Effects of Spirulina, a Blue-Green Alga, on Bone Metabolism in Ovariectomized Rats and Hindlimb-Unloaded Mice

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The safety and effectiveness were examined of the spirulina alga on bone metabolism in ovariectomized estrogen-deficient rats and hindlimb-unloaded mice. The dosage range was from an amount equal to that recommended in so-called health foods for humans (0.08 g/kg BW/day) to a 100-fold higher dose. The bone mineral density (BMD) of the whole femur and tibia of ovariectomized rats in the any spirulina-treated groups was not significantly different from that of the ovariectomized group, although BMD of the distal femur and proximal tibia was significantly lower in the spirulina-treated groups than in the ovariectomized group after a 6-week-experimental period. BMD of the femur and tibia was not affected by treatment with any dose of spirulina in hindlimb-unloaded mice. These results suggest that the intake of spirulina decreased BMD in the trabecular bone of rodents under estrogen-deficient conditions.

Key words: spirulina; osteoporosis; hindlimb-unloading; bone; health food

Spirulina alga, a blue-green alga, is a traditional food for some Mexican and African peoples and has recently become the subject of extensive research interest because of its high concentrations of nutrients and functional ingredients. Spirulina represents one of the richest sources of plant protein (60–70%) and is also a good source of vitamin B and such minerals as iron and magnesium.1,2) It is also believed to be effective for reducing the risk of life-style related diseases such as diabetes in humans3) and has reduce the incidence of hyperlipidemia by decreasing serum lipids in rats.4) In addition, it may also enhance the regression of oral carcinoma5) and antibody production and have antioxidative and antivirus activities,6) reasons why it is now used as a functional food source in many countries.

Specific marine plants have been attracting attention for their ability to improve bone metabolism, since they are rich in minerals and growth factors.7) Spirulina is also rich in protein and minerals, and has also attracted attention due to its ability to stimulate mineral absorption by its effect on intestinal microflora.8) This evidence suggests that spirulina has the ability to affect calcium and bone metabolism, although no study has so far assessed the activity of spirulina on bone metabolism in vivo. The green alga has also been studied as one of the potential sources of macronutrients as well as a suitable source for obtaining oxygen in the space environment.9,10) Since bone loss is a major problem with long-term space flight, it also seems to be important to assess the effects of spirulina on bones in appropriate systems.

We have been examining the effects of spirulina on bone metabolism by using animal models with osteopenia. In the present study, we adopted the ovariectomized rat, which is a model for postmenopausal osteoporosis induced by estrogen deficiency, and the hindlimb-unloaded mouse, which is a suitable animal model for unloaded skeletal conditions such as the bed-ridden status of aged people and astronauts in space flight. The former is a model for osteopenia induced by increased bone turnover, and the later is a model for osteopenia induced by decreased bone turnover.

