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Relationship between urinary phytoestrogen levels and *Z* score of lumbar vertebral bone mineral density in Japanese postmenopausal women

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Abstract

Phytoestrogens are attracting much attention for their anticancer effects, their reduction of menopausal symptoms, and their preventive effects against osteoporosis in the human body, resulting from the consumption of foods that contain phytoestrogens at high concentrations. The effects of 2 phytoestrogens, genistein and daidzein, have been reported, but little data have been published on the effects of equol, coumesterol, and other phytoestrogens. In the present study, using high-performance liquid chromatogaphy, we measured the urinary phytoestrogen levels of 80 postmenopausal Japanese women and simultaneously determined the bone mineral density (BMD) in lumbar vertebrae L2 through L4 (L2-L4BMD), measured the levels of the bone biochemical markers and calciotropic hormones, and also studied the relationships of these with urinary phytoestrogens. Our results showed the following values for phytoestrogens in grams per milligram of creatinine: daidzein, 3.11 ± 3.09; genistein, 2.74 ± 5.02; equol, 6.29 ± 11.44; coumesterol, 0.51 ± 0.77; *O*- desmethylangolensin (DMA), 1.59 ± 5.19; formononetin, 0.11 ± 0.19; and biochanin A, 0.08 ± 0.19. When these were converted to logarithms, log (genistein) was the only one to show a statistically significant positive correlation ($r = 0.29$, $P < .05$) with the *Z* score of L2-4BMD. Log (DMA) showed a negative correlation ($r = -0.36$, $P < .013$) with the urinary deoxypyridinoline level. The levels of osteocalcin and the calciotropic hormones showed no correlation with urinary phytoestrogen levels. The fact that there were correlations between the genistein level and *Z* score of bone density, and between DMA and biochemical markers of bone metabolism, suggested that phytoestrogens may have an effect on bone metabolism in Japanese women. Genistein and DMA might be involved in the inhibition of bone resorption in terms of the correlation between phytoestrogens levels and BMD.

Keywords: Genistein; DMA; Lumbar bone mineral density; Elderly Japanese women

1. Introduction

Soya beans have a high content of isoflavones, which are phytoestrogens [1]. In comparison with Western societies, of which most members are white by race, the Japanese people, who are Asians, consume large amounts of these beans. Reports are now appearing that suggest that such a diet may affect arteriosclerosis, osteoporosis, and aging [2]. The bone mineral density (BMD) of the proximal radius and ulna in postmenopausal women rose significantly, by 4.1% over 6 months with 57 mg/d and by 3.0% with 85.5 mg/d of isoflavone containing genistein, daidzein, formononetin, and biochanin [3]. In perimenopausal women who consumed a diet with enough soya beans to supply 80 mg of isoflavones for 24 weeks, the BMD was 5.6% higher than that in the control group [4]. Also, when tablets containing isoflavones extracted from clover were administered to postmenopausal women, 57 mg of isoflavones per day increased the BMD of

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the radius by 4.7% [5]. However, 90 mg of isoflavones given to premenopausal women did not result in any increase in BMD [6]. A significant increase in osteocalcin (9.3%) and a decrease in urinary type I collagen N-telopeptide (NTx) (13.9%) were found in normal postmenopausal women after a 12-week consumption of 60 mg/d of soy foods [7]. In contrast, although soy isoflavones do affect bone turnover, the magnitudes of change were so small that it is unlikely to be clinically relevant [8]. Thus, the contribution of isoflavones to bone metabolism is still a matter of some controversy. We have reported that a positive correlation exists between the intake of soy protein and BMD [9]. On the basis of those results, because soya beans contain large amounts of phytoestrogens (ie, isoflavones), we investigated the relationship between the urinary concentration of each of the phytoestrogens and BMD.

2. Subjects and methods

2.1. Subjects

We studied 80 elderly Japanese women who were outpatients who underwent diagnosis and/or treatment for osteoporosis at the Tokyo Metropolitan Geriatric Medical Center. Thirty of these patients had an osteoporosis *T* score of −2.5 or less in lumbar spine. All excluded taking drugs for osteoporosis, estrogen preparations, bisphosphonate, alphacalcidol, ipriflavone, or menaquinone. No patients with bone fractures were included. Before participation, the informed consent of all subjects was obtained by the Ethics Committee of the Tokyo Metropolitan Geriatric Medical Center.

