

Effect of *Sargassum horneri* Extract on Circulating Bone Metabolic Markers: Supplemental Intake Has an Effect in Healthy Humans

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The extract of *Sargassum horneri* (*S. horneri*) has been shown to have an anabolic effect on bone components due to stimulating bone formation and to inhibiting bone resorption in rat femoral tissues *in vitro* and *in vivo*. This study was undertaken to determine the effect of supplemental intake of the water-solubilized *S. horneri* extract on circulating bone metabolic markers in healthy humans. Thirty-six volunteers, aged 20–60 years (16 men and 20 women), were enrolled in this study. Volunteers were divided into three groups; placebo tablet without *S. horneri* extract (5 men and 7 women), tablet containing *S. horneri* extract at 300 mg/day (6 men and 7 women) or 900 mg/day (5 men and 6 women). Placebo or *S. horneri* extract tablet was ingested once a day for 4 or 8 weeks. Bone-specific alkaline phosphatase and γ -carboxylated osteocalcin are serum bone markers of bone formation, and bone tartrate-resistant acid phosphatase (TRACP) and *N*-telopeptides of type I collagen are markers of bone resorption. Serum bone-specific alkaline phosphatase or γ -carboxylated osteocalcin concentration was not significantly changed after the intake of *S. horneri* extract (300 or 900 mg/day) for 4 or 8 weeks. Serum bone TRACP activity was significantly decreased after the intake of *S. horneri* extract (300 or 900 mg/day) for 8 weeks. Serum *N*-telopeptides of type I collagen concentration was significantly decreased after the intake of *S. horneri* extract (900 mg/day) for 8 weeks. Meanwhile, serum calcium, inorganic phosphorus, and other biochemical findings were not changed after the intake of *S. horneri* extract (300 or 900 mg/day) for 4 or 8 weeks. This study demonstrates that the prolonged intake of *S. horneri* extract has inhibitory effects on bone resorption in humans.

Key words — marine algae, *Sargassum horneri*, bone resorption, bone metabolism, osteoporosis

INTRODUCTION

Bone loss with increasing age induces osteoporosis,^{1–3)} and it may be due to increased bone resorption and decreased bone formation. 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. Food factors may help to prevent bone loss with increasing age.^{5–7)} There are growing evidences that food factors may

have an important role in the prevention of osteoporosis.^{6,7)}

Among various marine algae, *Sargassum horneri* (*S. horneri*) extract has an anabolic effect on bone calcification in rat femoral tissues *in vitro* and *in vivo*.⁸⁾ *S. horneri* extract has been demonstrated to stimulate osteoblastic bone formation and inhibit osteoclastic bone resorption *in vitro* using rat femoral-diaphyseal and -metaphyseal tissues.^{9,10)} It is found that the active components, which have a stimulating effect on bone calcification and a suppressing effect on osteoclastic bone resorption, are different.¹¹⁾ The active component in stimulating bone calcification seems to be nearby molecular weight (MW) 1000, while the active component of *S. horneri* extract in inhibiting osteoclastic cell formation is at more than MW 50000.¹¹⁾ The intake of *S. horneri* extract may have a preventive effect on bone loss with increasing age,¹²⁾ and it also has

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a preventive effect on bone loss in streptozotocin-diabetic rats *in vivo*.¹³⁾ Thus the intake of *S. horneri* extract may have a preventive effect on osteoporosis.

The present study, moreover, was undertaken to determine whether the prolonged intake of *S. horneri* extract tablet has an effect on bone metabolism in healthy humans; this was estimated using the analysis of circulating bone metabolic markers.

MATERIALS AND METHODS

***S. horneri* Extracts**—The marine alga *S. horneri* was seasonally gathered from the coast at Iwate Prefecture (Japan), and was freeze-dried and

powered.^{8,9)} The fresh marine alga gathered was homogenized in distilled water with a Physcotron homogenizer, and the homogenate was centrifuged at $5500 \times g$ in a refrigerated centrifuge for 10 min. The $5500 \times g$ supernatant fraction was pooled for freeze-drying. Powder of the water-solubilized extract, which contains active components in stimulating bone formation and in inhibiting bone resorption, was made as tablet to use in experiments.

