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RESEARCH ARTICLES

One-year soy isoflavone supplementation prevents early postmenopausal bone loss but without a dose-dependent effect

Hui-Ying Huang[a,b], Hsiao-Ping Yang[c,*], Hui-Ting Yang[a], Tung-Chuan Yang[d], Ming-Jer Shieh[e], Shih-Yi Huang[e,*]

[a]*School of Pharmaceutical, Taipei Medical University, Taipei 110, Taiwan*
[b]*School of Nutrition, China Medical University, Taichung 404, Taiwan*
[c]*Department of Obstetrics and Gynecology, Taipei Medical University Hospital, Taipei 110, Taiwan*
[d]*Department of Obstetrics and Gynecology, China Medical University Hospital, Taichung 404, Taiwan*
[e]*School of Nutrition and Health Sciences, Taipei Medical University, Taipei 110, Taiwan*

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Abstract

It is believed that soy isoflavone has much potential effectiveness on the postmenopausal status; however, the optimal dose for preventing postmenopausal bone loss still remains unclear. This open-labeled, self-controlled pilot study was undertaken to determine the effect of 1-year supplementation of different high dosages of soy isoflavone in postmenopausal Taiwanese women. Forty-three women aged 45–67 years were enrolled and randomly assigned into a control (C), 100 mg/day isoflavone (IF100) and 200 mg/day isoflavone (IF200) groups for 1 year. Dual-energy X-ray absorptiometry and other related biochemical markers of bone metabolism were measured. Results indicated that the decrease in bone mineral density (BMD) was significant for lumbar vertebrae L1–3, L1–4 and the femur neck in the C group; surprisingly, the BMD of L1–3 was significantly elevated in the IF100 group; however, there were no consistent responses in the IF200 group. No significant change except loss of the bone mineral content of Ward's triangle ($P=.003$) was found in the IF200 group after treatment. The percentage change at L1–3 was less ($P=.04$) in the IF200 group when compared to the IF100 group. A relatively uniform direction of bone formation in expanding the weight and area with different rates of change resulted in different BMD changes. Both indicated a change of bone formation patterns with the higher-dose supplement. A protective effect of IF100 on estrogen-related bone loss was observed. A lack of a benefit such as high safety in the IF200 group for 1-year administration was ensured and lacked undesirable side effects.

Keywords: Postmenopausal women; Soy isoflavone; Bone mineral density (BMD); Bone mineral content (BMC)

1. Introduction

Postmenopause is known to be an important phase in the development of osteoporosis, associated fractures and further complications, that is, death since bone density begins to gradually determinate [1]. Among these bone complications, women with a hip fracture were more than twice as likely to die, even after the prefracture health status was taken into account. Such conditions in Taiwan slightly differ. Taiwanese women used to be known to have femoral neck bone mineral density (BMD) values of 0%–15% lower than those of Caucasians. The vertebral fracture rate was 18% for women, whereas the hip fracture rate was 0.203% in 1996, which was lower than that in Caucasians and similar to those in Chinese and other Asian women [2–5]. However, the incidence appeared to be as high as 505 per 100,000 in a recent nationwide survey in Taiwan [6]. As Caucasian females suffer from serious declines in BMD after age 40 years, Shaw [7] reported a yearly decrease of 0.01 g/cm^2 in women beyond 40 years old in Taiwan.

Although osteoporotic women in Taiwan respond to antiresorption agents better than do Caucasians [8], perimenopausal or postmenopausal women who are at risk of future osteoporosis still require effective preventive measures. The positive effect of soy has been confirmed epidemiologically. Among different cohorts of women in the US, femoral neck BMD was 12% greater in the highest versus lowest tertile of soy intake, with median genistein

* Corresponding authors. Tel.: +886 2 27361661x6550; fax: +886 2 27373112.
E-mail addresses: sihuang@tmu.edu.tw (S.-Y. Huang), alb.peter@msa.hinet.net. (H.-P. Yang).

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intakes representing soy intakes of 3511 and 7151 μg/day for Chinese and Japanese, respectively [9]. Another evaluation showed women with the highest nonsupplemented daily intake of dietary genistein had 18% lower N-terminal telopeptide [urinary N-terminal telopeptide/creatinine ratio (NTx)] concentrations and significant higher spinal BMD than those soy-free consumers [10].

Among studies using higher daily doses, a 3-month trial of daily intake of 65 and 130 mg soy protein isolates was undertaken by Wangen et al. [11]. Bone-specific alkaline phosphatase (BAP) activity was found to decrease in both groups, and no change in urinary deoxypyridinoline (Dpd) was observed. The response differed in premenopausal women who showed increases in Dpd in both groups. Six months of a 110 mg/day soy isoflavone trial conducted by Harkness et al. [12] revealed greater improvements in spinal BMD at L2 and L3. Isoflavone dosages used in all these studies were no higher than 150 mg/day, and few with longer durations were carried out. Is 100 mg/day isoflavone (IF100) for Asia women really an optimal dosage for osteoporosis prevention? Is the effect of isoflavone on bone still the same after a longer duration? Thus, the aim of this study was to evaluate the preventive effect of 1-year, high-dose isoflavone supplementation on bone densities in postmenopausal females in Taiwan.

