

Marine alga *Sargassum horneri* active component: Prevention of obese diabetic bone loss

Masayoshi Yamaguchi*

Department of Hematology and Medical Oncology, Emory University School of Medicine, Atlanta, USA

Abstract

Osteoporosis with its accompanying decrease in bone mass is widely recognized as a major public health problem. Food and nutritional factors may play a role in the prevention of bone loss with aging. This, however, is poorly understood. Among marine algae of *Undaria pinnatifida*, *Sargassum horneri*, *Eisenia bicyclis*, *Cryptonemia scmitziana*, *Gelidium amansii*, and *Ulva pertusa* Kjellman that were gathered seasonally, *Sargassum horneri* (*S. horneri*) was found to reveal a unique anabolic effect on bone. *S. horneri* active component revealed a stimulatory effect on osteoblastic bone formation and a suppressive effect on osteoclastic bone resorption *in vitro*, thereby increasing bone mass. Moreover, *S. horneri* active component suppressed adipogenesis in bone marrow cells *in vitro*. Intake of *S. horneri* active component revealed a preventive effect on bone loss in animal models for type 1 diabetes. Moreover, intake of *S. horneri* active component was demonstrated to reveal anabolic effects on bone metabolism in healthy human. *S. horneri* active component, a new osteogenic factor, may be a useful in the improvement of obese diabetic osteoporosis.

Introduction

Bone is a dynamic tissue that preserves skeletal size, shape, and structural integrity and regulates mineral homeostasis. Bone homeostasis is maintained through a balance between osteoblastic bone formation and osteoclastic bone resorption. Aging and numerous pathological processes induce decrease in bone formation and increase in bone resorption, leading to osteoporosis that is a devastating bone disease [1]. Osteoporosis, which is induced with increase in bone loss, is widely recognized as a major public health problem. The most dramatic expression of the disease is represented by fractures of the proximal femur for which the number increases as the population ages [2].

Currently, obesity and diabetes are a major health problem worldwide with growing in prevalence. Obesity and diabetes induce secondary diseases with various pathophysiological states including cardiovascular disease, neural disturbance, kidney disease, cancer and osteoporosis. Osteoporosis associated with obesity and diabetes has also been noticed [3-6]. Diabetes is seen frequent in the elderly and therefore frequently coexists with osteoporosis. The incidence of metabolic disease, including obesity and obese type 2 diabetes, has been increased to epidemic levels in recent years [5]. Type 1 and obese type 2 diabetes have been associated with increased fracture risk [4]. Thus, obesity, diabetes and osteoporosis are closely related. One of the shared features is that osteoblasts and adipocytes differentiate from a common precursor cell in the bone marrow mesenchymal stem cells [7,8]. There is an inverse relationship between differentiation of mesenchymal stem cells to osteoblasts and adipocytes [7,8]. It may be important to prevent and improve obese diabetic osteoporosis in clinical aspects.

There is growing evidence that the supplementation of nutritional and food factors may have preventive effects on bone loss that is induced in animal model of osteoporosis and in human subjects [9-12]. Functional factors in food and plants, which regulate on bone homeostasis, have been to be worthy of notice in maintaining of bone health and prevention of bone loss with increasing age [13-15]. The effect of marine algae on bone metabolism is poorly understood.

Undaria pinnatifida, *Sargassum horneri*, *Eisenia bicyclis*, *Cryptonemia scmitziana*, *Gelidium amansii*, and *Ulva pertusa* Kjellman are applied as food materials. Among these marine algae, *Sargassum horneri* (*S. horneri*) was found to have a unique anabolic effect on bone [16]. *S. horneri* extract has been found to have a stimulatory effect on osteoblastic bone formation and a suppressive effect on osteoclastic bone resorption *in vitro*, thereby increasing bone mass. Moreover, *S. horneri* active component was found to reveal suppressive effects on adipogenesis in bone marrow cells [17]. Intake of *S. horneri* active component revealed a preventive effect on bone loss in animal models for type 1 diabetes [18]. This review has been written to outline our recent advances that have been made concerning preventive effects of *S. horneri* active component on osteoporosis.