Experimental Procedures—Thirty-six volunteers, aged 20–60 years (16 men and 20 women), who were judged to be healthy with no abnormal liver or kidney function as assessed by standard clinical and biochemical data, were enrolled as volunteers in this study. Informed consent was obtained from all before enrollment. The intake of other foods with marine alga was prohibited during

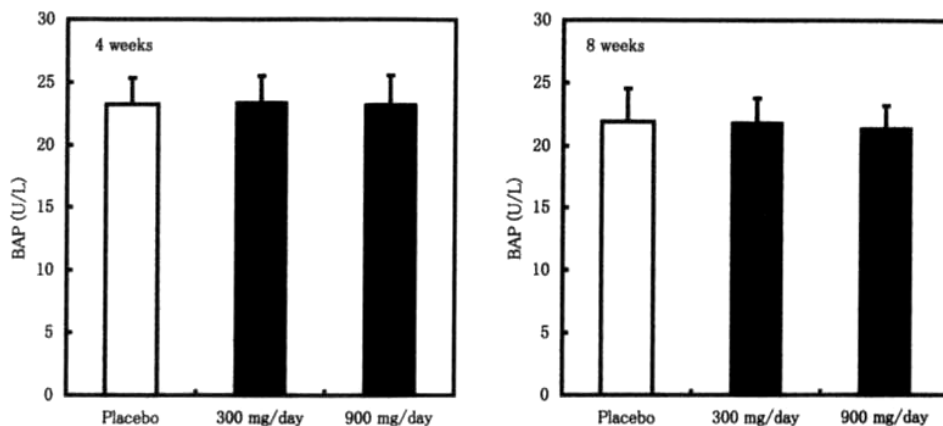


Fig. 1. Changes in Serum Bone-Specific Alkaline Phosphatase (BAP) Activity Following Intake of *S. horneri* Extract

S. horneri extract (placebo, 300, or 900 mg/day) was given to volunteers once daily for 4 or 8 weeks. Thirty-six subjects were divided into three groups of 12, 13, or 11 subjects for the intake of *S. horneri* extract (placebo, 300, or 900 mg/day), respectively. Each value is the mean \pm SEM of each group subjects. There were no significant differences among the groups compared with the value obtained after placebo intake.

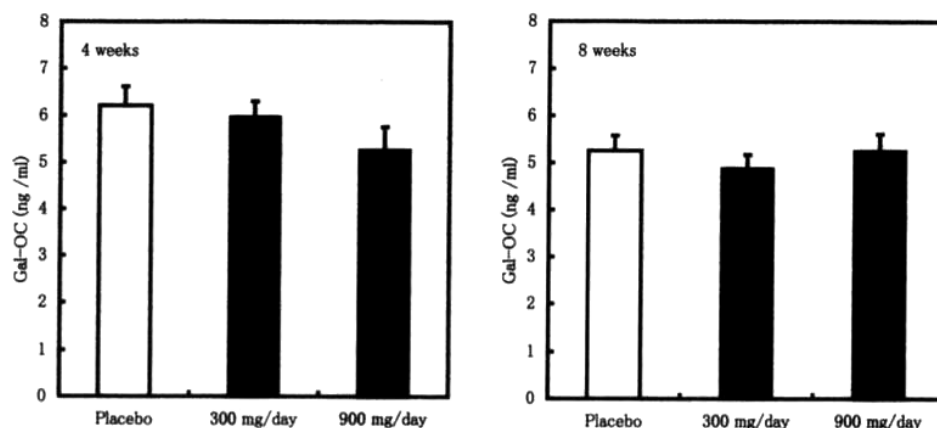


Fig. 2. Changes in Serum γ -Carboxylated Osteocalcin (Gal-OC) Concentrations Following Intake of *S. horneri* Extract

The procedure for the intake of *S. horneri* extract is described in Fig. 1. Each value is the mean \pm SEM of each group subjects. There were no significant differences among the groups compared with the value obtained after placebo intake.

the experimental period.