Materials and Methods

Animals and diets. To assess the safety and the effects of spirulina intake on bone mass, 18-wk-old growing female Wistar rats were purchased from Clea Japan (Tokyo, Japan) and fed on an AIN-93G diet11) with low calcium (0.2%) for a 1-week adaptation period before the study. The rats were then sham-treated or ovariectomized (OVX), the OVX rats being randomly assigned to four diet groups of 6 rats each, and pair-fed on a control diet or spirulina-containing diets with 0.2% calcium. Spirulina plantensis was purchased from Toyo Enzyme (Chiba, Japan) and contained protein (60%), carbohydrates (17%), fat (7%), ash (10% including 0.35% of Ca), and water (6%); it was fed at 0.08 g/kg body weight (BW)/day (SPI-OVX group), 0.8 g/kg body weight (BW)/day (SPI-OVX group), 0.8 g/kg body weight (BW)/day (SPI-OVX group), 0.8 g/kg body weight (BW)/day (SPI-OVX group), 0.8 g/kg body weight (BW)/day (SPI-OVX group), 0.8 g/kg body weight (BW)/day (SPI-OVX group), 0.8 g/kg body weight (BW)/day (SPI-OVX group), 0.8 g/kg body weight (BW)/day (SPI-OVX group), 0.8 g/kg body weight (BW)/day (SPI-OVX group), 0.8 g/kg body weight (BW)/day (SPI-OVX group), 0.8 g/kg body weight (BW)/day (SPI-OVX group), 0.8 g/kg body weight (BW)/day (SPI-OVX group), 0.8 g/kg body weight (BW)/day (SPI-OVX group), 0.8 g/kg body weight (BW)/day (SPI-OVX group), 0.8 g/kg body weight (BW)/day (SPI-OVX group), 0.8 g/kg body weight (BW)/day (SPI-OVX group), 0.8 g/kg body weight (BW)/day (SPI-OVX group), 0.8 g/kg body weight (BW)/day (SPI-OVX group), 0.8 g/kg body weight (BW)/day (SPI-OVX group), 0.8 g/kg body weight (BW)/day (SPI-OVX group), 0.8 g/kg
Bone mineral density. The femora and tibiae of the rats or mice were carefully removed at the time of necropsy, and the right femur and tibia of each rat were used for the analysis of BMD and bone area by dual energy X-ray absorptiometry (DXA; DCS-600R, Aloka, Tokyo, Japan). Bone mineral content of the femora was closely correlated with the ash weight ($r = 0.978$). Whole body BMD of each rat was assessed by DXA with a QDR-4500A scanner (Hologic, Walsom, MA, USA), each rats being carefully placed in the same position, with the whole body settled on the center the table. The in vivo reproducibility for values was evaluated by measuring the coefficient of variation (CV) five times after repositioning the rats. The same researcher conducted all DXA scans and analyses.

Serum biochemical markers. The total cholesterol (TC) and triglyceride (TG) concentrations in the serum, and GOT and GPT were measured by enzymatic colorimetric methods (cholesterol C-test, Wako Pure Chemicals, Osaka, Japan; triglyceride E-test, Wako Pure Chemicals; GOT UV-test, Wako Pure Chemicals; GPT UV-test, Wako Pure Chemicals).

Statistical analysis. Each data value is presented as the mean ± standard error of the mean (SEM). The significance of differences among the groups was determined by a one-way analysis of covariance (ANCOVA) followed by Fisher’s protected least-significant-difference test. To adjust for possible confounding, covariates of body weight and food intake were used in the analysis. Statistical analyses were performed by the SAS program, and differences are considered significant at the level of $p < 0.05$.

Results

Effects of spirulina intake on the body and tissue weight of growing rats

The initial body weight did not differ among the five groups, but the weights of the OVX animals, except for those in the E2 group, were significantly higher than those of the sham group 6 weeks after the experiment (Table 1). The intake of spirulina for 6 wk did not affect the body weight of the OVX rats, although the food intake of the animals in the groups fed with spirulina was significantly greater than that of animals in the sham and OVX groups. The uterine weight was significantly decreased by ovariectomy ($p < 0.05$), indicating that the rats were estrogen deficient, and the intake of spirulina did not affect this change (data not shown). The serum biochemical markers for liver, GOT and GPT, were not affected by the intake of spirulina at any dose (Table 2). Serum total cholesterol in the SPI-OVX group was significantly higher than that in the sham group, but triglyceride was significantly low in the SPI10-OVX...
Splen and uterus (Table 3). The effects of spirulina intake on the bone mass of the femur in these
OVX rats (Table 2). Serum calcium in the OVX group was significantly lower than that in the sham
group, and the rats treated with the highest dose of spirulina showed lower serum calcium than that in the
OVX rats (Table 2).

Total BMD of the femur in any spirulina-treated group was not significantly different from that in the
OVX group (Fig. 1A), although the BMD of the distal femur in the SP1- and SP10-OVX groups was
significantly lower than that in the OVX group (p < 0.05) (Fig. 1B). No dose-response effect of
spirulina on BMD of the distal femur was apparent in the OVX rats. BMD at the middle and proximal
regions of the femur in these groups did not differ from that in the OVX group (data not shown). Treatment with E2 prevented the decrease of BMD in the OVX rats (Figs. 1A and B).

