Anabolic Effect of Marine Alga *Sargassum Horneri* Extract on Bone Components in the Femoral-diaphyseal and -metaphyseal Tissues of Young and Aged Rats *in Vivo*

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The effect of *Sargassum horneri* on bone components in the femoral-diaphyseal and -metaphyseal tissues of young and aged rats was investigated. Rats were orally administered a water-solubilized extract (2.5, 5, and 10 mg/100 g body weight) of *S. horneri* once a day for 7 or 14 days. Calcium content, alkaline phosphatase activity and deoxyribonucleic acid (DNA) content in the femoral-diaphyseal and -metaphyseal tissues of young male (4 weeks old) rats was significantly increased by the administration of *S. horneri* extract (2.5, 5, and 10 mg/100 g) for 7 days. Moreover, these bone components in the femoral-diaphyseal and -metaphyseal tissues of aged female (50 weeks old) rats were significantly increased by the administration of *S. horneri* extract (10 mg/100 g) for 14 days. Meanwhile, body weight and serum calcium, zinc and inorganic phosphorus concentrations of female aged rats were not significantly altered by the administration of *S. horneri* extract (10 mg/ 100 g) for 14 days. The present study demonstrates that the oral intake of the water-solubilized extract of *S. horneri* can exhibit an anabolic effect on bone components of young rats *in vivo*, and that this effect is also seen in aged rats. The intake of *S. horneri* extract may have a preventive effect on bone loss with increasing age.

Key words —— bone metabolism, *Sargassum horneri*, marine alga, osteoporosis, rat femur

INTRODUCTION

Bone mass decreases with increasing age.[1–3] Osteoporosis with a decrease in bone mass is widely recognized as a major public health problem.[4] The most dramatic expression of this disease is represented by fractures of the proximal femur. Nutritional factors can help to prevent bone loss with increasing age.[5]

Recent studies have shown that isoflavones and saponins in soybean have an anabolic effect on bone metabolism.[6–9] Also, menaquinone-7, an analogue of vitamin K_2, which is abundant in fermented soybean (*natto*),[10] has been shown to have a preventive effect on bone loss induced by ovariectomy in rats.[11,12] These factors have been shown to stimulate osteoblastic bone formation and inhibit osteoclastic bone resorption,[13–16] thereby increasing bone mass. Nutritional factors may be important in the prevention of bone loss with increasing age.

More recently, it has been shown that, amoung various marine algae, *Sargassum horneri* extract has an anabolic effect on bone calcification in rat femoral tissues *in vivo* and *in vitro*.[17] *S. horneri* extract has been demonstrated to stimulate osteoblastic bone formation[18] and inhibit osteoclastic bone resorption[19] *in vitro* using rat femoral-diaphyseal and -metaphyseal tissues. The action of *S. horneri* extract on bone metabolism has not been fully clarified, however.

The present study was thus undertaken to determine the effect of the prolonged oral administration of *S. horneri* extract on bone metabolism in young and aged rats. The administration of water-solubilized *S. horneri* extract was found to have an anabolic effect on bone components *in vivo*.

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MATERIALS AND METHODS

Marine Alga Extracts —— The marine alga *S. horneri* was seasonally gathered from the coast at Shimoda (Shizuoka prefecture, Japan), and was freeze-dried and powdered. The fresh marine alga gathered was homogenized in distilled water with a Physcotron homogenizer, and the homogenate was centrifuged at 5500 *g* in a refrigerated centrifuge for 10 min.[18] The 5500 *g* supernatant fraction was pooled for freeze-drying. Powder of the water-solubilized extract was dissolved in ice-cold distilled water for use in experiments. This solution of the water-solubilized extract of *S. horneri* was digested by the addition of nitric acid for 24 hr at 110°C. Calcium and zinc concentrations were determined by atomic absorption photometry.