The washout and intake periods of *S. horneri* extract tablets were 4 and 8 weeks, respectively. Volunteers were divided into three groups; placebo tablet without *S. horneri* extract (5 men and 7 women), tablet containing *S. horneri* extract at 300 mg/day (6 men and 7 women), or 900 mg/day (5 men and 6 women). Placebo or *S. horneri* extract tablet was sequentially given once daily for 4 or 8 weeks.

Blood samples were collected from each between 9:00 and 11:30 (morning) on the day prior to intake (control), and 4 and 8 weeks after the start of intake. Serum samples were prepared between 30 and 60 min after blood sampling and then stored at -80°C until assayed.

Analytical Procedures—The serum bone-specific alkaline phosphatase activity was assayed using a METRA BAP EIA kit (Quidel, San Diego, CA, U.S.A.).¹⁴⁾ Serum γ -carboxylated osteocalcin was assayed using a Gla type osteocalcin (γ -carboxylated osteocalcin) EIA kit (Takara Shuzo, Shiga, Japan).¹⁵⁾ Serum bone tartrate-resistant acid phosphatase (TRACP) activity was assayed using a Bone TRACP Assay EIA kit (SBA Sciences, Turku, Finland).¹⁶⁾ Serum bone *N*-telopeptides of type I collagen was measured using an OSTEOMARK NTx Serum EIA kit (Mochida Pharmaceutical Co., Ltd., Tokyo, Japan).¹⁷⁾

Serum total protein, nitrogen urea, creatinine, uric acid, total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, triglyceride, total bilirubin, glutamic-

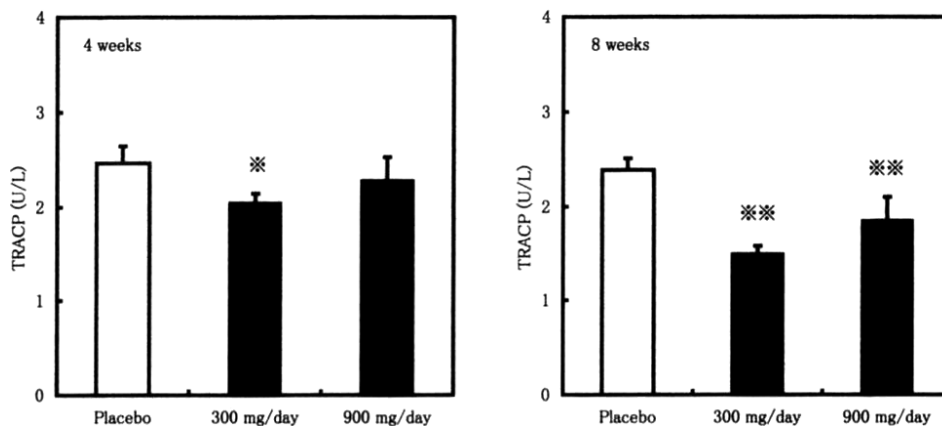


Fig. 3. Changes in Serum Bone TRACP Activity Following Intake of *S. horneri* Extract

The procedure for the intake of *S. horneri* extract is described in Fig. 1. Each value is the mean \pm SEM of each group subjects. * $p < 0.05$ or ** $p < 0.01$ compared with the value obtained after intake of placebo.