Bone homeostasis and osteoporosis

Bone metabolism plays a physiological role to maintain skeletal structure and to regulate mineral homeostasis. Bone remodeling and modeling underpin development and maintenance of the skeletal system [19]. Bone modeling is responsible for growth and mechanically induced adaptation of bone, and it requires that the processes of bone formation and bone removal (resorption). Process of bone remodeling that makes bone unique among organs and tissues, and add so many levels of complexity with respect to interactions along remodeling sequence by systemic influences (hormones), stress action (physical activity/weight bearing), and growth factors and cytokines produced from the bone cells or factors that come from nearby cells in the

Correspondence to: Dr. Masayoshi Yamaguchi, Department of Hematology and Biomedical Oncology, Emory University School of Medicine, 1365 C Clifton Road, NE, Atlanta, GA 30322, USA, **E-mail:** yamamasa1155@yahoo.co.jp

Key words: *S. horneri* extract, osteoblastic bone formation, osteoclastic bone resorption, adipogenesis, obese diabetes, osteoporosis

Received: August 14, 2014; **Accepted:** August 16, 2014; **Published:** August 19, 2014

marrow tissues. Bone homeostasis, which maintains bone mass, is skillfully regulated through a delicate balance between osteoblastic bone formation and osteoclastic bone resorption [20-22]. Bone acts as major storage site for growth factors, which are produced by osteoblasts, diffuse into newly deposited osteoid and are stored in the bone matrix including insulin-like growth factors (IGF-I and II), transforming growth factor- β 1 (TGF- β 1), platelet-derived growth factor (PDGF), or bone morphogenic proteins (BMPs) [23,24]. These bone-derived factors, which can be liberated during subsequent periods of bone resorption, act in an autocrine, paracrine, or delayed paracrine fashion in the local microenvironment of the bone surface.

Osteoporosis is a disease characterized by loss in bone density and bone strength and deterioration of bone microarchitecture, resulting in increased risk for bone fractures. The most dramatic expression of osteoporosis is represented by fractures of the proximal femur for which the number increases as the population ages [2]. Osteoporosis is a common metabolic disease and generally affects people at an advanced age and suffering from other chronic diseases. It is more common in women and a significant loss of bone mass after menopause begins. Bone mass is dramatically reduced after menopause, which depresses secretion of ovarian hormone (estrogen) in women [1]. Deficiency of estrogen advances osteoclastic bone resorption. This is very important as a primary osteoporosis. Postmenopausal osteoporosis is the archetypal osteoporotic condition in women after menopause. Osteoporosis is a major cause of increased morbidity and mortality affecting the aging population. It has been estimated that osteoporosis affects at least 200 million women worldwide, one third of women aged between 60 and 70 years and two thirds over 80 years [2,25]. In 1995, incidence of osteoporotic fractures in the U.S. was about 1.5 million, of which 750 000 vertebral fractures, 250,000 hip fractures, 250,000 fractures in the wrist fracture and 250 000 other locations. According to a recent World Health Organization report, osteoporosis has become a global health problem with a disease incidence and mortality rate similar to that of cardiovascular diseases, cancer and diabetes [3-6]. Osteoporosis is widely recognized as a major public health threat.

***S. horneri* active component stimulates osteoblastic bone formation**

Marine algae *S. horneri* [*Sargassum horneri* (Turner) C. Agardh] was seasonally gathered from the coast at Shimoda (Shizuoka Prefecture, Japan) and Miyako (Iwate Prefecture, Japan), and it was freeze-dried and powdered [16]. The gathered fresh marine algae were homogenized in distilled water and are centrifuged at 5500 g in a refrigerated centrifuge for 10 minutes. The 5500 g supernatant fraction was pooled for freeze-drying. The powder of the water-solubilized extract was dissolved in ice-cold distilled water for use in the experiments. The water-solubilized extract from *S. horneri* was purified through the method of membrane fractionation to collect active component of various molecular weights.

The direct effect of *S. horneri* active component on bone formation and mineralization has been examined using bone tissues and MC3T3 preosteoblastic cells *in vitro* [26,27]. Preosteoblastic cells were differentiated into mineralizing osteoblasts in the presence of *S. horneri* active component in culture with mineralizing medium for 21 days *in vitro* [27]. *S. horneri* active component (10 and 25 μ g/ml) was found to potentially enhance mineralization in both MC3T3 cells [27]. The *S. horneri* extract (25-100 μ g/ml of medium) did not have a significant effect on cell number of MC3T3-E1 cells with culture [27]. *S. horneri* active component did not appear to mediate direct toxic effects on the

cultures as cells were proliferated robustly over the culture period, and were still alive and visibly attached to the plate at the end of the experiment [27].