2. Materials and methods

2.1. Subjects

This study recruited 43 menopausal women aged 67 years and under, who had experienced at least 1–13 years of menopause according their own statements. They were recruited between February 2002 and May 2003 from China Medical University Hospital (Taichung, Taiwan). Serum follicular-stimulating hormone (FSH) was the primary measurement to confirm the menopausal hormone status of subjects. Subjects whose FSH level was over 40 IU/L were excluded from the study. Other exclusion criteria included women under hormone therapy, taking any medication including herbal medicine and with a drinking or smoking history, drug allergy history, connective tissue disease, gastrointestinal diseases, hepatic disease, hyperthyroidism or hypothyroidism, Cushing's syndrome, diabetes mellitus, acromegaly, premature ovarian failure, gonadal agenesis, thromboembolism, bedridden condition, any bone or marrow disease or cancer. All subjects in the trial were notified of the study contents, and informed signed consent was obtained; a complete medical history and physical examination were then taken before initiating the study.

2.2. Study protocol

The study was an open-labeled, self-controlled study to examine how bone density reacts to different high doses of isoflavone supplementation for 12 months. Forty-three postmenopausal women were assigned into one of the three dietary supplement groups: a control (C) group with a regular diet, an IF100 group and 200 mg/day isoflavone (IF200) group with two tablets daily to provide 100 mg soy isoflavone and two tablets twice daily to provide 200 mg soy isoflavone supplement with a regular diet.

The isoflavone tablets used in the study were kindly provided by Chia-Hsin Food and Synthetic Fiber, Taipei, Taiwan. The tablet is made of 125 mg soy protein extracts containing 50 mg isoflavone (35.5 mg genistein and 14.5 daidzein). Subjects were instructed to take two tablets once or twice daily before breakfast and/or dinner with water and were encouraged to maintain their regular dietary habits and daily activities during the study. Forty-two cases completed this 1-year study.

2.3. Clinical analysis

2.3.1. Baseline assessment and sample collection

A medical history and anthropometrical measurements were taken. Overnight fasting blood and second voided urine samples were taken before and after the end of the 1-year isoflavone intervention. Blood collected was centrifuged at $3000\times g$ for 10 minutes at 4°C, and serum samples were separated and stored in −80°C refrigerator until analysis. Urine samples were collected and stored at −4°C for further tests.

2.3.2. Blood hormone, lipid profiles and other basic biochemical analyses

Serum estradiol-17β (E_2) and serum intact parathyroid hormone were quantified by radioimmunoassay methods with commercial kits (Nichols Institute Diagnostics). Serum total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglyceride, glutamate oxalacetate transaminase, glutamate pyruvate transaminase, alkaline phosphatase (ALP), blood urine nitrogen, creatinine (Cr), uric acid and glucose were assessed by colorimetrically enzymatic commercial kits (Randox Laboratories, UK) with an autoanalyzer.

2.3.3. Bone density assessments

Dual-energy X-ray absorptiometry (DEXA, Lunar DPXL, Lunar) was used to measure the BMD of the L1 to L4 lumbar vertebrae, the femur neck, Ward's triangle and trochanter. The bone mineral content (BMC) and projected bone area (BA) of the femur neck, Ward's triangle and trochanter were also calculated.

2.3.4. Markers of bone turnover

In addition to changes in BMD, biochemical markers of osteoblast and osteoclast activity and other related parameters were also measured to investigate bone turnover. To clarify whether isoflavone affected bone calcium metabolism and thus influenced activation of osteoblasts, serum phosphorus, serum total calcium and serum BAP were