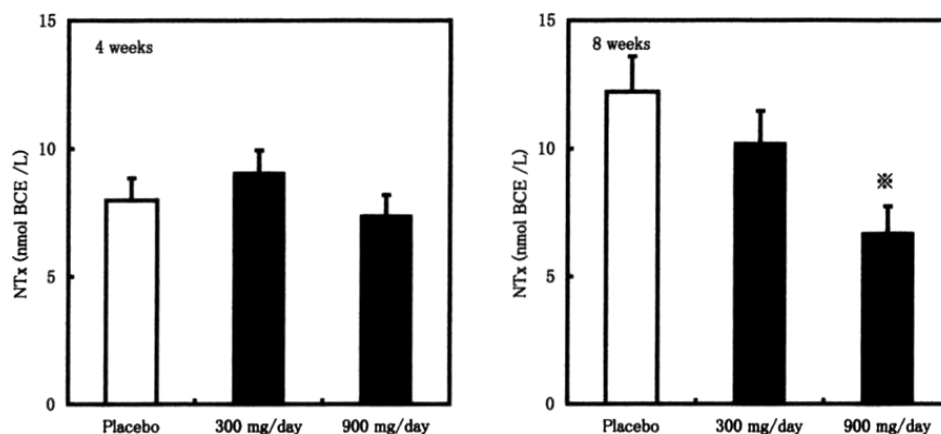


Fig. 4. Changes in Serum Concentration of *N*-Telopeptide of Type I Collagen (NTx) Following Intake of *S. horneri* Extract

The procedure for the intake of *S. horneri* extract is described in Fig. 1. Each value is the mean \pm SEM of each group subjects. * $p < 0.01$ compared with the value obtained after intake of placebo.

oxaloacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT), alkaline phosphatase, acid phosphatase, γ -glutamyltransferase (γ -GTP), amylase, calcium, inorganic phosphorus, and glucose levels were determined using kits.

Statistical Analysis— Differences in values after the intake of placebo and *S. horneri* extract tablet were estimated using Student's *t*-test. A paired *t*-test was used for differences in values between placebo and the intake of *S. horneri* extract tablet after each intake period. We also used multiple analysis of variance (ANOVA) to compare the treatment groups. Values of *p* less than 0.05 were considered to represent statistically significant differences.

RESULTS

Changes in Bone Metabolic Markers Following the Intake of *S. horneri* Extract in Humans

Thirty-six volunteers, aged 20–60 years (16 men and 20 women), were enrolled in this study. Volunteers were divided into three groups; placebo tablet without *S. horneri* extract (5 men and 7 women), tablet containing *S. horneri* extract at 300 mg/day (6 men and 7 women), or 900 mg/day (5 men and 6 women). Placebo or *S. horneri* extract tablet was sequentially given once daily for 4 or 8 weeks.

Serum bone-specific alkaline phosphatase activity (Fig. 1) or γ -carboxylated osteocalcin concentration (Fig. 2) was not significantly changed after the intake of *S. horneri* extract (300 or 900 mg/day) for 4 or 8 weeks.

Serum bone TRACP activity was significantly decreased after the intake of *S. horneri* extract (300 mg/day) for 4 weeks (Fig. 3). TRACP activity was significantly decreased after the intake of *S. horneri* extract (300 or 900 mg/day) for 8 weeks (Fig. 3).

Serum *N*-telopeptides of type I collagen concentration was significantly decreased after the intake of *S. horneri* extract (900 mg/day) for 8 weeks (Fig. 4).

Effects of Intake of *S. horneri* Extract on Other Markers

Serum total protein, nitrogen urea, creatinine, uric acid, total cholesterol, LDL cholesterol, HDL cholesterol, triglyceride, total bilirubin, GOT, GPT, alkaline phosphatase, acid phosphatase, γ -GTP, amylase, calcium, inorganic phosphorus, and glucose levels were not significantly changed after the intake of *S. horneri* extract (300 or 900 mg/day) for 8 weeks (Table 1).

Hematological changes (including the number of leukocytes, red blood cell, and thrombocytes) were not observed after the intake of *S. horneri*

