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ORIGINAL ARTICLE

Soy protein consumption and bone mass in early postmenopausal Chinese women

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Abstract Recent interest has been shown in the potential beneficial effects of phytoestrogens on bone health. As the early years of menopause are a period of rapid bone loss, and the risk for osteoporosis increases substantially, the habitual intake of soy protein and isoflavones may play a role in the retardation of bone loss. This paper reports the results of the baseline cross-sectional analysis of the association between dietary soy protein intake and bone mineral density/content in a population-based study of Chinese women. The sample comprised 454 healthy Chinese women (mean age 55.1 ± 3.57) within the first 12 years of postmenopause. We estimated the dietary intake of soy protein and isoflavones, and other key nutrients, including dietary protein and calcium, using the quantitative food frequency method. Bone mineral density (BMD) and content (BMC) at the spine, hip and total body were measured with a dual energy X-ray densitometer (Hologic 4500A). Soy protein consumption was categorized as quartiles of intake, and related to BMD values at the spine and hip, and BMC of total body. Stratified analyses were carried out among women within or at least 4 years postmenopausal. We observed few differences in BMD/BMC values among the intake quartiles in women within the first 4 years of menopause. However, among the later postmenopausal women, we noted a dose-response relationship with increasing higher BMD values at the trochanter, intertrochanter as well as the total hip and total body with increasing soy protein intake quartiles ($P < 0.05$ from tests for trend). The BMD values differed by about 4–8% between the first and fourth soy protein intake quartiles. Though women from the fourth intake quartile had a 2.9% higher BMD value compared with those from the first intake quartile, the difference was not statistically significant. Stepwise multiple linear regression analyses showed the association between soy intake quartiles and hip BMD as well as total body BMC values remained after adjusting for body weight, which was retained in the final model. Analyses based on soy isoflavones content yielded similar results. This study demonstrated that, among women after the initial few years postmenopausal, soy protein/isoflavones intake had a modest but significant association with hip BMD as well as total body BMC. The effects of soy protein and soy isoflavones on bone health should be further explored in populations with habitual dietary soy intake.

Keywords Bone mineral content · Bone mineral density · Chinese postmenopausal women · Soy protein

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Introduction

Although the incidence of hip fractures is increasing in many parts of Asia, the rates are still lower than those reported in Western populations [1]. It has been postulated that soy intake may have potential beneficial effects on bone health. Data from rodent studies are supportive of such a hypothesis and suggest that at optimal dosages, soy isoflavonoid phytoestrogens are beneficial in maintaining or modestly improving bone mass [2, 3]. Data from human studies are limited and have shown inconsistent results. A 6-month trial in postmenopausal women reported that subjects consuming 90 mg of isoflavones contained in soy protein have a 2% increased bone mass compared with those on 56 mg of

intake contained in the same protein level [4]. Another study by Alekel et al. [5] revealed that soy protein isolate containing 80 mg of isoflavones, attenuated bone loss in the lumbar region of perimenopausal women. Both studies used milk protein as the control groups. However, a 9-month trial by Gallagher et al. [6] found no effect of soy isoflavones on bone mineral density in early postmenopausal women when compared with a control group supplied with alcohol-washed soy protein.

Soy is part of the regular diet in the Asian populations. The limited published data on soy-bone relationship in these populations have generally revealed a positive role of phytoestrogens on bone health. A 3-year longitudinal study of 116 young premenopausal Chinese women aged 30–40 years has noted the potential role of soy food intake in the maintenance of bone mass [7]. A cross-sectional study in 995 premenopausal Japanese women aged 40–49 reported a positive soy-bone association [8]. Cross-sectional studies also observed a positive soy effect on bone in postmenopausal Chinese [9] and Japanese [10, 11] women.

We conducted a population-based study, aimed at investigating the relation between soy protein consumption and bone mass in early postmenopausal Chinese women. This paper reports the baseline association between quartiles of soy protein intake and bone mineral density at the various bone sites and total body while controlling for other confounding factors such as dietary calcium, protein intake, body weight and years since menopause.

Materials and methods

The study population consisted of 454 healthy women aged 48–62 years recruited from community subjects residing in housing estates in Shatin. Stratified-cluster sampling method was used to select the housing estates in the Shatin district of Hong Kong. Method of recruitment included both door-to-door as well as written invitations placed in mailboxes. Women within the specified age range were screened for eligibility. Respondents who were taking hormonal replacement therapy and those who had malabsorption syndromes, chronic liver kidney diseases, parathyroid and thyroid diseases, gastric operation or cancer were excluded from the study. Women who had undergone oophorectomy and/or hysterectomy were also excluded because of inability to determine menopausal status. We confined the subjects to women within the first 12 years of menopause. After initial screening for eligibility, we invited the eligible women to the Prince of Wales Hospital (a regional teaching hospital) for structured face-to-face interviews, bone mineral density and anthropometric measurements. The structured interview included medical history, basic demographic characteristics, dietary intake and lifestyle variables.