Animals —— Male and female Wistar rats (conventional) weighing 100–120 g (4 weeks old) or female Wistar rats weighing 200–250 g (50 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 at a room temperature of 25°C, with free access to distilled water.

Administration Procedures —— The water suspension (2.5, 5, and 10 mg/ml/100 g body weight) of the powder of a water-solubilized extract of marine alga *S. horneri* was orally administered to rats through a stomach tube once daily for 7 or 14 days. Control rats received distilled water (1.0 mg/100 g body weight) orally. The animals were killed 24 hr 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 and analyzed immediately. Serum calcium was determined by the method of Willis.[20] Serum inorganic phosphorus was measured by the method of Taussky and Shon.[21]

The diaphyseal and metaphyseal tissues were dried for 16 hr at 110°C, weighed, and then dissolved in nitric acid solution. Calcium was determined by atomic absorption spectrophotometry.[22] Calcium content in bone tissues was expressed as milligrams per gram of dry bone.

To assay alkaline phosphatase activity, the 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 × *g* for 5 min, was used to measure enzyme activity. An enzyme assay was carried out under optimal conditions. Alkaline phosphatase activity was determined by the method of Walter and Schutt.[23] Enzyme activity was expressed as micromol of *p*-nitrophenol liberated per minute per milligram of protein. Protein concentration was determined by the method of Lowry *et al*.[24]

To measure bone DNA content, the diaphyseal and metaphyseal tissues were shaken with 4.0 ml ice-cold 0.1 N NaOH solution for 24 hr after homogenization of the bone tissues.[25] After alkali extraction, the samples were centrifuged at 1000 × *g* for 5 min, and the supernatant was collected. DNA content in the supernatant was determined by the method of Ceriotti[26] and expressed as the amount of DNA (mg)/g wet weight of bone tissue.

Statistical Analysis —— The significance of difference between values was estimated by Student's *t*-test. *p*-Values of less than 0.05 were considered to indicate statistically significant differences.

RESULTS

Effect of Administration of *S. Horneri* Extract on Bone Component in Young Male Rats

The body weight of young male (4-week-old) rats was not significantly altered by the oral administration of the water-solubilized extract (2.5, 5, and 10 mg/100 g body weight) of *S. horneri* for 7 days (data not shown). Serum calcium, zinc and inorganic phosphorus concentrations were also not significantly altered by *S. horneri* extract administration (data not shown).

Calcium content in the water-solubilized extract (10 mg/ml) of *S. horneri* was 23.5 μg, while zinc content was not detected. Calcium content (Fig. 1), alkaline phosphatase activity (Fig. 2), and DNA content (Fig. 3) in the femoral-diaphyseal and -metaphyseal tissues of young male rats were significantly increased by oral administration of the water-solubilized extract (2.5, 5, 10 mg/100 g) from *S. horneri* for 7 days.

Effect of Administration of *S. Horneri* Extract on Bone Component in Aged Female Rats

The body weight and serum calcium, inorganic phosphorus and zinc concentrations of aged (50-week-old) rats were not significantly altered by oral administration of the water-solubilized extract (10 mg/ 100 g body weight) for 14 days. (Table 1).

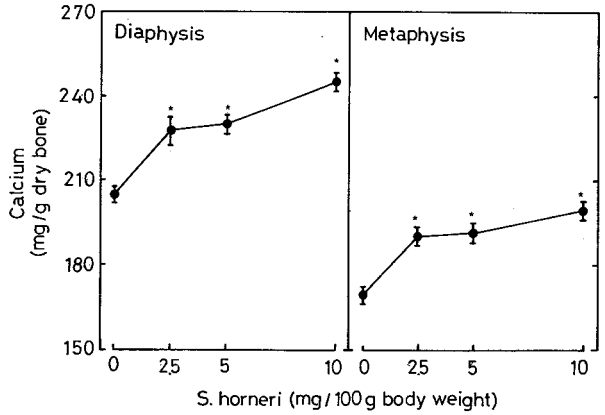

Fig. 1. Change in Calcium Content in the Femoral-Diaphyseal and -Metaphyseal Tissues of Young Male Rats Orally Administered Water-solubilized Extract of *S. Horneri in vivo*

Rats were orally administered the water-solubilized extract (2.5, 5, and 10 mg/ml/100 g body weight) once daily for 7 days, and the animals were killed 24 hr after the last administration. Each value is the mean ± S.E.M. of six rats. $*p < 0.01$ compared with the control (none) value.