Table 1. Serum Metabolic Findings Following Intake of *S. horneri* Extract

Serum level	Placebo	300 mg/day	900 mg/day
Total protein (g/dl)	7.4 \pm 0.45	7.2 \pm 0.43	7.4 \pm 0.33
Urea nitrogen (mg/dl)	12.7 \pm 3.3	7.2 \pm 0.43	15.1 \pm 3.4
Creatinine (mg/dl)	0.67 \pm 0.10	0.72 \pm 0.18	0.69 \pm 0.09
Uric acid (mg/dl)	4.8 \pm 1.3	4.8 \pm 1.1	4.8 \pm 1.8
Total cholesterol (mg/dl)	211.7 \pm 24.6	205.0 \pm 29.0	194.6 \pm 18.4
LDL-cholesterol (mg/dl)	122.4 \pm 19.0	120.4 \pm 21.4	111.8 \pm 17.7
HDL-cholesterol (mg/dl)	72.8 \pm 20.4	67.3 \pm 14.1	67.5 \pm 10.4
Triglyceride (mg/dl)	75.9 \pm 48.1	67.3 \pm 14.1	71.9 \pm 64.5
Total bilirubin (mg/dl)	0.58 \pm 0.24	0.74 \pm 0.37	0.62 \pm 0.23
GOT (IU/l)	19.1 \pm 3.2	19.2 \pm 3.1	22.6 \pm 3.2
GPT (IU/l)	21.0 \pm 17.0	18.6 \pm 8.0	20.7 \pm 25.0
Alkaline phosphatase (IU/l)	202.1 \pm 59.2	187.7 \pm 44.7	202.7 \pm 33.3
γ -GTP (IU/l)	24.6 \pm 18.9	24.9 \pm 16.9	22.0 \pm 25.2
Amylase (IU/l)	69.4 \pm 21.2	73.9 \pm 18.6	75.5 \pm 16.8
Calcium (mg/dl)	9.0 \pm 0.25	9.0 \pm 0.37	8.9 \pm 0.35
Phosphorus (mg/dl)	3.2 \pm 0.53	2.9 \pm 0.29	2.9 \pm 0.40
Glucose (mg/dl)	87.8 \pm 6.9	90.2 \pm 9.4	91.6 \pm 8.2
Acid phosphatase (IU/l)	11.3 \pm 2.4	9.9 \pm 1.4	10.4 \pm 2.3

S. horneri extract was given to volunteers for 8 weeks. Sixty subjects were divided into three groups of 12, 13, or 11 subjects. Each value is the mean \pm SEM. Data were not significant as compared with that of placebo.

extract (300 or 900 mg/day) for 8 weeks (data not shown). Urinary biochemical findings (including calcium, inorganic phosphorus, creatinine, protein, and glucose) were not significantly changed after the intake of *S. horneri* extract (300 or 900 mg/day) for 8 weeks (data not shown). Blood pressure was not significantly changed after the intake of *S. horneri* extract (300 or 900 mg/day) for 8 weeks (data not shown).

The intake of *S. horneri* extract (300 or 900 mg/day) for 8 weeks did not have any negative effects.

DISCUSSION

Bone loss with aging may be due to decreased bone formation and increased bone resorption. Chemical factors in food and plants may help to prevent bone loss due to increasing age. Marine alga *S. horneri* extract has been shown to have stimulatory effects on bone formation and inhibitory effects on bone resorption in rat femoral tissues *in vitro* and *in vivo*.^{8–13} This study, moreover, demonstrates that the supplemental intake of *S. horneri* extract has an effect on circulating bone metabolic markers in healthy humans.

The intake of *S. horneri* extract for 8 weeks was found to have suppressive effects on serum TRACP activity and N-telopeptides of type I collagen level. These are bone metabolic markers that are increased in osteoclasts with bone resorption.¹⁸ This finding suggests that the intake of *S. horneri* extract has an inhibitory effect on bone resorption in humans.

Serum bone-specific alkaline phosphatase activity or γ -carboxylated osteocalcin is bone formation markers. These proteins are produced in osteoblasts.¹⁹ These bone formation markers were not significantly changed after the intake of *S. horneri* extract (300 or 900 mg/day) for 8 weeks. A significant increase in bone formation markers may be caused after the prolonged intake of *S. horneri* extract (300 or 900 mg/day). It is speculated that the supplemental intake of *S. horneri* extract reveals suppressive effects on bone resorption, and later it exhibits stimulatory effects on bone formation in humans, thereby increasing bone mass. Presumably, the supplemental intake of *S. horneri* extract has a preventive effect on bone loss with increasing age. Further study is needed in demonstrating the effect in menopausal women.

The intake of *S. horneri* extract (900 mg/day)

for 8 weeks did not have a significant alteration in other biochemical markers for the metabolic function of organs, suggesting that the intake does not have toxic effects in humans.

In conclusion, it has been demonstrated that the intake of *S. horneri* extract has suppressive effect on serum bone resorption markers in humans.

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