The assessment of dietary soy protein intake was based on a quantitative food frequency questionnaire (FFQ). The FFQ has been previously validated against the 3-day 24-hr dietary recalls in 51 perimenopausal women ($r = 0.68$, $P < 0.001$). In addition, commonly available soy products identified from market survey were included in the FFQ. Thus the FFQ contained the following soy food/products: soft tofu, hard tofu, tofu pudding, fried tofu, pressed tofu, soy vegetarian items, curd sheet, tofu-rope, tofu-pop, soy bean sprout, soy bean (including fresh and canned), soy milk, soy powder, soy paste and fermented tofu. Reliability check of soy protein intake based on the FFQ after 3 and 9 months were $r = 0.60$, $P < 0.001$ and $r = 0.62$, $P < 0.001$ among 46 and 369 subjects, respectively.

We asked the participants to report the average intake of food per week or month (depending on the frequency intake), using the previous 12 months prior to the interview as a reference period. Foods with frequency of intake less than once per month, or 12 times per year, were ignored. Pictures of food items in the reference portion sizes were provided as visual aids. Soy protein and other nutrients were derived from food composition tables and published data [12, 13, 14, 15, 16]. The amount of soy isoflavones intake was also estimated [13, 14, 15, 16]. The study received approval from the Chinese University of Hong Kong, Faculty of Medicine Ethics Committee. All women signed the written informed consent form before being enrolled in the study.

Bone measurements

Bone mass measurements were performed by means of dual energy X-ray densitometry (Hologic 4500A) at the lumbar vertebrae (L2–L4), the left hip and the total body. The coefficient of variation for the measurements of the lumbar spine BMD based on the spine phantom was 0.39%. The in-vivo reproducibility of the machine was 1.53%, 1.72%, 1.15%, 4.86%, and 1.2% for the spine, femoral neck, trochanter, intertrochanter and whole body, respectively.

Statistical analysis

For the investigation of the relation between soy protein intake and bone mass, the dependent variables were the BMD or BMC measurements at the spine, various hip sites and total body. We performed regression plot and simple regression analysis with soy protein and BMD as continuous variables. Since non-linear relationship was found, soy protein or isoflavone intake was categorized as quartiles, and treated as linear ordinal variables. As the first 3–4 years since menopause characterizes a rapid decline in bone mass [17], we performed the analyses separately for women who were less than 4 years postmenopausal, and women who were 4 years or more since menopause. One-way ANOVA and tests for trend were used to compare the mean BMD/BMC values among the different intake quartiles, and for the dose-response relationship between soy intake and BMD values. Because of potential colinearity among the dietary variables, stepwise multiple regression analyses were used to examine the independent effects of soy protein intake quartiles (method = enter) as well as the other main determinants (methods = stepwise, F-to-enter = 0.05, F-to-remove = 0.1) on BMD and BMC. The other independent variables [18, 19, 20] included body weight, years since menopause, dietary calcium intake, total protein intake, energy intake as well as the soy protein-calcium interaction. Body weight, instead of other anthropometric measurements, was used in the models as it had the strongest correlation with BMD values. SPSS for Windows (Release 10.1, SPSS Inc., Chicago, USA) was used for the analysis.

Results

About 8% each of the women had no formal or post-secondary education, 40% had primary and 44% secondary education. About two-thirds of the study subjects were housewives. Only 2.2% of the women smoked, and 4% were occasional alcohol drinkers. Forty percent of the study population were at least 4 years postmenopausal, with a range up to 11.5 years. Table 1 shows the distribution of the characteristics of the study subjects. The mean age of the study subjects was 55.1 years (standard deviation = 3.57), and the