Table 1
Basic and biochemical characteristics of subjects

Variable	C group ($n=12$)		IF100 group ($n=15$)		IF200 group ($n=15$)	
	Initial	Final	Initial	Final	Initial	Final
Age (year)	51.2±4.2	NA	53.9±1.8	NA	51.9±1.5	NA
BMI (kg/m^2)	23.9±0.9	NA	22.9±0.5	NA	23.8±0.7	NA
Body fat (%)	26.3±1.5[a]	NA	22.6±1.6	NA	19.7±2.1[a]	NA
Time since menopause (year)	4.4±1.2	NA	5.6±1.3	NA	3.1±0.9	NA
GOT (U/ml)	30.7±3.2	30.8±3.1	24.5±2.3	18.4±1.39	26.8±2.0	24.3±2.9
GPT (U/ml)	21.4±4.9	22.1±4.7*	20.7±4.1	15.1±1.8	23.1±3.9	29.0±6.6
BUN (mg/dl)	15.4±1.2	15.3±1.3	13.5±0.6	12.3±0.6	14.0±0.7	12.6±1.2
Cr (mg/dl)	0.92±0.06	0.94±0.05	0.70±0.06	0.84±0.05*	0.71±0.06	0.86±0.02*
Uric acid (mg/dl)	3.34±0.39	3.43±0.38*	4.33±0.36	4.01±0.19	4.65±0.69	4.14±0.20
Cholesterol (mg/dl)	217.3±11.7	217.3±11.5	206.1±12.4	202.0±11.5	206.5±10.5	210.3±6.1
HDL (mg/dl)	33.9±2.6	34.4±2.7	41.5±3.24	40.3±2.9	46.7±4.9	40.13±2.6
LDL (mg/dl)	145.8±10.4	145.6±10.6	150.2±14.2	158.7±10.8	152.1±9.8	161.5±8.6
TG (mg/dl)	104.4±18.3	103.5±18.1	101.8±16.2	94.1±13.6	95.5±12.3	99.7±17.4
Plasma glucose (mg/dl)	114.7±9.7	108.9±7.4	114.5±10.9	93.3±6.4*	93.3±3.5	89.5±7.7
E_2 (pg/ml)	49.6±43.5	45.4±69.1	44.1±59.0	26.3±28.5	32.7±21.1	12.2±25.5

Values are presented as the mean ± S.E.M. BUN, blood urine nitrogen; GOT, glutamate oxalacetate transaminase; GPT, glutamate pyruvate transaminase; NA, not available; TG, triglyceride.

* $P<.05$ in the same group after treatment.

examined with commercial kits (Sigma Diagnostics, St. Louis, and Alkphase-B, Metra Biosystems) using an automatic analyzer (ALCYON 300i, Abbott Laboratories). Another two markers, urinary deoxypyriddinoline and NTx, were also assessed to evaluate the state of bone loss. The urinary type I collagen cross-linked N-telopeptide (NTx) released by osteoclasts was measured in spot urine with a by commercial ELISA kit (Osteomark, Ostex International) to measure the bone resorption changes. Urinary Dpd was also measured with an ELISA kit (Quidel), and the value was expressed as the ratio of urinary Dpd to Cr level (Dpd/Cr).

2.4. Statistical analysis

All values are presented as the mean ± S.E.M. The data were analyzed using WSE STATA/SE8.0 software for Windows (StataCorp LP). Paired and unpaired t tests and one-way analysis of variance were conducted to identify potential effects of treatments. The level of significance was set at $P<.05$. In addition, linear regressions of levels of observed indicators of the initial groups, as well as percentage changes of various relevant independent variables, were performed. Variables in the regression models were selected so that either a meaningful causal effect was implied or the potential relations were consistent with the existing literature.

3. Results

3.1. Initial analysis and basic characteristics

No relation was exhibited in comparison of the initial BMI and body fat% with any of the initial BMD, BMC or BA readings ($n=42$). The percentage change in BMI was not related to that of body fat percentage. The lack of relation between BMI, body fat% and other bone mineral measurements was probably due to the relative small range of individual BMI levels since 36 of the 42 women had a BMI of 18–24 kg/m^2.

3.1.1. Biochemical and hematological markers

The biochemical parameters are shown in Table 1. Although there were several significant differences seen in the initial parameters, all of them were within normal limits and the percentage changes were all nonsignificant. The percentage changes of hematological profiles were also all nonsignificant as expected (data not shown). Supplementation of 200 mg/day isoflavone (IF200) in our study showed no adverse effects to known biochemical and hematological markers. However, no beneficial effects on lipid profiles were noted at the same time.

3.1.2. Serum E_2

There were no significant differences among the three groups when comparing the initial and final serum E_2. However, decreases in the serum level were significant in both the C and IF200 groups, while the change was slightly less significant in the IF100 group ($P=.06$). The percentage decreases of serum E_2 1 year after treatment was slightly significant when comparing the IF200 and C groups (−0.69% vs. −0.03%, $P<.1$). No relationship was found when comparing initial body fat% or BMI with serum E_2. The initial E_2 was not linearly related to the initial BMD, BMC or BA. There were also no relationships between percentages the change of serum E_2 with any in the bone density measures.