Bone morphogenic proteins (BMPs) such as BMP-2 are anabolic agents that signal through the Smad signaling pathway [24]. The effect of *S. horneri* extract on basal and BMP-2-induced Smad activation has been shown using a Smad 4-luciferase reporter responsive to all Smad species [27]. *S. horneri* active component did not have a direct effect on basal Smad activation. *S. horneri* active component (50 or 100 μ g/ml) enhanced Smad-activation induced by BMP-2. Also, *S. horneri* active component (25 -100 μ g/ml) enhanced Smad-activation induced by TGF- β 1 [27]. NF- κ B activation is a potent inhibitory to osteoblast differentiation, and TNF- α -induced NF- κ B activation leads to Smad suppression in MC3T3 osteoblast precursors [28,29]. Whether *S. horneri* active component is able to prevent TNF- α -induced NF- κ B activation in MC3T3 osteoblast precursors was examined [27]. *S. horneri* active component (25-100 μ g/ml of medium) prevented TNF- α -induced NF- κ B activation, although *S. horneri* active component did not have a direct effect on basal NF- κ B activation. Thus, *S. horneri* active component has been shown to stimulate osteoblastic differentiation and mineralization *in vitro*. TGF- β 1- and BMP-2-induced activation of Smad signaling respectively plays an important role in the early commitment and differentiation of osteoblasts [30]. *S. horneri* active component was found to enhance BMP-2- or TGF- β 1-induced Smad activation with the dose-dependency [27]. *S. horneri* extract-induced Smad enhancement may be an important to stimulate osteoblastic differentiation and mineralization.

NF- κ B signaling has been shown to downregulate osteoblast differentiation [28,29]. One major mechanism appears to involve the intersection of NF- κ B with the Smad signaling pathway [30]. NF- κ B signaling in osteoblasts intersects with and disrupts Smad signaling by promoting production of Smad7, an inhibitor of TGF- β - and BMP-induced R-Smad activation [30]. TNF- α further antagonizes BMP signaling by upregulating Smad ubiquitination regulatory factor 1 (Smurf1), promoting proteasomal degradation of bone morphogenetic signaling proteins [31]. Multiple suppressors of NF- κ B activation are capable of rescuing the inhibitory effect of TNF- α on BMP-2 and/or TGF- β 1-induced Smad activation [28]. *S. horneri* active component was found to suppress TNF- α -induced NF- κ B activation in preosteoblastic MC3T3-E1 cells [27]. This finding provides a possible molecular mechanism by which *S. horneri* active component stimulates osteoblastic bone formation.

The anabolic effect of *S. horneri* active component on bone tissues *in vitro* has been found. Rat femoral-metaphyseal tissues were cultured in a medium containing water-solubilized extract (25 and 50 μ g/ml) obtained from *U. pinnatifida*, *S. horneri*, *E. bicyclis*, or *C. scmitziana* *in vitro* [16,26]. Bone calcium content was significantly elevated in the presence of *S. horneri* active component (25 and 50 μ g/ml). No effect was seen in the extracts of other marine algae. In addition, water suspensions (5%) of marine alga powder were orally administered once daily for 7 days. Bone calcium content was significantly increased after the administration of *U. pinnatifida*, *S. horneri*, *E. bicyclis*, or *C. scmitziana* [16]. Also, bone alkaline phosphatase activity, which is an enzyme for calcification [32], was significantly enhanced with the administration of *S. horneri* or *G. amansii*. Thus, *S. horneri* active component has a unique anabolic effect on bone calcification *in vitro* and *in vivo* [16]. The effects of *S. horneri* active component in increasing calcium content, alkaline phosphatase activity, and deoxyribonucleic acid (DNA) content in the femoral-diaphyseal and

-metaphyseal tissues *in vitro* was completely abolished in the presence of cycloheximide, an inhibitor of protein synthesis [16]. Anabolic effect of *S. horneri* active component may be resulted from newly synthesized protein components.

***S. horneri* active component suppresses osteoclastic bone resorption**

S. horneri extract has been shown to suppress osteoclastic bone resorption. To examine the effect of *S. horneri* active component on osteoclast formation, RAW264.7 osteoclast precursors were differentiated into mature osteoclasts by stimulation with RANKL in the presence or absence of *S. horneri* active component with the dose range of 5 to 100 µg/ml of medium [27]. The *S. horneri* active component did not have effects on cell number of preosteoclasts (RAW267.4 cells) with culture. *S. horneri* extract (100 µg/ml) did not have a toxicity to the precursors suppressing their proliferation over 7 days of culture [27]. *S. horneri* active component (25–100 µg/ml) significantly suppressed osteoclast formation induced by RANKL [27].

The NF-κB signal transduction pathway is essential for the generation of osteoclasts [20,21]. The effect of *S. horneri* active component on NF-κB activation by RANKL in osteoclast precursors has been examined. RAW 264.7 cells were transfected with an NF-κB reporter and stimulated with RANKL to induce NF-κB activity in the presence or absence of *S. horneri* active component (5-100 µg/ml) [27]. *S. horneri* active component did not have a significant effect on basal NF-κB activity [27]. However, RANKL-induced increase in NF-κB activity was significantly blunted in the presence of *S. horneri* active component [27].

