

Prolonged Intake of Isoflavone- and Saponin-Containing Soybean Extract (*Nijiru*) Supplement Enhances Circulating γ -Carboxylated Osteocalcin Concentrations in Healthy Individuals

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The effect of *nijiru*, which is a by-product of the processing of soybeans to make the fermented soybeans called *natto*, on circulating blood chemistry levels related to calcium and bone metabolism in healthy individuals was investigated. Twelve volunteers (six men and six women) were received *nijiru* twice a day for 60 days at a dose of 1500 mg (6 tablets) per day. The serum γ -carboxylated osteocalcin concentration was significantly increased by the intake of *nijiru* in both men and women to about 2-fold that in the control group. The serum calcium concentration was significantly decreased by *nijiru* supplementation in women, and the serum inorganic phosphorus concentration was significantly reduced in both men and women. However, the intake of *nijiru* did not have a significant effect on serum glucose, nitrogen urea, albumin, free cholesterol, triglyceride, high-density lipoprotein cholesterol, and γ -glutamyltranspeptidase concentrations in men or women, indicating that liver and renal function is not affected by *nijiru* supplementation. The results of the present study suggest that the intake of isoflavone- and saponin-containing *nijiru* can stimulate the γ -carboxylation of osteocalcin, which plays an important role in bone formation and mineralization, in healthy individuals.

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INTRODUCTION

It is known that bone mass decreases in both men and women with increasing age. The decrease in bone mass is due to increased bone resorption and to decreased bone formation. Osteoporosis with a decrease in bone mass is widely recognized as a major public health problem. The most dramatic expression of this disease is represented by fractures of the proximal femur, of which the incidence increases as the population ages.^{1,2)} A deficiency in both micronutrients and macronutrients appears to be strongly implicated in the pathogenesis and consequences of hip fracture in the osteoporotic elderly.³⁾ Nutritional factors may prevent bone loss with increasing age, although this has not been fully clarified.

Daidzein and genistein, natural isoflavonoid phytoestrogens found in Leguminosae, have an anabolic effect on bone metabolism in rats.⁴⁻⁶⁾ Isoflavones including daidzin, daidzein, genistin, and genistein are present in soybeans high concentrations. Daidzin and genistin are hydrolyzed to daidzein and genistein, respectively, by β -glucosidase in the gastrointestinal tract. Genistein and daidzein have been demonstrated to stimulate bone formation and to inhibit bone resorption, thereby increasing bone mass.⁷⁻¹³⁾

Nijiru is produced in the processing of the fermented soybean preparation *natto*, and contains significant quantities of isoflavone and saponin. The intake of isoflavone- and saponin-containing *nijiru* has been found to have an anabolic effect on bone components in rats, suggesting its role in the prevention of osteoporosis.¹⁴⁾ Soybean saponin has been demonstrated to have an anabolic effect on bone component *in vitro* and *in vivo*.¹⁵⁾ Moreover, the prolonged intake of *natto* supplemented with *nijiru* containing isoflavone and saponin has a preventive effect on ovariectomy-induced bone loss, suggesting that it may have a role in the prevention of osteoporosis.¹⁶⁾ Thus soybean-based foods may be useful for the prevention of osteoporosis with increasing age.

The present study was undertaken to determine the effect of supplemental *nijiru* intake on bone metabolic marker in healthy individuals over the long term.

MATERIALS AND METHODS

Materials — Soybeans were boiled under 1.5 atmospheric pressure for 40 min at 160°C, and the *nijiru* produced was then freeze-dried. The isoflavone content was measured after removal from the *nijiru* powder by extraction with 80% hot ethanol solution. This ethanol solution was filtered, and the filtrate was subjected to reverse-phase high-performance liquid chromatography. The isoflavone concentration was expressed as micrograms per gram of *nijiru* powder. The calcium content in the *nijiru* powder was measured by atomic absorption spectrophotometry after digestion with HNO₃. The composition of saponin, daidzin, daidzein, genistin, genistein, and calcium in *nijiru* prepared from soybeans was 320, 1800, 12, 1600, 15, and 820 µg/g dry powder, respectively. One tablet contained 250 mg of *nijiru* dry powder.