3.2. Bone density and BMC changes

3.2.1. Lumbar spine BMD

Changes in the lumbar spine BMD varied within the C group. The decrease level in BMD was significant at L1–4

Table 2
Changes of BMD values of the lumbar vertebrae

	C group ($n=12$)			IF100 group ($n=15$)			IF200 group ($n=15$)		
	Initial	Final	Change (%)	Initial	Final	Change (%)	Initial	Final	Change (%)
L1	0.95±0.06	0.94±0.06	−1.35	0.95±0.04	0.93±0.04*	−1.43	0.99±0.03	0.97±0.03	−0.68
L2	1.04±0.06	1.03±0.06	−1.76	1.05±0.05	1.06±0.05	1.19	1.07±0.03	1.06±0.02	−0.33
L3	1.10±0.06	1.08±0.07	−2.29	1.11±0.05	1.11±0.05	−0.07	1.12±0.03	1.10±0.02	−0.90
L4	1.13±007	1.11±0.07	−1.42	1.17±0.06	1.18±0.05	1.46	1.11±0.03	1.15±0.03	4.65
L1–2	1.00±0.06	0.98±0.06	−1.66	1.00±0.04	1.00±0.05	−0.04	1.03±0.03	1.02±0.03	−0.70
L1–3	1.04±0.06	1.02±0.06	−2.04[a]	1.00±0.05	1.04±0.05**	4.00[ab]	1.06±0.03	1.05±0.02	−0.50[b]
L1–4	1.06±0.06	1.04±0.06*	−1.92[c]	1.09±0.05	1.10±0.05	0.67[c]	1.07±0.03	1.08±0.02	1.69
L2–3	1.07±0.06	1.05±0.07	−2.07	1.08±0.05	1.07±0.05	−0.83	1.09±0.03	1.08±0.02	−0.53
L2–4	1.09±0.06	1.07±0.07	−1.88[d]	1.13±0.05	1.14±0.05	1.15[d]	1.09±0.03	1.10±0.02	0.99
L3–4	1.11±0.06	1.10±0.07	−1.89	1.15±0.05	1.16±0.05	0.72	1.11±0.03	1.12±0.02	1.50

Values are presented as the mean ± S.E.M. Percentage change in the same row with different superscript letters indicate a significant difference at $P<.05$ between the two groups. Change (%), the mean of the percentage of change.

* In the same row, indicates a significant difference at $P<.05$ in the same group after treatment.

** In the same row, indicates a significant difference at $P<.01$ in the same group after treatment.

($P=.03$), with an significant change of the average for L1 to L4 ($P=.03$). The drop was also significant for L1–3 ($P=.05$) and slightly less so for L2, L1–2 and L2–4 ($P<.1$). On the other hand, the BMD value of L1 in the IF100 group also fell significantly after 1 year, whereas L1–3 rose significantly ($P=.0006$). None of the other measures in this group exhibited significant changes. Falling short of our expectations, supplementation with 200 mg/day did not result in better performance. None of the values changed significantly (Table 2). Percentage changes in the lumbar spine BMD were further checked as well. All the percentage changes were negative in the C group. Significant values were found for L1–3, L1–4 and L2–4 when comparing the percentage increases in the IF100 group and the decreases in the C group (Table 2). The decrease in L1–3 in the IF200 group was, however, significant compared with the respective increase in the 100 mg group. In addition, no percentage change in the IF200 group compared to the C group was significant.

3.2.2. Hip BMD

In the C group, significant decreases were found in changes of the femur neck BMD level ($P=.0001$), whereas those in the IF100 and IF200 groups showed no significant differences (Table 3). This protection effect on the femur neck of isoflavone supplementation was confirmed by measuring the percentage change in the femur neck BMD in each group. The percentage tended to increase in the IF100 (0.66% vs. −2.32%, $P<.1$) and significantly increase in the IF200 group (0.24% vs. −2.32%, $P<.05$) compared to the C group. Although these percentage increases in the IF100 and IF200 groups did not exhibit dose-dependent responses, there was a significant percentage increase in BMD in the femur neck when comparing the IF200 to the C group, implying a greater possible protective effect of 200 mg/day to the femur neck (Table 3). A positive linear relationship was found in the initial levels of BMD between the trochanter and Ward's triangle ($n=42$, $P<.001$, adjusted $R^2=0.72$) and between the neck and trochanter ($n=42$,