S. horneri active component with dose-dependency, which did not have cell toxicity, was found to have a suppressive effect on RANKL-stimulated osteoclastogenesis [27]. RANKL, the key osteoclastogenic cytokine, is central to formation of osteoclasts, the cells that resorb bone, through NF-κB signaling [20,21]. *S. horneri* active component suppressed RANKL-induced NF-κB activation in osteoclast precursor cells [27]. This finding coincided with the observation that *S. horneri* extract suppresses RANKL-induced osteoclastogenesis [27]. *S. horneri* active component may suppress osteoclastogenesis through suppression of NF-κB activation.

The effect of *S. horneri* active component on mineralization in MC3T3-E1 cells with culture for 21 days was observed at 10 µg/ml [27]. However, the effects of *S. horneri* active component on Smad activity in MC3T3-E1 cells and on NF-κB activity in RAW 264.7 cells with culture for 24 hours were observed at 25 µg/ml [27]. The effects of *S. horneri* active component on osteoclastogenesis in RAW 264.7 cells with culture for 6 days were also revealed at 25 µg/ml [27]. The effect of *S. horneri* active component on osteoblastogenesis and osteoclastogenesis may be observed with lower doses in the culture for longer periods.

Suppressive effects of *S. horneri* active component on bone resorption using femoral tissues *in vitro* have been demonstrated [33]. Parathyroid hormone (PTH) and prostaglandin E₂ (PGE₂) are known to induce osteoclastic bone resorption [34]. PTH- and PGE₂-induced decreases in bone calcium content were inhibited after culture with *S. horneri* active component (10, 25, and 50 µg/ml) [33]. Also, *S. horneri* active component suppressed PTH- or PGE₂-induced increase in medium glucose consumption and lactic acid production by the bone tissues [33]. Moreover, *S. horneri* active component blocked PTH-induced increase in acid phosphatase activity in the diaphyseal and metaphyseal tissues [33]. These findings indicate that *S. horneri* active

component has a direct suppressive effect on bone resorption in tissue culture *in vitro*.

S. horneri active component possesses a potent Smad activation and anti-NF-κB activity and may have promise for development into an antiosteoporotic material capable of promoting new bone formation while simultaneously reducing bone resorption. *S. horneri* active component may be usefulness as a tool in the prevention of osteolysis with various pathophysiological states.

***S. horneri* active component suppresses adipogenesis in bone marrow cells**

S. horneri active component has been found to reveal suppressive effects on adipogenesis, which is involved in obesity [17]. Mouse bone marrow cells were cultured for 3 days in differentiation medium (containing dexamethasone and 3-isobutyl-1-methylxanthine) with either vehicle or *S. horneri* active component (5, 10, 25, and 50 µg/ml of medium per well) for 48 hours, and then the cells were cultured in the presence of insulin or *S. horneri* active component (5, 10, 25, and 50 µg/ml of medium per well) for an additional 4 days [17]. Adipogenesis was markedly enhanced after culture with medium containing insulin [17]. This enhancement was clearly suppressed in the presence of *S. horneri* active component (10-50 µg/ml of medium) [17]. Thus, *S. horneri* active component was found to cause suppression of adipogenesis in bone marrow culture *in vitro*. Culture with *S. horneri* active component (10-50 µg/ml of medium), which was added at early stage of differentiation of bone marrow cells to adipocytes, was also found to cause a significant suppression of adipogenesis in bone marrow culture *in vitro* [17]. Thus, *S. horneri* active component was found to suppress processes of the differentiation from bone marrow mesenchymal stem cells to pre-adipocytes and from pre-adipocytes to mature adipocytes.