In the case of the tibia, the decrease in BMD was particularly at in the proximal region in the SP1-
and SP10-OVX groups (Fig. 2). No dose-response effect of spirulina on BMD in the proximal region of the
tibia apparent in the OVX rats, and BMD in the middle and distal regions of the tibia in the SP1- and
SP10-OVX groups did not differ from that in the OVX group (data not shown).

The effects of spirulina intake on the bone mass of the tail-suspended mice

Mice were tail-suspended and fed on diets containing spirulina at doses 20- and 100-fold higher than the
recommended dose as health food for 3 weeks. Spirulina did not affect the weights of the body, liver, kidney,
spleen and uterus (Table 3).

Figure 3 shows the effects of spirulina on BMD of the

<table>
<thead>
<tr>
<th>Total body weight (g)</th>
<th>Sham</th>
<th>OVX</th>
<th>SP1-OVX</th>
<th>SP10-OVX</th>
<th>SP50-OVX</th>
<th>E2</th>
</tr>
</thead>
<tbody>
<tr>
<td>week 6</td>
<td>257.0 ± 4.1</td>
<td>256.8 ± 6.7</td>
<td>256.7 ± 5.8</td>
<td>258.0 ± 6.0</td>
<td>259.7 ± 5.4</td>
<td>261.2 ± 5.5</td>
</tr>
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</table>

Each value is the mean ± SEM, n = 6. Means with different letters differ at p < 0.05.

<table>
<thead>
<tr>
<th>Table 1. Effects of Spirulina Intake on the Body and Tissue Weight, and on the Food Intake of Ovariectomized Rats</th>
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<tr>
<th>Table 2. Effects of Spirulina Intake on Biochemical Markers in the Serum of Ovariectomized Rats</th>
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<tr>
<th>GOT (U/1)</th>
<th>GPT (U/1)</th>
<th>TC (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>Serum Ca (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>OVX</td>
<td>SP1-OVX</td>
<td>SP10-OVX</td>
<td>SP50-OVX</td>
</tr>
<tr>
<td>129.8 ± 19.2</td>
<td>163.6 ± 28.3</td>
<td>127.7 ± 13.8</td>
<td>156.0 ± 22.8</td>
<td>133.0 ± 18.9</td>
</tr>
<tr>
<td>34.5 ± 5.57b</td>
<td>38.6 ± 3.47b</td>
<td>35.1 ± 2.32b</td>
<td>34.0 ± 2.78b</td>
<td>30.4 ± 1.83b</td>
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<tr>
<td>87.0 ± 5.82b</td>
<td>88.6 ± 6.79b</td>
<td>110.3 ± 8.58b</td>
<td>97.3 ± 6.31b</td>
<td>97.4 ± 3.83b</td>
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<tr>
<td>96.2 ± 21.7b</td>
<td>85.6 ± 32.5b</td>
<td>69.7 ± 7.4b</td>
<td>37.0 ± 10.2b</td>
<td>64.4 ± 24.2b</td>
</tr>
<tr>
<td>10.8 ± 0.14b</td>
<td>10.3 ± 0.12b</td>
<td>10.3 ± 0.08b</td>
<td>10.2 ± 0.09b</td>
<td>9.96 ± 0.11b</td>
</tr>
</tbody>
</table>

Each Values is the mean ± SEM, n = 6. Means with different letters differ at p < 0.05.

Fig. 1. Effects of Spirulina Intake on BMD of the Whole Femur (A) and Distal Femur (B) in Ovariectomized Rats Fed a Control Diet or with Various Amounts of Spirulina in Their Diet (0.08 g/kg BW/day, SP1-OVX: 0.8 g/kg BW/day, SP10-OVX: and 4.0 g/kg BW/day, SP50-OVX) or 17β-Estradiol (E2) for 6 Weeks.

Each value is the mean ± SEM for 6 rats in each dietary group. Means with different letters differ at p < 0.05.
The data in this study show that the intake of spirulina at doses in a range from equal to 50-fold the daily intake recommended by the manufacturers of health foods decreased BMD of the distal femur and proximal tibia in estrogen-deficient rats. On the other hand, spirulina at doses 20- and 100-fold the recommended daily intake did not affect BMD of the femur and tibia in skeletal unloaded-mice, although it decreased BMD in the mice equipped for tail-suspension.