Table 3
Relative bone parameter changes in different regions of the femur

Variable	C group ($n=12$)			IF100 group ($n=15$)			IF200 group ($n=15$)		
	Initial	Final	Change (%)	Initial	Final	Change (%)	Initial	Final	Change (%)
BMD (g/cm^2)									
Neck	0.78±0.03	0.76±0.02**	−2.32[a]	0.80±0.03	0.81±0.03	−0.66	0.85±0.03	0.85±0.02	−0.24[a]
Ward's triangle	0.65±0.04	0.65±0.04*	−0.78	0.71±004	0.71±0.04	−0.28	0.77±0.04	0.77±0.03	−0.38
Trochanter	0.69±0.04	0.68±0.04*	−1.71	0.71±0.03	0.71±0.03	−0.59	0.74±0.02	0.73±0.02	−0.70
BMC (g)									
Neck	3.78±0.16	3.79±0.17*	−0.35	4.17±0.17	4.30±0.24	−4.85	4.19±0.18	3.97±0.24*	−3.96
Ward's triangle	1.74±0.14	1.80±0.17*	−3.17	1.82±0.15	1.78±0.10	−7.96	1.96±0.08	1.73±0.09*	−1.42
Trochanter	7.10±0.56	6.71±0.64*	−6.41[bc]	6.62±0.32	6.73±0.34	−5.27[b]	6.09±0.36	6.39±0.29*	−3.20[c]
Area (cm^2)									
Neck	4.88±0.13	4.96±0.19*	−1.40	4.96±0.11	5.07±0.16	−3.42	5.16±0.20	4.93±0.19	−4.18
Ward's triangle	2.67±0.15	2.79±0.21*	−3.69[d]	2.35±0.13	2.32±0.09	−6.65[d]	2.73±0.06	2.50±0.07	−0.89
Trochanter	10.19±0.46	9.65±0.54*	−5.57[ef]	8.94±0.36	9.17±0.30	−6.48[e]	8.61±0.43	9.08±0.31	−4.08[f]

Values are presented as the mean ± S.E.M. Percentage change in the same row with different superscript letters indicate a significant difference at $P<.05$ between the two groups for each variable.

* In the same row, indicates a significant difference at $P<.05$ in the same group after treatment.

** In the same row, indicates a significant difference at $P<.01$ in the same group after treatment.

Table 4
Changes in selected bone markers before and after isoflavone administration

Variables	C group ($n=12$)			IF100 group ($n=15$)			IF200 group ($n=15$)		
	Initial	Final	Changes (%)	Initial	Final	Changes (%)	Initial	Final	Changes (%)
Serum ALP (U/L)	59.1±5.3	58.3±5.21	−1.34	48.9±3.1	69.4±5.23	54.3	43.0±2.8	69.1±4.50*	65.7
Dpd/μCr (nM/mM)	4.04±0.52	4.24±0.46*	4.13	3.62±0.39	7.24±1.05**	136.5	4.17±0.48	8.55±0.93**	181.8
NTx (nM BCE/mM Cr)	6.35±5.33	NA	NA	2.61±0.40	3.07±0.84	16.3	3.98±0.98	4.61±1.63	11.9
BAP (U/L)	15.3±2.3	NA	NA	17.5±1.5	14.3±1.4*	−17.3	16.6±1.3	16.3±1.3	−0.05
Serum calcium (mg/dl)	10.1±0.2	9.98±0.11	−0.64	8.58±0.56	9.94±0.28	26.0	8.30±0.50	9.42±0.36*	17.9
Serum phosphorus (mg/dl)	2.53±0.19	3.11±0.24	9.36	2.71±0.15	3.21±0.15*	25.0	2.57±0.16	3.09±0.10**	26.1
Urine calcium (mg/dl)	74.6±7.9	73.3±7.9*	−2.70	143.0±12.6	114.6±12.6	−14.3	162.0±25.7	114.8±15.5	−22.9
IPTH (pg/ml)	37.2±5.1	37.1±5.1	−0.32	32.4±3.5	33.4±2.4	02.89	35.6±6.10	37.2±5.1	4.58

Values are presented as the mean ± S.E.M. IPTH, intact parathyroid hormone.
* $P<.05$ in the same group after treatment.
** $P<.01$ in the same group after treatment.

$P<.001$, adjusted $R^2=0.70$). However, the BMD of Ward's triangle and the trochanter did not change significantly after treatment in any of the three groups. Between the initial levels of BMD in the femur neck and Ward's triangle, there was a significant negative linear relationship ($n=42$, $P<.001$, adjusted $R^2=0.84$). The relationship between the percentage changes of the two in the C and IF100 groups, however, did not exhibit any significance, whereas in the IF200 group, there was a significant positive linear relationship ($P<.01$, adjusted $R^2=0.48$).

3.2.3. Femur bone BMC and BA

There were positive linear relationships between the initial levels of the BA and BMC in the femur neck ($P<.001$, adjusted $R^2=0.50$), the trochanter ($P<.001$, adjusted $R^2=0.70$) and Ward's triangle ($P<.001$, adjusted $R^2=0.50$). Moreover, there was also positive linear relationship between the percentage changes in the BMC and BA of the femur neck, the trochanter and Ward's triangle in all treatment groups ($P<.05$, adjusted $R^2=0.51$–0.94, except $P=.21$ in the trochanter of the IF100 group). These were further rechecked and confirmed by comparing the percentage changes in all subjects irrespective of their group ($P<.001$, adjusted $R^2=0.60$–0.63). The positive linear relationship of BMC and BA among all percentage changes in the femur neck and trochanter indicated a rather-uniform pattern of bone mineralization with or without isoflavone treatment at these sites.