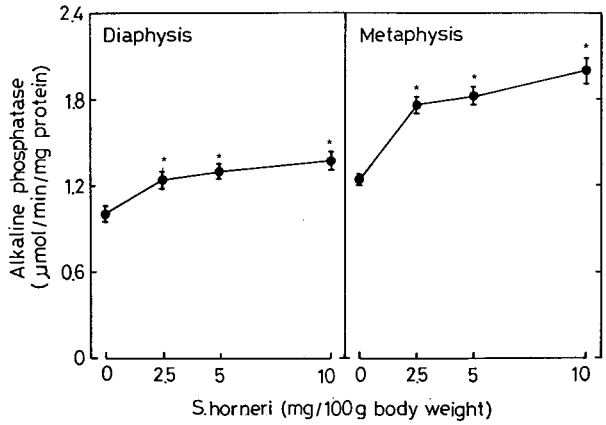

Fig. 2. Change in Alkaline Phosphatase Activity in the Femoral-Diaphyseal and -Metaphyseal Tissues of Young Male Rats Orally Administered Water-solubilized Extract of *S. Horneri in vivo*

The procedure of administration was described in the legend of Fig. 1. Each value is the mean ± S.E.M. of six rats. $*p < 0.01$ compared with the control (none) value.

Calcium content (Fig. 4) in the femoral-diaphyseal and -metaphyseal tissues was not altered by increasing age. Alkaline phosphatase activity (Fig. 5) and DNA content (Fig. 6) in the femoral-diaphyseal and -metaphyseal tissues were significantly decreased by increasing age.

The oral administration of the water-solubilized extract (10 mg/100 g) of *S. horneri* to young and aged female rats for 14 days caused a significant increase in calcium content (Fig. 4), alkaline phosphatase activity (Fig. 5), and DNA content (Fig. 6) in the femoral-diaphyseal and -metaphyseal tissues.

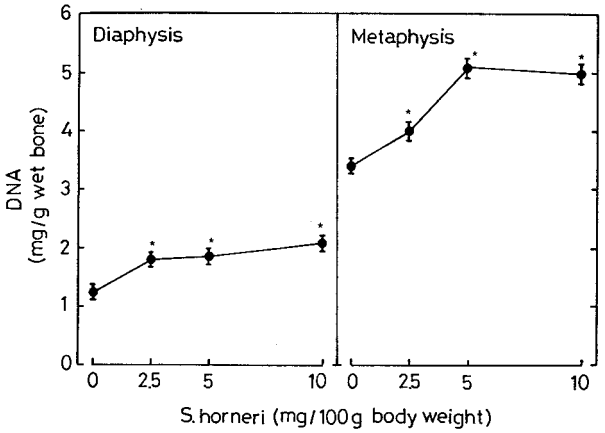

Fig. 3. Change in DNA Content in the Femoral-Diaphyseal and -Metaphyseal Tissues of Young Male Rats Orally Administered Water-solubilized Extract of *S. Horneri in vivo*

The procedure of administration was described in the legend of Fig. 1. Each value is the mean ± S.E.M. of six rats. $*p < 0.01$ compared with the control (none) value.

