Oral Administration of β-Cryptoxanthin Induces Anabolic Effects on Bone Components in the Femoral Tissues of Rats in vivo

Satoshi UCHIYAMA,a Takashi SUMIDA,b and Masayoshi YAMAGUCHI*a
aLaboratory of Endocrinology and Molecular Metabolism, Graduate School of Nutritional Sciences, University of Shizuoka; 52–1 Yada, Shizuoka 422–8526, Japan: and b Research & Development, Ehime Beverage Inc.; 478 Anjoyomachi, Matsuyama 791–8603, Japan. Received September 16, 2003; accepted October 16, 2003

Bone loss with aging induces osteoporosis, which is widely recognized as a major public health problem. 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 factors are needed to prevent bone loss with increasing age. Chemical factors in food may help to prevent bone loss with aging. The chemicals in food which act on bone metabolism, however, are poorly understood.

Recent studies have shown that isoflavones (increasing genistein and daidzein), which are contained in soybean, have a stimulatory effect on bone formation, thereby increasing bone mass. Also, menaquinone-7, an analogue of vitamin K2 which is essential for the γ-carboxylation of osteocalcin of bone matrix protein, is abundant in fermented soybean. Menaquinone-7 has been demonstrated to stimulate osteoblastic bone formation and to inhibit osteoclastic bone resorption in vitro. The supplementation of isoflavones and vitamin K2 has a preventive effect on bone loss induced by ovariectomy in rats, which is an animal model of osteoporosis. Food chemical factors thus play a role in bone health and may be important in the prevention of bone loss with increasing age.

Carotenoids are present in fruit and vegetables. The effects of carotenoids on bone metabolism, however, have not yet been clarified. Recently, it has been shown that β-cryptoxanthin has a unique anabolic effect on bone calcification in vivo. Lutein, lycopene, and β-carotene do not have an effect on bone calcification in vivo. β-Cryptoxanthin has a direct stimulatory effect on bone formation and an inhibitory effect on bone resorption in cultured in vitro. The present study was undertaken to determine the effect of the administration of β-cryptoxanthin on bone components in growing rats in vivo. The oral administration of β-cryptoxanthin, which was isolated from Satsuma mandarin, was found to induce anabolic effects on bone components in vivo.

Key words β-cryptoxanthin; bone formation; bone metabolism; rat femur

MATERIALS AND METHODS

Chemicals Dulbecco’s modified Eagle’s medium (DMEM) (high glucose, 4.5 g/dl) and a penicillin–streptomycin solution (5000 units/mg penicillin and 5000 μg/ml streptomycin) were obtained from Gibco Laboratories (Grand Island, NY, U.S.A.). Bovine serum albumin (BSA), cycloheximide, and corn oil were obtained from Sigma Chemical (St. Louis, MO, U.S.A.). β-Cryptoxanthin (100% purity) was supplied from Ehime Beverage Inc. (Matsuyama, Japan). All other chemicals were reagent grade from Wako Pure Chemical Industries (Osaka, Japan). All water used was glassdistilled.

Animals Male Wistar rats (4 weeks old) were obtained from Japan SLC (Hamamatsu, Japan). The animals were fed commercial laboratory chow (solid) containing 57.4% Ca and 1.1% P for 7 d at room temperature of 25°C and had free access to distilled water.

Bone Culture The femurs were removed aseptically after exsanguination and soaked in ice-cold 0.25 M sucrose solution. The femur was cleaned of soft tissue and marrow, and the diaphysis and metaphysis (not containing epiphyseal tissue) were separated. The femoral-diaphyseal and femoral-metaphyseal tissues were cut into small pieces. Femoral-diaphyseal or femoral-metaphyseal fragments were cultured for 48 h in 35-mm dishes in 2.0 ml of medium consisting of DMEM (high glucose, 4.5 g/dl) supplemented with 0.25% BSA plus antibiotics (100 units of penicillin and 100 μg of streptomycin/ml of medium). In the experiments, bone tissue was cultured for 48 h in a medium containing either vehicle or compound (including 0.1% ethanol). Cultures were maintained at 37°C in a water-saturated atmosphere containing 5% CO2 and 95% air.

Administration Procedures β-Cryptoxanthin was dis-
solved in corn oil at a concentration of 10, 25, or 50 μg/ml. β-Cryptoxanthin (10, 25, or 50 μg/ml/100 g body weight) was orally administered to rats through a stomach tube once daily for 7 d. Control rats received corn oil (1 ml/100 g body weight) orally. The animals were killed 24 h after the last administration by cardiac puncture under light ether anesthesia, and the blood and femur were removed immediately.

**Analytical Procedures** Blood samples were centrifuged for 30 min after collection, and the serum was separated. Serum calcium levels were determined using the method of Willis. Serum inorganic phosphorus levels were measured using the method of Taussky and Shon.