The percentage increases in BMC of the trochanter in the IF100 or IF200 group were significant compared with the percentage decrease in the C group (BMC 5.27% or 3.20% vs. −6.41%, Table 3). When comparing the percentage change in BA of the trochanter, the IF100 and IF200 groups, both had significant percentage increases compared to the decrease in the C group (6.48% and 4.08% vs. −5.57%). Both changes were greater in the IF100 group than in the IF200 group, although without significance. These results suggested a greater bone quantity (BMC and BA) at the femur trochanter under isoflavone treatment without a significant dose-dependent effect.

The BMC and BA of Ward's triangle exhibited a linear relation in the initial analysis ($n=42$, $P<.001$, adjusted $R^2=0.4$).The BMC o f Ward's triangle significantly decreased in the IF100 group ($P<.1$), whereas that in the IF200 group decreased without clinical significance. However, the percentage changes in BMC and BA at Ward's triangle showed positive linear relationships in the C and IF100 groups ($P=.005$, adjusted $R^2=0.56$; $P=.001$, adjusted $R^2=0.5$), whereas in the IF200 group, these two exhibited a negative linear relationship ($P<.001$, $R^2=0.7$). It is possible that a different pattern of bone mineralization with uncorrelated BMC and BA changes exists at Ward's triangle under IF200 supplementation, which differs from the response seen at the femur neck and trochanter region.

3.3. Biomarkers of bone turnover

As an adjuvant diagnostic tool of osteoporosis, the bone markers we chose here did not change in the expected directions. Nor were the initial levels related to the initial BMD. There was an unpredictable error that caused the loss of some files. The final data of these two parameters in the C group were lost. Table 4 shows the changes in the concentration and their percentage changes for each parameter.

Although the urinary NTx showed no significant changes in the IF100 and 200 groups, there was a significant negative relationship found between the final NTx and percentage changes of BMD for most of the lumbar vertebrae in the IF100 group, including L1–2 ($P=.047$, adjusted $R^2=0.58$), L1–3 ($P=.046$, adjusted $R^2=0.59$), L1–4 ($P=.029$, adjusted $R^2=0.69$) and L2–4 ($P=.035$, adjusted $R^2=0.64$).

There were significant increases in urinary Dpd concentrations in the C group. The urinary Dpd/Cr and serum ALP both significantly increased in the IF100 and IF200 groups. The percentage changes in urinary Dpd/Cr were significantly higher in both treated groups than in the C group. When comparing the linear relation between final urinary Dpd/Cr concentration and bone density, only a weak negative relation was noted in the percentage change of Ward's triangle BMD (P=.05, adjusted R^2=0.2) in the IF100 group. No other relationship could be identified.

There was no relationship between the initial data of bone markers and the initial BMDs. However, a significant relationship between the initial serum calcium and ALP (P<.001, adjusted R^2=0.4) was found. There was a significant linear relationship between ALP and BAP only after 1 year of treatment but not at the initial levels. The serum BAP decreased significantly in the IF100 group but not in the IF200 group. The percentage decrease in BAP was significantly greater in the IF100 group than in the IF200 group. This indicated possible less bone formation activity after taking IF100 for 1 year compared to 200 mg/day, which was opposite to the real changes in BMD in the isoflavone groups. Interestingly, a positive linear relation was only exhibited in the IF100 group, that the percentage changes of BMD at femur neck and trochanter were both positive related to those of BAP (P=.018, adjusted R^2=0.38; P=.015, adjusted R^2=0.41). At the same time, the final BAP was negatively related to the percentage change in BMD of the femur neck (P=.021, adjusted R^2=0.34) and Ward's triangle (P=.017, adjusted R^2=0.36) in the IF200 group.

The final serum ALP was negatively related to the percentage change in BMD of the femur neck, Ward's triangle and trochanter and all the measures in lumbar vertebrae (P<.001, adjusted R^2=0.5–0.6) irrespective of group. It appeared that serum ALP exhibited good and nonspecific relationships with both the proximal femur bone and the lumbar vertebrae.

4. Discussion

Metabolic changes in bone catabolism appear pronounced in the perimenopausal period, even though there is still a certain amount of estrogen supply systemically. Women in the perimenopausal period showed the greatest bone loss and are thus considered to be in a crucial phase of developing osteoporosis [1]. Although higher bone density does not assure a lower fracture risk, a smaller loss of bone density of the hip and spine in early postmenopausal women is still prudent to prevent those fractures affecting life quality.

Fractures are one of the most common causes of disability in older women. Most types of fractures increase as bone density declines and gradually becomes osteoporotic. Osteoporosis is thus diagnosed by a low BMD measurement that contributes to increased fracture risk. The BMD of the proximal femur was reported to have the highest diagnostic sensitivity for both spinal and femoral fractures in postmenopausal women [13,14]. The femoral neck BMD is effective in predicting osteoporotic fracture risk within the first decade after diagnosis.