Experimental Procedures — Twelve adults aged 17–58 years (six men and six women), who were judged to be healthy with no abnormal liver or kidney function as assayed by standard biochemical data, were enrolled as volunteers in this study. Informed consent was obtained from all. The intake of other foods with an abundance of vitamin K, which stimulates production of γ -carboxylated osteocalcin, was prohibited during the study. All volunteers were 1500 mg (6 tablets) of *nijiru* powder twice a day for 60 days. Blood samples were collected at 10:00 on the day prior to the beginning of administration and 60 days thereafter. Serum γ -carboxylated osteocalcin, calcium, and inorganic phosphorus levels and other biochemical levels were measured using routine methods.

Analytical Procedures — Serum samples were obtained by centrifugation (2500 rpm for 5 min) between 20 and 40 min after blood collection, and then stored at –20°C until assayed. The serum γ -carboxylated osteocalcin concentration was assayed using a Gla-type Osteocalcin (Gla-OC) EIA Kit (Takara Shuzou, Shiga, Japan).¹⁷ Serum calcium and inorganic phosphorus concentrations were determined using KIT (Wako Junyaku, Osaka, Japan). Serum glucose, nitrogen urea, albumin, free-cholesterol, triglyceride, high-density lipoprotein (HDL) cholesterol, and γ -GTP were assayed using KIT.

Statistical Analysis — Differences in values before and after the intake of *nijiru* tablets were estimated using Student's *t*-test. *p* values of less than 0.05 were considered statistically significant.

Table 1. Change in Serum Calcium and Inorganic Phosphorus Concentrations Following Intake of Isoflavone- and Saponin-Containing Soybean Extract in Healthy Individuals

Serum level	Control (baseline)	At day 60
Calcium (mg/dl)		
All	9.00±0.09	8.61±0.21
Men	9.18±0.12	9.02±0.29
Women	8.81±0.08	8.28±0.25*
Inorganic phosphorus (mg/dl)		
All	3.36±0.12	4.02±0.16***
Men	3.12±0.15	3.89±0.27**
Women	3.60±0.15	4.22±0.07**

Each value is the mean ± S.E.M. of 12 (both men and women) or 6 (men or women) individuals. **p* < 0.05, ***p* < 0.025, and ****p* < 0.01 compared with the baseline value.

Table 2. Serum Metabolic Findings Following Intake of Isoflavone- and Saponin-Containing Soybean Extract in Healthy Individuals

Serum level	Control (baseline)	At day 60
Glucose (mg/dl)	100.3±4.00	91.7±4.67
Nitrogen urea (mg/dl)	14.1±0.96	13.4±1.08
Albumin (g/dl)	5.1±0.11	5.1±0.08
Free cholesterol (mg/dl)	49.0±4.61	44.8±2.23
Triglyceride (mg/dl)	56.9±5.68	67.2±8.06
HDL cholesterol (mg/dl)	55.3±3.36	59.1±3.30
γ -GTP (IU/l)	32.9±4.81	29.7±5.06

Each value is the mean ± S.E.M. of 12 healthy volunteers.

RESULTS

The changes in serum calcium and inorganic phosphorus concentrations in healthy individuals before and after administration of *nijiru* are shown in Table 1. The serum calcium concentration was significantly decreased with the intake of *nijiru* tablets in women, although not seen in men. The serum inorganic phosphorus concentration was significantly reduced in both men and women at 60 days compared with baseline.

The effect of *nijiru* tablet intake on serum metabolic findings is shown in Table 2. There was no significant alteration in glucose, nitrogen urea, albumin, free-cholesterol, triglyceride, HDL cholesterol, and γ -GTP levels in either men or women.