2.2. Determination of urinary phytoestrogen concentrations by high-performance liquid chromatography

The high-performance liquid chromatography (HPLC) conditions and method of Franke et al [11] were followed. Briefly, the first morning urine was collected and was kept until examination after being mixed with oxalic acid buffer. Urinary phytoestrogens were first extracted with a Sep-Pak C18 cartridge, which were then hydrolyzed with glucuronidase/sulfatase and analyzed for each of the components using a Nova-Pak C18 analytical column. Elution was carried out in 2 gradient systems. In an acetonitrile–acetic acid gradient system, elution was started with 20% acetonitrile concentration in 10% aqueous acetic acid for 16 minutes, then with a linear gradient 20% to 70% for 16 minutes, and, finally, with a linear gradient of 70% to 20% for 10 minutes. In the methanol/acetonitrile/dichloromethane–acetic acid system, elution was started with 5% of methanol/acetonitrile/dichloromethane (10/5/1, v/v/v) in 10% acetic acid for 5 minutes, then with a linear gradient of 5% to 45% for 20 minutes, 45% to 70% for 6 minutes, and 70% to 5% for 3 minutes, and, finally, 5% for 15 minutes. In each case, the flow rate was 0.8 mL/min. Ultraviolet light at a wavelength of 280 nm (and 260 nm) was used for detection. Urinary genistein, daidzein, equol, formononetin, coumesterol, biochanin A, and *O*-desmethylangolensin (DMA) were quantified with reverse-phase chromatography using both solvent gradient systems, and the results were corrected using urinary creatinine. The recovery rates were at least 90%, and inter- and interassay variances were 10% or less.

Table 1
Baseline characteristics

Age (y)	66.9 ± 7.3
BMI (kg/m^2)	21.8 ± 2.9
L2-L4BMD (g/cm^2)	0.790 ± 0.135
T score	−2.6 ± 1.1
Z score	−0.6 ± 1.0
IPTH (pmol/L)	5.1 ± 1.8
1,25(OH)2D (ng/mL)	28.8 ± 12.5
IOC (ng/mL)	8.3 ± 3.1
dpd (nmol/mmol · Cr)	5.9 ± 1.8
Uca/Cr	0.22 ± 0.16

All values are shown as mean ± SD. The *T* score, −2.6, is low. Uca indicates urine calcium; Cr, creatinine.

Table 2
Baseline of phytoestrogen value

Daidzein	1.380 ± 1.368
Genistein	1.142 ± 2.104
Equol	2.941 ± 4.784
Coumesterol	0.214 ± 0.324
DMA	0.701 ± 2.273
Formononetin	0.046 ± 0.060
Biochanin A	0.031 ± 0.075

Values are in micromoles per millimoles of creatinine. The highest level is that of equol.

2.3. Determination of calciotropic hormones and biochemical markers

Serum calcium was measured using the OCPC (orthocresolphthalien complexon) method after being frozen for 1 to 2 months. The plasma level of parathyroid hormone (1-84 intact-parathyroid hormone [IPTH]) was assayed by the immunoradiometric method (Allegro Intact PTH, Nichols Institute, San Juan Capistrano, Calif). 1,25-Dihydrocholecalciferol ($1,25(OH)_2D_3$) was determined with radioimmunoassay (Immunodiagnostic Systems, Bolton, UK), and urinary deoxypyridinoline (udpd) with ELISA (Metra Biosystems, Mountain View, CA) after collection of the first morning's urine. Osteocalcin was also measured by ELISA (Kokusai Shiyaku, Kobeshi, Japan). Intra-assay and interassay variances in the measurements were 6.9% and 3.5% for IPTH, 8.8% and 8.7% for $1.25(OH)_2D_3$, 4.8% and 5.4% for intact osteocalcin, and 7.5% and 10.1% for udpd, respectively.

2.4. Bone mineral density measurement

Bone mineral density in the lumbar spine was measured by dual-energy x-ray absorptiometry (IPX- IQ, GE Lunar Corp, Madison, Wis). *Z* score in BMD was an adjusted value by ethnicity, age, and body weight. The coefficient of variation of image quality in BMD measurement was 0.7% in spine L2 through L4 [10].