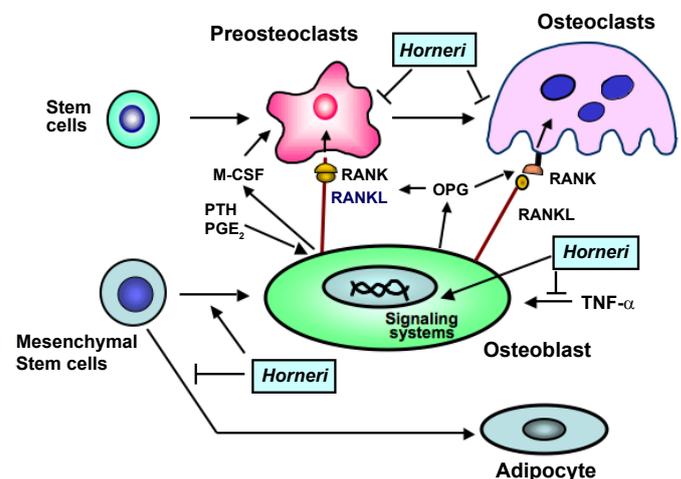


Figure 1. Involvement of *S. horneri* active component in bone homeostasis and adipogenesis. RANKL is produced by bone resorbing factors (PTH, PGE₂ and other) in osteoblasts and binds RANK to activate osteoclastogenesis. Osteoprotegerin (OPG), a natural antagonist of RANKL, inhibits binding of RANKL to RANK. Macrophage-colony stimulating factor (M-CSF) stimulates formation of preosteoclasts from stem cells. *S. horneri* active component stimulates osteoblastic bone formation and suppresses osteoclastic bone resorption, thereby increasing bone mass. *S. horneri* active component enhances bone growth factors (TGF-β1 and BMP-2)-induced Smad activation in osteoblasts and suppresses TNF-α- and RANKL-enhanced NF-κB activation in preosteoclasts and osteoclasts, suggesting a possible molecular mechanism of osteogenic effects of *S. horneri* active component. Moreover, bone marrow mesenchymal stem cells are differentiated to adipocytes *S. horneri* active component suppresses adipogenesis from bone marrow mesenchymal stem cells, suggesting an involvement in the prevention of obesity and its related bone loss.

S. horneri active component suppresses adipogenesis and may lead to prevention of obesity. Intake of *S. horneri* active component may be useful tool in the prevention and therapy on obesity and obesity-induced type 2 diabetes.

S. horneri active component, which contains less than molecular weight 3000, has been shown to stimulate osteoblastic bone formation and suppress osteoclastic bone resorption in cloned bone cells but not bone marrow cells [27]. *S. horneri* active component, which contains less than molecular weight 3000, reveals suppressive effects on the differentiation from bone marrow mesenchymal stem cells to adipocytes, thereby inhibiting adipogenesis. *S. horneri* active component may be useful tool in the prevention and treatment of obesity with enhancement of adipogenesis.

Characterization of active component in *S. horneri*

S. horneri active component has been found to stimulate osteoblastic bone formation and to suppress osteoclastic bone resorption. Those active components have been found to be present in *S. horneri* extract obtained from various coasts in both Japan (Shimoda and Iwate, Japan) and China [35]. The active component of *S. horneri* extract in stimulating bone calcification has been found to be near molecular weight (MW) 3000 [27]. Meanwhile, the active component of *S. horneri* extract in inhibiting osteoclastic cell formation was less than MW 3000 and over MW 50000 [23,33]. These components were stable under heat treatment. It is speculated that the active component in stimulating bone calcification is a chemical but not peptide, and that the component over MW 50000 in inhibiting bone resorption may be a polysaccharide. These active components obtained from the coasts of Iwate (Japan) or China showed an identical molecular weight. The active component, which stimulates osteoblastogenesis and suppresses osteoclastogenesis, was found to be present in the components less than MW 3000 of *S. horneri* extract using preosteoblastic cells and RAW267.4 cells *in vitro* [27]. Active component that stimulates osteoblastic bone formation and suppresses osteoclastic bone resorption may be identical.

We found the existence of 4 chemicals in *S. horneri* active component (less than MW 3000) using the analysis with liquid chromatography mass spectrophotometry system (LCMS-IT-TOF; Shimadzu, Kyoto, Japan). These chemicals were identified as 1,3,5-tris(oxolan-2-ylmethyl)-1,3,5-triazinane (MW 339), 5-phenyl-2-[2-(5-phenyltetrazol-2-yl)ethyl] tetrazole (MW 318), 3-(hexadecylamino) propane-1,2-diol (MW 316), and 2-(2-hydroxyethyl)-tridecylamino ethanol (MW 288). These chemicals may have an effect on osteoblastogenesis and/or osteoclastogenesis. It is possible that the combination of these compounds reveals a potential anabolic effect on bone.

S. horneri active component improves diabetic bone loss *in vivo*

The anabolic effects of *S. horneri* active component on bone components in the femoral tissues of young and aged rats *in vivo* have been demonstrated [36]. Calcium content, alkaline phosphatase activity, and DNA content in the femoral-diaphyseal and -metaphyseal tissues of young male (4-week-old) rats were increased after the administration of *S. horneri* active component (25, 50, and 100 mg/kg) for 7 days [36]. Moreover, these bone components in the femoral-diaphyseal and -metaphyseal tissues of aged female (50-week-old) rats were increased after the administration of *S. horneri* active component (100 mg/kg) for 14 days, suggesting a preventive effect on bone loss with increasing age [36].