In our study, compared to the general loss of BMD in the C, the BMD of L1–3 and BMC of the trochanter significantly increased in the IF100 group. Results of BMD and BMC of the spine and proximal femur led to a satisfactory bone-sparing, potentially remediative effect of the spine and proximal femur (neck and trochanter) among postmenopausal subjects in this study. Nonetheless, the relative decrease in the BMC at Ward's triangle in the IF100 group (P<.1) and the nonsignificant decrease in the IF200 group implied a poor protective effect on the trabecular bone by isoflavone. The administration of IF100 is comparable to the known effective dose. These results are quite similar to those of Harkness et al. [12] who showed a greater spinal BMD at L2 and L3 with 110 mg/day. Arjmandi et al. [15] reported no significant changes in total hip BMD, BMC and lumbar BMD with 60 mg/day isoflavone administration. However, Chen et al. [16] concluded that a positive effect of soy isoflavone supplementation only existed among women with a lower initial baseline BMC in the higher-dose group (80 mg/day) compared with the 40 mg/day dose by stratified analyses. From the point of view of bone catabolism, it is worth noting that the potential dose of soy isoflavone to prevent bone loss can be achieved by a dosage as high as 80 mg/day. Daily intake of 90–100 mg/day isoflavone (30–40 g/day soy protein) was suggested in attenuating postmenopausal bone loss [17].

Surprisingly, the intake of IF200 did not exert a dose-dependent effect in our study except for a better percentage change in the neck BMD, which was significant compared to the C but not to the IF100 group. No additional benefit in BMD by IF200 was found even when comparing all of the other percentage changes within the two treated groups. Furthermore, the loss of BMC at Ward's triangle was significant in the IF200 group, while none of the lumbar spine measures increased. Based on these clinical results, supplementation using a dose as high as IF200 did not show a positive effect on BMD and may be a worse choice than 100 mg/day with respect to its bone-sparing effect. Fluctuation of the response with 200 mg/day for which the (percentage change ranged from 41.07% to −26.76% at the femur neck and 25.15% to −14.91% at L1–4) was the main cause of the nonsignificant results in the study. These variations seemed to be due to individualized responses to the high dose of isoflavone. It has been reported in cell culture that genistein acts like estrogen, stimulating osteogenesis and inhibiting adipogenesis at low concentrations ($\leq$1 μM) but acts as a ligand of PPARγ, leading to down-regulation of osteogenesis and up-regulation of adipogenesis at higher concentrations ($>$1 μM) [18]. Although the highest genistein amount in our study was 71 mg in a single oral dose, it proved to be within the maximal rate of

absorption up to 150 mg [19], and some confounding factors that affect the result should be clarified. The intersubject variations in response to soy reflect subject-specific differences in gut microfloral species. The transit time in the colon is another factor that affects individual resorption of both genistein and daidzein [20].

Changes in concentrations of bone markers (such as Dpd, BAP and NTx) in this study do not support results of previous studies that isoflavone prevents bone loss. According to our data, it was significant that bone turnover increased while bone formation decreased and resorption increased after 1 year of isoflavone administration. Reduced formation of whole-body bone was thus suspected by the presentation of markers, an indication of the necessity for longer periods of study. By using 60 mg/day of isoflavone, Arjmandi et al. [15] showed that both lumbar BMD and BMC significantly decreased in the soy and C groups but no changes were seen in the total hip measures. The BAP was elevated in both groups without significance, and the urinary Dpd (bone resorption marker) did not change [18]. Wangen et al. [11] evaluated these effects using 1.00 and 2.01 mg/kg body wt/day isoflavone-rich soy food diets, averaging 65 and 130 mg/day for 3 months, BAP decreased with both dosages in menopausal women, with trends toward decreased levels of osteocalcin, IGFI and IGFBP3 with increasing isoflavone consumption. No evidence demonstrated consistency in bone markers in these studies.

Another marker of bone resorption, NTx, showed no significant changes. However, we did find positive linear relationships between the final BAP and the percentage changes in BMD at the femur neck and trochanter in the IF100 group and negative relationships between the final NTx and most of the percentage changes in the lumbar spine BMD with the dose of 100 mg/day but none at femur. These relationships imply that the linkage between changes in bone markers and BMD were the same as those mentioned in the literature. It is believed that different bone markers might represent bone turnover in certain regions. Such relationships were lacking in the IF200 group. This is comparable to the complex change in the pattern of bone formation seen with the DEXA measures. Data from the final NTx in the IF100 group that only related significantly to the spine were supported by Krall et al. [21] who pointed out that individuals whose values of both osteocalcin and NTx in the lowest quartiles had mean femoral neck BMD values 11% higher than individuals with marker values in the highest quartiles. This suggestion again implies that specific relations exist between changes in bone markers and the site of increased bone turnover and that changes in the level of NTx specifically reflect turnover in the lumbar spine but not in the femur.