Discussion

The data in this study show that the intake of spirulina at doses in a range from equal to 50-fold the daily intake recommended by the manufacturers of health foods decreased BMD of the distal femur and proximal tibia in estrogen-deficient rats. On the other hand, spirulina at doses 20- and 100-fold the recommended daily intake did not affect BMD of the femur and tibia in skeletal unloaded-mice, although it decreased BMD in the mice equipped for tail-suspension.
Spirulina is one of the most concentrated natural sources of nutrients; it contains all the essential amino acids, and is rich in beta-carotene, minerals, and other natural photo-chemicals. This alga is used not only as a good source of nutrients, but also as an ingredient of functional foods. For example, aqueous extracts from spirulina inhibited the replication of HIV-1, herpes simplex, the influenza A virus in a human-derived T-cell line and peripheral blood mononuclear cells, and also exhibited anti-cancer effects. C-phycocyanin in spirulina is involved in antioxidative and radical-scavenging activity. Spirulina is one of the most common ingredients of so-called health foods in Japan that are intended to prevent lifestyle-related diseases such as diabetes and hyperlipidemia in humans, although these are insufficient clinical data on these effects. It has been reported that spirulina feeding, accompanied by an increase in lipoprotein lipase activity, improved the dietary hyperlipidemia caused by a high-fructose diet. In the present study, the serum total cholesterol level in the SP1-OVX group was significantly higher than that in the sham and OVX groups. However, since higher doses of the alga did not affect the serum total cholesterol level, it seems that this was not an original effect of spirulina. On the contrary, spirulina tended to reduce the triglyceride level in OVX rats fed with a normal diet. Taken together with these findings, it is conceivable that the alga might have affected serum lipids only in the hyperlipidemic condition.

Osteoporosis is one of the most common disorders associated with aging and results in bone fracture, this being a substantial public health problem. Specific marine plants have recently attracted attention for their ability to improve bone metabolism, since they are rich in minerals and growth factors. An epidemiological study in Taiwan has shown a seaweed diet to be a significant variable in a multiple linear regression analysis of BMD. Furthermore, spirulina has gained attention as a source of nutrients including proteins and minerals for space flight. It therefore seems to be important to examine the effects of spirulina on bone metabolism in appropriate models. In the present study, the alga did not prevent bone loss in the OVX rats. BMD of the distal femur and proximal tibia in rats, which are rich in trabecular bone, was in fact decreased by the spirulina treatment. One of the reasons for this seems to be the high content of minerals other than calcium in spirulina, since it contains 0.66% magnesium and 1% phosphorus in addition to 0.35% calcium. In fact, the serum concentration of calcium in the SP50-OVX rats was significantly lower than that in the OVX group. However, no dose dependence of spirulina was apparent in this study, so the exact mechanism responsible for this inhibitory effect on BMD is not clear. One possible mechanism is that minerals other than calcium inhibit calcium absorption in the small intestine. In particular, phosphate could form a precipitate with calcium in the digestive tract and inhibit calcium absorption. Further examinations is needed to clarify the exact mechanism for the effects of spirulina on bone metabolism in future studies.

The green alga has been studied as one of the potential sources of macronutrients and as a suitable source for obtaining oxygen in a space habitat. Since skeletal unloading induces osteopenia in the loaded bones, tail-suspended animal models simulate the conditions of lack of mechanical stress on bones such as that in spaceflight. The unloaded condition in this model is also appropriate to the bed-ridden status of aged people. Spirulina did not affect the BMD of either the femur or tibia in the unloaded mice, although the high doses of spirulina decreased BMD of the femur and tibia in the loaded mice equipped for tail-suspension. It is likely that BMD of the unloaded mice was too low to respond to the change in food components. Loss of bone in the loaded group given a high dose of spirulina might have been caused by the high mineral content other from calcium and/or by a high level of protein leading to calcium exclusion in the kidneys.

In conclusion, spirulina intake induced trabecular bone loss under estrogen-deficient and some stress-bearing conditions in rodents. The component in spirulina responsible for the observed effects needs to be identified in future studies.

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References