The changes in serum γ -carboxylated osteocalcin concentration after 60 days of *nijiru* intake are shown in Fig. 1. Serum γ -carboxylated osteocalcin increased significantly in individuals of both sexes.

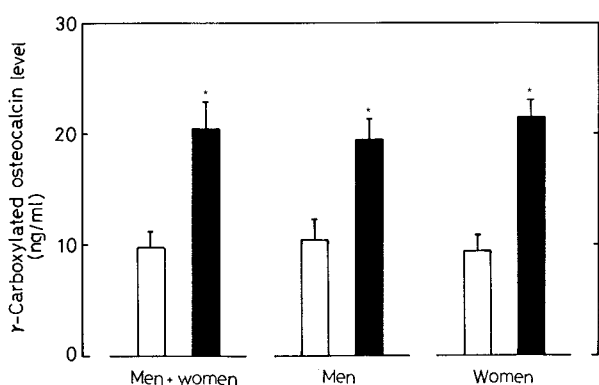


Fig. 1. Changes in Serum γ -Carboxylated Osteocalcin Concentrations Following Intake of Isoflavone- and Saponin-Containing Soybean Extract

Each value is the mean \pm S.E.M. of 12 (men and women) or 6 (men or women) individuals. * $p < 0.01$ compared with the baseline value. White bars, control; black bars, after *nijiru* intake.

DISCUSSION

Nutritional factors may be significant in preventing bone loss with increasing age. Soybean isoflavone has been shown to have an anabolic effect on bone metabolism in rats, suggesting its role in prevention of osteoporosis.⁴⁻¹³ *Nijiru* has been demonstrated to have a stimulatory effect on bone formation and mineralization in normal rats and an inhibitory effect on bone loss in ovariectomized rats.¹⁴⁻¹⁶ We found that the intake of *nijiru* supplement causes a significant increase in circulating γ -carboxylated osteocalcin concentration in healthy individuals.

Osteocalcin is a bone matrix protein containing γ -carboxyglutamic acids, which is synthesized in osteoblasts of bone tissues.^{18,19} Noncarboxylated osteocalcin cannot bind to hydroxyapatite in mineralized tissues.²⁰ *Nijiru* contains large quantities of isoflavones (genistin, genistein, daidzin, and daidzein).¹⁴ These isoflavones have a stimulatory effect on protein synthesis in osteoblastic MC3T3-E1 cells.⁷⁻⁹ The intake of *nijiru* supplement caused a significant increase in the circulating γ -carboxylated osteocalcin concentration in healthy individuals. Presumably isoflavones, which are contained in *nijiru* supplement, stimulate the production of γ -carboxylated osteocalcin in the osteoblasts of bone tissue in healthy individuals. In addition, *nijiru* contains large amounts of saponin,¹⁴ which has been shown to stimulate bone mineralization.¹⁵ It is possible that saponin has a stimulatory effect on the production of γ -carboxylated osteocalcin. The intake of *nijiru* supplement may therefore stimulate bone

mineralization in healthy individuals.

The intake of *nijiru* supplement caused a significant decrease in serum calcium and inorganic phosphorus concentrations in healthy volunteers. This decrease may be partly involved in bone mineralization, which is related to an increase in γ -carboxylated osteocalcin following the intake of *nijiru*.

Serum findings associated with liver and kidney functions were not significantly altered by the intake of *nijiru* supplement in healthy individuals. This indicates that prolonged intake of *nijiru* does not influence metabolic functions of the liver and kidney in such individuals. In addition, the body weight of the healthy volunteers did not change significantly with prolonged intake of *nijiru* (data not shown). From these observations, it is assumed that *nijiru* is a safety and healthy dietary supplement.

Nutrition may have a role in the prevention of bone loss with increasing age. Numerous elements in food have been shown to have an anabolic effect on bone metabolism.^{21,22} The isoflavone and saponin in *nijiru* may be related to an anabolic effect on bone mineralization in healthy individuals. The intake of *nijiru* as a supplement may thus have a role in the prevention of age-related bone loss.

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