Intake of *S. horneri* active component has been shown to reveal a preventive effect on bone loss in a diabetes state [18]. Diabetes has been shown to induce bone loss [4,37,38]. Streptozotocin (STZ) induces decrease in insulin secretion in pancreatic cells and causes type 1 diabetes. The oral administration of *S. horneri* active component (100 mg/kg body weight) to STZ (60 mg/kg body weight)-diabetic rats was found to have a preventive effect on bone loss with diabetes *in vivo* [18]. This finding suggests that the dietary intake of *S. horneri* active component has a preventive effect on bone loss in diabetes state. When the femoral tissues obtained from STZ-diabetic rats were cultured in medium containing *S. horneri* active component, the femoral calcium content and alkaline phosphatase activity were increased *in vitro* [18]. Alkaline phosphatase is related to bone calcification [32]. *S. horneri* active component has a stimulatory effect on bone formation [16,27] and a suppressive effect on bone resorption *in vitro* [27,33]. Thus, suppressive effects of *S. horneri* active component on diabetes-induced bone loss may be resulted from a stimulatory effect on bone formation and a suppressive effect on bone resorption.

Interestingly, oral administration of *S. horneri* active component (100 mg/kg body weight) to STZ-diabetic rats was found to reveal preventive effects on the decrease in body weight and the increase in serum glucose and triglyceride levels induced in the diabetic state [18]. This was the first time finding. Intake of *S. horneri* active component has restorative effects on serum biochemical finding with diabetes *in vivo*.

Thus, intake of *S. horneri* active component was demonstrated to reveal preventive effects on bone loss, hyperglycemia and hyperlipidemia in type 1 diabetes [18]. *S. horneri* active component in preventing bone loss induced with diabetic state may be identical to the component that prevents an elevation of serum glucose and triglyceride levels with diabetes. This remains to be elucidated.

S. horneri active component reveals anabolic effects on bone metabolism in human subjects

Supplemental intake of *S. horneri* active component has been shown to reveal anabolic effects on bone metabolism in human subjects [39]. This study was undertaken to determine the effect of supplemental intake of *S. horneri* active component on circulating bone metabolic markers in healthy human [39]. Thirty-six volunteers, aged 20-60 years (16 men and 20 women), were enrolled. Volunteers were divided into three groups; placebo tablet without *S. horneri* active component (5 men and 7 women), tablet containing *S. horneri* active component at 300 mg/day (6 men and 7 women) or 900 mg/day (5 men and 6 women) [39]. Placebo or *S. horneri* active component tablet was ingested once a day for 4 or 8 weeks [39]. The intake of dietary *S. horneri* active component (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 [39].

Bone-specific alkaline phosphatase [40] and γ -carboxylated osteocalcin [41] are serum bone markers of bone formation, and bone tartrate-resistant acid phosphatase (TRACP) [42] and N-telopeptides of type I collagen [43] 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* active component (300 or 900 mg/day) for 4 or 8 weeks [39]. Serum bone TRACP activity was significantly decreased after the intake of *S. horneri* active component (300 or 900 mg/day) for 8

weeks [39]. Serum N-telopeptides of type I collagen concentration was significantly decreased after the intake of *S. horneri* active component (900 mg/day) for 8 weeks [39]. Meanwhile, serum calcium, inorganic phosphorus, and other biochemical findings were not changed after the intake of *S. horneri* active component (300 or 900 mg/day) for 4 or 8 weeks [39]. Thus, the prolonged intake of *S. horneri* active component has inhibitory effects on bone resorption in humans was demonstrated.

S. horneri active component was found to reveal a stimulatory effect on osteoblastic bone formation and an inhibitory effect on osteoclastic bone resorption. Supplemental intake of *S. horneri* active component reveals suppressive effects on bone resorption and later may reveal stimulatory effects on bone formation in humans, thereby increasing bone mass. Supplemental intake of *S. horneri* active component may have preventive and restorative effects on osteoporosis with increasing age and post-menopausal women.

Prospect

Bone loss is induced with increasing aging and numerous pathological processes including cardiovascular disease, neural disturbance, kidney disease, inflammatory states and cancer, leading to osteoporosis. Currently, bone loss with obese diabetes is widely recognized as a major public health problem. Functional food factors may reveal potential effects to improve bone loss with various pathologic states. Among various marine algae that are applied to food, *S. horneri* active component was found to reveal a unique anabolic effect on bone mass due to suppressing adipogenesis and osteoclastic bone resorption and stimulating osteoblastic bone formation. Supplemental intake of *S. horneri* active component may be usefulness in the prevention and improvement of bone loss in various pathologic states including obese diabetes. Development of this functional food biomaterial will be expected in clinical fields.