DISCUSSION

Of various marine algae (*Undaria pinnatifida*, *Sargassum horneri*, *Eisenia bicyclis*, *Cryptonemia scmitziana*, *Gelidium amansii*, and *Ulva pertusa* Kjellman) which were gathered seasonally, *S. horneri* extract has been demonstrated to have a unique stimulatory effect on bone calcification *in vitro* and *in vivo*.[17] Moreover, among *S. horneri*, *S. ringgoldianum* Harvey, and *S. yamadae* Yoshida et T. Konno extract, which belongs to *Sargassum*, *S. horneri* extract has been shown to stimulate bone formation *in vitro*.[18] Thus, of various marine algae, *S. horneri* extract had a specific anabolic effect on calcification *in vitro*. The anabolic effect of *S. horneri* extract on bone calcium content may be the result of a stimulatory effect on bone formation and an inhibitory effect on bone resorption *in vitro*.[18,19] Furthermore, the present study demonstrates that the prolonged oral administration of water-solubilized *S. horneri* extract to young and aged rats caused an anabolic effect on bone components in the femoral-diaphyseal and -metaphyseal tissues *in vitro*. The intake of dietary supplementation with *S. horneri* extract may have a preventive effect on bone loss with increasing age.

The body weight of aged rats was not signifi-

Table 1. Body Weight and Serum Calcium, Zinc and Inorganic Phosphorus Concentrations in Female Aged Rats Orally Administered *S. Horneri* Extract

Treatment	Body weight (g)	Serum concentration		
		Calcium (mg/dl)	Zinc (μg/dl)	Inorganic phosphorus (mg/dl)
Control	211.2±3.75	9.96±0.13	151.0±11.4	5.22±0.34
S. Horneri extract	215.4±5.52	10.15±0.18	167.3±22.4	4.80±0.42

Rats (50 weeks old) were orally administered the water-solubilized extract (10 mg/100 g body weight) of *S. horneri* for 14 days. Each value is the mean ± S.E.M. of six rats. Data were not significant.

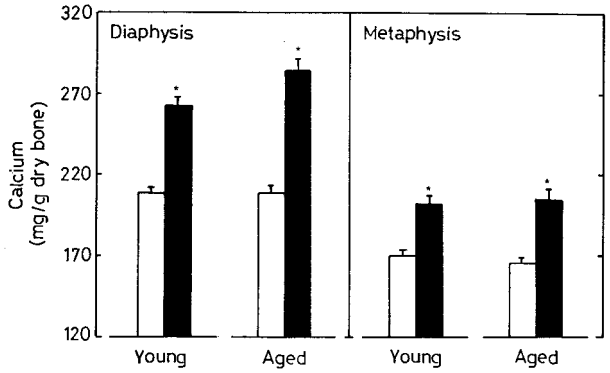

Fig. 4. Change in Calcium Content in the Femoral- Diaphyseal and -Metaphyseal Tissues of Female Young and Aged Rats Orally Administered Water-solubilized Extract of *S. Horneri in vivo*

Rats were orally administered a water-solubilized extract (10 mg/ml/100 g body weight) once daily for 14 days, and the animals were killed 24 hr after the last administration. Each values is the mean ± S.E.M. of six rats. $*p < 0.01$ compared with the control (none) value. White bars, control (none); black bars, *S. horneri* extract.

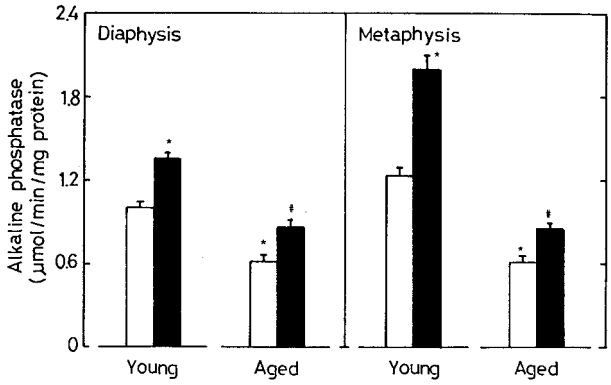

Fig. 5. Change in Alkaline Phosphatase Activity in the Femoral-Diaphyseal and -Metaphyseal Tissues of Female Young and Aged Rats Orally Administered Water-solubilized Extract of *S. Horneri in vivo*

