose and triglyceride levels in diabetes *in vivo*.

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