Acknowledgement

S. horneri active component was supplied through Masaaki Yokoyama, MS and Toru Matsumoto, MS, Biomaterial Department, Maruhachi Muramatsu, Inc., Yaizu, Shizuoka, Japan.

References

- Weitzmann MN, Pacifici R (2006) Estrogen deficiency and bone loss: an inflammatory tale. *J Clin Invest* 116: 1186-1194. [Crossref]
- Johnell O, Kanis JA (2006) An estimate of the worldwide prevalence and disability associated with osteoporotic fractures. *Osteoporos Int* 17: 1726-1733. [Crossref]
- Walker-Bone K (2012) Recognizing and treating secondary osteoporosis. *Nat Rev Rheumatol* 28: 480-492. [Crossref]
- Leslie WD, Rubin MR, Schwartz AV, Kanis JA (2012) Type 2 diabetes and bone. *J Bone Miner Res* 27: 2231-2237. [Crossref]
- Nielson CM, Srikanth P, Orwoll ES (2012) Obesity and fracture in men and women: an epidemiologic perspective. *J Bone Miner Res* 27: 1-10. [Crossref]
- Weilbaecher KN, Guise TA, McCauley LK (2011) Cancer to bone: a fatal attraction. *Nat Rev Cancer* 11: 411-425. [Crossref]
- Minguell JJ, Erices A, Conget P (2001) Mesenchymal stem cells. *Exp Biol Med* (Maywood) 226: 507-520. [Crossref]
- Muruganandan S, Roman AA, Sinal CJ (2009) Adipocyte differentiation of bone marrow-derived mesenchymal stem cells: cross talk with the osteoblastogenic program. *Cell Mol Life Sci* 66: 236-253. [Crossref]
- Bonjour JP, Schurch M-A, Rizzori R (1996) Nutritional aspects of hip fracture. *Bone* 18: 1395-1445.
- Yamaguchi M (2006) Regulatory mechanism of food factors in bone metabolism and prevention of osteoporosis. *Yakugaku Zasshi* 126: 1117-1137. [Crossref]
- Yamaguchi M (2010) Nutritional Factors and Osteoporosis Prevention. *Nova Science Publishers, Inc.*, New York.
- Yamaguchi M (2010) Role of nutritional zinc in the prevention of osteoporosis. *Mol Cell Biochem* 338: 241-254. [Crossref]
- Yamaguchi M (2002) Isoflavone and bone metabolism: Its cellular mechanism and preventive role in bone loss. *J Health Sci* 48: 209-222.
- Yamaguchi M (2012) Nutritional factors and bone homeostasis: synergistic effect with zinc and genistein in osteogenesis. *Mol Cell Biochem* 366: 201-221. [Crossref]
- Yamaguchi M (2012) Role of carotenoid β -cryptoxanthin in bone homeostasis. *J Biomed Sci* 19: 36. [Crossref]
- Yamaguchi M, Hachiya S, Hiratsuka S, Suzuki T (2001) Effect of marine algae extract on bone calcification in the femoral-metaphyseal tissues of rats: Anabolic effect of *Sargassum horneri*. *J Health Sci* 47: 533-538.
- Yamaguchi M, Matsumoto T (2014) Marine algae *Sargassum horneri* bioactive factor suppresses adipogenesis in mouse bone marrow culture *in vitro*. *OA Biotechnology* 3: 7.
- Uchiyama S, Yamaguchi M (2003) Preventive effect of marine alga *Sargassum horneri* extract on bone loss in streptozotocin-diabetic rats *in vivo*. *J Health Sci* 49: 149-155.
- Raggatt LJ, Partridge NC (2010) Cellular and molecular mechanisms of bone remodeling. *J Biol Chem* 285: 25103-25108. [Crossref]
- Zaidi M, Blair HC, Moonga BS, Abe E, Huang CL (2003) Osteoclastogenesis, bone resorption, and osteoclast-based therapeutics. *J Bone Miner Res* 18: 599-609. [Crossref]
- Chambers TJ, Fuller K (2011) How are osteoclasts induced to resorb bone? *Ann N Y Acad Sci* 1240: 1-6. [Crossref]
- Baron R, Vignery A, Horowitz M (1984) Lymphocytes, macrophages and the regulation of bone remodeling. *Bone Miner Res* 2: 175-243. [Crossref]
- Canalis E, McCarthy T, Centrella M (1988) Growth factors and the regulation of bone remodeling. *J Clin Invest* 81: 277-281. [Crossref]
- Chen G, Deng C, Li YP (2012) TGF- β and BMP signaling in osteoblast differentiation and bone formation. *Int J Biol Sci* 8: 272-288. [Crossref]
- Davidge Pitts CJ, Kearns AE (2011) Update on medications with adverse skeletal effects. *Mayo Clin Proc* 86: 338-343. [Crossref]
- Uchiyama S, Yamaguchi M (2002) Stimulatory effect of *Sargassum horneri* extract on bone formation in rat femoral-diaphyseal and -metaphyseal tissues *in vitro*. *J Health Sci* 48: 148-153.
- Yamaguchi M, Matsumoto T (2012) Marine algae *Sargassum horneri* bioactive factor stimulates osteoblastogenesis and suppresses osteoclastogenesis *in vitro*. *OA Biotechnology* 1: 3.
- Li Y, Li A, Strait K, Zhang H, Nanes MS, et al. (2007) Endogenous TNF alpha lowers maximum peak bone mass and inhibits osteoblastic Smad activation through NF-kappaB. *J Bone Miner Res* 22: 646-655. [Crossref]
- Chang J, Wang Z, Tang E, Fan Z, McCauley L, et al. (2009) Inhibition of osteoblastic bone formation by nuclear factor-kappaB. *Nat Med* 15: 682-689. [Crossref]
- Eliseev RA, Schwarz EM, Zuscik MJ, O'Keefe RJ, Drissi H, et al. (2006) Smad7 mediates inhibition of Saos2 osteosarcoma cell differentiation by NFkappaB. *Exp Cell Res* 312: 40-50. [Crossref]
- Guo R, Yamashita M, Zhang Q, Zhou Q, Chen D, et al. (2008) Ubiquitin ligase Smurf1 mediates tumor necrosis factor-induced systemic bone loss by promoting proteasomal degradation of bone morphogenetic signaling proteins. *J Biol Chem* 283: 23084-92. [Crossref]
- Yohay DA, Zhang J, Thraikill KM, Arthur JM, Quarles LD (1994) Role of serum in the developmental expression of alkaline phosphatase in MC3T3-E1 osteoblasts. *J Cell Physiol* 158: 467-475. [Crossref]