The procedure of administration was described in the legend of Fig. 4. Each value is the mean ± S.E.M. of six rats. $*p < 0.01$ compared with the control (none) value. $\#p < 0.01$, compared with the control value from aged rats. White bars, control (none); black bars, *S. horneri* extract.

cantly altered by the prolonged oral administration of water-solubilized *S. horneri* extract, suggesting that the administration did not have a toxic effect. Also, serum calcium and inorganic phosphorus concentrations were not significantly changed by the administration of *S. horneri* extract to aged rats. The *S. horneri* extract administration-induced increase in the femoral calcium content may be not related to calcium-regulating hormones (calcitonin, parathyroid hormone, and 1,25-dihydroxyritamin D_3). Presumably, the anabolic effect of *S. horneri* extract administration to aged rats results from a direct action of the extract active component on bone tissues. *S. horneri* extract has been shown to have a stimulatory effect on bone formation and an inhibitory effect on bone resorption using rat femoral-diaphyseal and -metaphyseal tissues *in vitro*.[18,19]

When bone components of young rats were compared to those of aged rats, increasing age induced a significant decrease in alkaline phosphatase activity, a marker enzyme of osteoblastic bone formation,[27] as well as decreased DNA content, an index of number of bone cells,[28] in the femoral-diaphyseal and -metaphyseal tissues. This observation suggests that increasing age induces a decrease in osteoblastic bone formation. The administration of *S. Horneri* extract to aged rats caused a significant increase in alkaline phosphatase activity and DNA content in the femoral-diaphyseal and -metaphyseal tissues. From these results, it is assumed that the active component of *S. horneri* extract stimulates osteoblastic bone formation in the femoral-diaphyseal and -metaphyseal tissues of aged rats.

Heat-treated *S. horneri* extracts (for 30 min at 80°C) have been shown to counteract a stimulatory effect on bone formation *in vitro*.[18] The active components of a water-solubilized extract of *S. horneri* are not related to trace elements. *S. horneri* extract solubilized with 20% ethanol had no effect on bone calcification *in vitro*.[17] The identification of active components remains to be elucidated.

In conclusion, it has been demonstrated that the

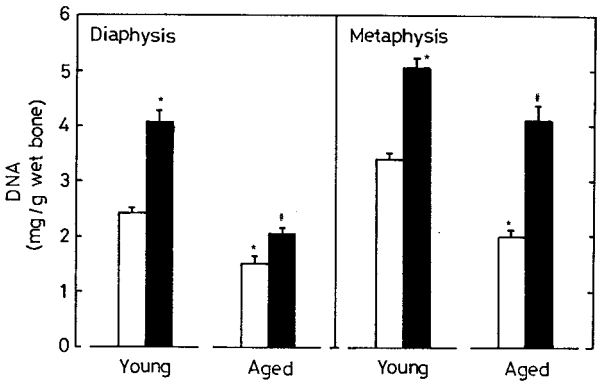

Fig. 6. Change in DNA Content in the Femoral-Diaphyseal and -Metaphyseal Tissues of Female Young and Aged Rats Orally Administered Water-solubilized Extract of *S. Horneri in vivo*

The procedure of administration was described in the legend of Fig. 4. Each value is the mean ± S.E.M. of six rats. *$p < 0.01$ compared with the control (none) value. #$p < 0.01$, compared with the control value from aged rats. White bars, control (none); black bars, *S. horneri* extract.

prolonged oral administration of *S. horneri* extract to young and aged rats has an anabolic effect on bone components in the femoral-diaphyseal and -metaphyseal tissues *in vivo*. The dietary intake of marine alga *S. horneri* extract may have a role in the prevention of bone loss with increasing age.

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