The diaphyseal and metaphyseal tissues were dried for 16 h at 110 °C. Calcium content was determined by atomic absorption spectrophotometry. Calcium content in bone tissue is expressed as milligrams/gram of dry bone.

To assay alkaline phosphatase activity, the diaphyseal and metaphyseal tissues were immersed in 3.0 ml of ice-cold 6.5 mM barbital buffer (pH 7.4), cut into small pieces, homogenized with a physcotron homogenizer, and disrupted for 60 s with an ultrasonic device. The supernatant centrifuged at 600×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. Enzyme activity was expressed as μmol of p-nitrophenol liberated/minute/milligram of protein. Protein concentration was determined using the method of Lowry et al.

To measure bone DNA content, the diaphyseal and metaphyseal tissues were shaken with 4.0 ml of ice-cold 0.1 M NaOH solution for 24 h after homogenization of the bone tissues. After alkali extraction, the samples were centrifuged at 10000×g for 5 min, and the supernatant was collected. DNA content in the supernatant was determined using the method of Ceriotti and expressed as the amount of DNA (mg/g) wet weight of bone tissue.

**Statistical Analysis** The significance of difference between values was estimated using Student’s t-test. p-values of less than 0.01 were considered to indicate statistically significant differences.

## RESULTS

**Effect of β-Cryptoxanthin on Bone Components in Tissue Culture in Vitro** Rat femoral-diaphyseal and femoral-metaphyseal tissues were cultured for 48 h in a medium containing either vehicle or β-cryptoxanthin (10⁻⁷ or 10⁻⁶ M) in the presence or absence of cycloheximide (10⁻⁶ M). The presence of β-cryptoxanthin (10⁻⁷ or 10⁻⁶ M) caused a significant increase in calcium content (Fig. 1) and alkaline phosphatase activity (Fig. 2) in the femoral-diaphyseal and femoral-metaphyseal tissues. These increases were completely prevented in the presence of cycloheximide (10⁻⁶ M), an inhibitor of protein synthesis. Thus β-cryptoxanthin had an anabolic effect on bone calcification in vitro.

**Effect of Administration of β-Cryptoxanthin on Bone Component in Rats in Vivo** The body weight of rats was not significantly altered by the oral administration of β-cryptoxanthin (10, 25, or 50 μg/100 g body weight) for 7 d (Table 1). Serum calcium and inorganic phosphorus concentrations were also not significantly changed by β-cryptoxanthin administration (Table 1).

The oral administration of β-cryptoxanthin (10, 25, or 50 μg/100 g body weight) to rats for 7 d caused a significant increase in calcium content (Fig. 3) and alkaline phosphatase activity (Fig. 4) in the femoral-diaphyseal and femoral-metaphyseal tissues. Femoral-diaphyseal DNA content was significantly increased by the administration of β-cryptoxanthin (25 or 50 μg/100 g body weight) for 7 d. A significant elevation of femoral-metaphyseal DNA content was also observed.
Effect on bone calcification in Femoral-Diaphyseal and -Metaphyseal Tissues of Rats

β-Cryptoxanthin (10, 25, or 50 μg/100 g body weight) was orally administered once daily for 7 d to rats. Each value is the mean±S.E.M. of six rats. *p<0.01 compared with the control (vehicle) value.

The oral administration of β-cryptoxanthin to rats for 7 d was found to induce anabolic effect on bone components in vivo. β-Cryptoxanthin administration significantly increased calcium content, alkaline phosphatase activity, and DNA content in the femoral-diaphyseal and femoral-metaphyseal tissues of rats. Alkaline phosphatase is an enzyme marker of osteoblasts, and the enzyme participates in bone mineralization. DNA content in bone tissues is an index of the number of bone cells. The administration of β-cryptoxanthin may stimulate osteoblastic bone formation in vivo.

The anabolic effect of β-cryptoxanthin was induced by oral administration for 7 d with the lowest dose of 10 μg/100 g body weight of β-cryptoxanthin, although the serum concentration of the carotenoid was not determined. This dose may have physiologic significance. It has been reported that the serum concentrations of β-cryptoxanthin increases due to consumption of vegetable juice in women to the range of 1.3×10⁻⁷ to 5.3×10⁻⁷ M. The anabolic effect of β-cryptoxanthin on bone calcification was observed at 10⁻⁷ and 10⁻⁶ M in vitro. Presumably, the supplemental intake of β-cryptoxanthin has a stimulatory effect on the bone formation rate in vivo.

In conclusion, it has been demonstrated that the oral administration of β-cryptoxanthin induces an anabolic effect on bone components in the femoral-diaphyseal and femoral-metaphyseal tissues of growing rats in vivo.

REFERENCES

(1999).