33. Uchiyama S, Yamaguchi M (2002) Inhibitory effect of marine alga *Sargassum horneri* extract on bone resorption in tissue culture *in vitro*. *J Health Sci* 48: 154-160.
34. Klein-Nulend J, Fall PM, Raisz LG (1990) Comparison of the effects of synthetic human parathyroid hormone (PTH)-(1-34)-related peptide of malignancy and bovine PTH-(1-34) on bone formation and resorption in organ culture. *Endocrinology* 126: 223-227. [[Crossref](#)]
35. Uchiyama S, Hashizume M, Hokari Y, Nakagawa T, Igarashi A, et al. (2004) Characterization of active component in marine alga *Sargassum horneri* extract in stimulating bone calcification *in vitro*. *J Health Sci* 50: 634-639.
36. Uchiyama S, Yamaguchi M (2002) 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*. *J Health Sci* 48: 325-330.
37. Hui SL, Epstein S, Johnston CC Jr (1985) A prospective study of bone mass in patients with type I diabetes. *J Clin Endocrinol Metab* 60: 74-80. [[Crossref](#)]
38. McNair P (1988) Bone mineral metabolism in human type 1 (insulin dependent) diabetes mellitus. *Dan Med Bull* 35: 109-121. [[Crossref](#)]
39. Matsumoto T, Hokari Y, Hashizume M, Yamaguchi M (2008) Effect of *Sargassum horneri* extract on circulating bone metabolic markers: Supplemental intake has an effect in health humans. *J Health Sci* 54: 50-55.
40. Levy JR, Murray E, Manolagas S, Olefsky JM (1986) Demonstration of insulin receptors and modulation of alkaline phosphatase activity by insulin in rat osteoblastic cells. *Endocrinology* 119: 1786-1792. [[Crossref](#)]
41. Price PA (1985) Vitamin K-dependent formation of bone Gla protein (osteocalcin) and its function. *Vitam Horm* 42: 65-108. [[Crossref](#)]
42. Halleen JM, Alatalo SL, Suominen H, Cheng S, Janckila AJ, et al. (2000) Tartrate-resistant acid phosphatase 5b: a novel serum marker of bone resorption. *J Bone Miner Res* 15: 1337-1345. [[Crossref](#)]
43. Clemens JD, Herrick MV, Singer FR, Eyre DR (1997) Evidence that serum NTx (collagen-type I N-telopeptides) can act as an immunochemical marker of bone resorption. *Clin Chem* 43: 2058-2063. [[Crossref](#)]