Moreover, the BAP was related to the percentage changes in BMDs of the neck and trochanter in the IF100 group. However, serum ALP was significantly related to changes in lumbar region. The decline of BAP in the IF100 group was significantly associated with increases in urinary Dpd and serum ALP in both treated groups. Nonetheless, these results were not comparable to the actual changes in BMD in the isoflavone groups. The reason why the BMD of the spine and femur remained relatively stable in spite of the increased bone turnover is not known. Since bone markers represent metabolism of whole-body bone, it is reasonable to assume that soy isoflavone maintains bone mass of the spine and proximal femur, while increasing the resorption of other regions of bones at the end of 1 year of supplementation. Bone turnover is known to occur most rapidly at skeletal sites rich in trabecular bone, such as the spine and proximal femur of the hip, since approximately 25% of trabecular bone is resorbed and replaced yearly, compared with only 3% of cortical bone. Thus, the tendency of decreases in trabecular-rich areas such as Ward's triangle in the two isoflavone groups might have produced more changes in the markers so that the resorption appeared more serious than what it actually was in general [3]. This provides an explanation for the stable BMD and increased bone turnover associated with the loss of Ward's triangle BMD in the two isoflavone-treated groups.

Other illnesses may also disturb the presentation of bone markers, such as osteoarthritis (OA) [22]. The significant increases of Dpd in urine in the three groups without relation to any specific bone change can possibly be referred to the cartilage distribution of Dpd. The deterioration in cartilage in aged women therefore might have contributed to the values of Dpd in the present study.

It was indicated by Whitham et al. [23] that the external quality assessment for bone markers, including BAP, NTx and urinary Dpd, revealed poor numerical agreement between commercial assays and between-laboratory precision. Nevertheless, several factors may affect bone-related biochemical markers. A cross-sectional study investigating nonsupplemented soy showed trends toward significant differences in NTx and higher spine bone density in women with the highest level of isoflavone consumption [10]. It was noted that evening calcium supplementation markedly suppresses the nocturnal increase in Dpd and NTx and reversed the usual nocturnal increase in the level of parathyroid hormone, although another study of daily 1000 mg calcium intake for 6 weeks increased only serum calcium, but not NTx or BAP, in early menopausal women [24].

There is still debate as to whether higher bone density really benefits Asian women. In addition, half of the hip fractures in women older than 75 year are associated with normal BMD; thus, other risk factors should be taken into account and improved, such as back muscle strength, quadriceps strength, foot coordination and gene [25,26]. Aside from the benefits of greater soy intake, the lower risk of hip fracture compared to Caucasians may also be a result of smaller body figure with shorter hip axis [27]. In addition, hip and spine OA in women is associated with reduced bone loss in the femoral neck or increased spinal BMD, respectively [28,29]. It is suggested that higher bone density increases pressure on the cartilage, hence deterio-

rating the joint [22]. It was thus probably more appropriate to maintain bone density at less than the mean minus one S.D. (osteopenic) rather than to increase it. Chinese women were found to have an increased trochanteric BA with age independent of weight or height and fewer declines in the density of the trochanter compared to the neck and Ward's triangle. These reflect a possible structural change at the proximal femur and imply differences in mechanical strengths in menopause comparing to premenopause [3,21]. However, in this study, we found only a significant increase in trochanteric BA and a decrease in Ward's triangle BA in the isoflavone groups but not in the C group. How these changes in BA affect the risk of hip fracture is worthy of further studies since it might be important to not only improve bone density but also promote structure more resistant to fractures.

The mean daily intake in the highest quartile in a known epidemiologic survey in Chinese was 20 g soy protein, which contributed roughly 40 mg isoflavone, a level far less than that used in most human studies [30]. The possible mechanism of adverse effects in long-term isoflavone intake as high as 200 mg still needs to be determined. Recent studies indicated that genistein and/or daidzein could induce cancers of reproductive organs in animals. Genistein, daidzein and their metabolites were also found to induce DNA damage in vitro [31]. However, 120 mg/g of soy isoflavones by SPI did not induce any change in endometrium in postmenopausal women [32]. On the other hand, they were also suggested to have anticarcinogenic effects [33,34]. Until adequate safety and efficacy studies are completed, isoflavone should be taken in as low a dosage as possible.

The quantity and density of bone decrease with age after menopause. There is a negative relationship between age or time elapsed since menopause and BMD at the spine or hip. This deterioration highlights the importance of early prevention of bone loss as soon as menopause is clinically established. Our study was able to identify the stable protective effect in early menopausal women produced by IF100 in an isolated form. In spite of proving the effect by DXA, the highly active turnover phenomenon shown after 1 year of supplementation by related bone markers indicates the necessity of studies longer than 1 year for high-dosage trials.

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