Table 1 Baseline characteristics of study subjects

Characteristics of study sample	All women ($n=454$)			<4 years ($n=269$)			≥ 4 years ($n=185$)			P-value (t-test)
	Mean ± SD	Range	Median	Mean ± SD	Range	Median	Mean ± SD	Range	Median	
Age	55.1 ± 3.57	48–63	54.80	53.4 ± 2.66	48.0–62.3	53.20	57.6 ± 3.23	49.0–63.0	58.30	< 0.001
Years since menopause	3.76 ± 1.98	0–11.49	3.37	1.60 ± 0.21	0–3.94	1.46	6.90 ± 0.71	4.17–11.49	6.67	< 0.001
Anthropometric data										
Height (cm)	153.1 ± 5.21	137.4–172.0	153.0	153.4 ± 5.48	137.4–172.0	153.2	152.6 ± 4.77	141.5–164.0	152.5	0.099
Weight (kg)	57.5 ± 8.95	32.7–94.3	56.4	57.3 ± 8.89	32.7–94.3	56.0	57.8 ± 9.05	38.8–85.4	57.4	0.615
Body mass index	24.6 ± 3.74	14.2–46.1	24.3	24.4 ± 3.75	14.2–46.1	24.2	24.8 ± 3.71	16.1–37.5	24.4	0.237
Dietary intake per day										
Energy (kcal)	1383.5 ± 459.28	237.1–3182.8	1297.4	1392.5 ± 457.9	237.1–3182.8	1291.0	1370.3 ± 462.2	546.7–2989.3	1370.3	0.613
Calcium (mg)	566.5 ± 268.87	107.5–1436.9	508.2	574.1 ± 266.5	107.5–1436.9	530.2	555.5 ± 272.7	134.1–139.6	480.5	0.472
Soy isoflavones (mg)	17.33 ± 19.67	0–161.6	11.42	18.32 ± 19.68	0–152.36	12.40	15.89 ± 19.61	0–161.6	10.03	0.197
Soy protein (g)	7.9 ± 9.05	0–76.9	5.04	8.3 ± 9.00	0–71.3	5.69	7.3 ± 9.11	0–76.9	4.47	0.235
Total protein (g)	58.3 ± 24.3	11.8–168.2	53.12	60.0 ± 25.5	11.8–168.2	53.47	55.9 ± 22.4	18.8–166.7	52.35	0.069
BMD measurements (g/cm^2)										
Spine	0.838 ± 0.131	0.522–1.316	0.834	0.856 ± 0.130	0.531–1.316	0.854	0.812 ± 0.129	0.522–1.224	0.807	< 0.001
Neck	0.681 ± 0.099	0.449–1.062	0.668	0.692 ± 0.099	0.467–1.062	0.681	0.665 ± 0.099	0.449–1.050	0.654	0.005
Trochanter	0.593 ± 0.096	0.329–1.029	0.584	0.599 ± 0.094	0.329–1.029	0.588	0.583 ± 0.099	0.401–0.956	0.573	0.085
Intertrochanteric	0.961 ± 0.138	0.612–1.438	0.955	0.974 ± 0.133	0.642–1.438	0.964	0.943 ± 0.143	0.612–1.389	0.932	0.021
Total hip	0.797 ± 0.114	0.522–1.224	0.785	0.807 ± 0.109	0.525–1.224	0.792	0.783 ± 0.119	0.522–1.173	0.771	0.029
Total body	0.959 ± 0.090	0.732–1.206	0.958	0.974 ± 0.088	0.732–1.206	0.973	0.938 ± 0.088	0.738–1.181	0.934	< 0.001
BMC measurements (g)										
Total body BMC	1619.0 ± 247.5	968.0–2402.4	1614.3	1658.7 ± 246.1	1035.4–2402.4	1656.2	1560.9 ± 238.5	968.0–2269.9	1559.5	< 0.001

Table 2 Mean soy protein intake (g/day) and mean BMD/BMC values at various sites by soy protein intake quartiles. Range of values within quartiles: 1st quartile: (0–2.73) g/day; 2nd quartile: (2.74–5.03) g/day; 3rd quartile: (5.04–9.58) g/day; 4th quartile:(9.59–76.9) g/day

Intake quartile	n	Intake (g/day)	BMD (g/cm^2)						BMC (g)
			Spine	Femoral neck	Trochanter	Intertrochanteric	Total hip	Total body	Total body
All women ($n=454$)									
1	113	1.38 ± 0.81	0.825 ± 0.118	0.668 ± 0.103	0.581 ± 0.098*	0.945 ± 0.145*	0.781 ± 0.118*	0.958 ± 0.088	1601 ± 255
2	114	3.81 ± 0.66	0.831 ± 0.133	0.677 ± 0.101	0.586 ± 0.100	0.953 ± 0.149	0.792 ± 0.120	0.951 ± 0.092	1598 ± 247
3	114	7.11 ± 1.32	0.853 ± 0.139	0.684 ± 0.096	0.598 ± 0.091	0.966 ± 0.125	0.802 ± 0.103	0.962 ± 0.095	1628 ± 259
4	113	19.41 ± 11.58	0.844 ± 0.133	0.694 ± 0.099	0.606 ± 0.095	0.981 ± 0.130	0.815 ± 0.111	0.966 ± 0.084	1649 ± 228
P-value (ANOVA)	–	< 0.001	0.364	0.271	0.194	0.219	0.132	0.642	0.364
P-value (test for trend)	–	< 0.001	0.149	0.049	0.031	0.037	0.018	0.336	0.097
Years since menopause <4 ($n=269$)									
1	62	1.32 ± 0.79	0.842 ± 0.115	0.683 ± 0.103	0.594 ± 0.100	0.971 ± 0.139	0.799 ± 0.114	0.982 ± 0.086	1659 ± 244
2	64	3.78 ± 0.70	0.873 ± 0.124	0.695 ± 0.097	0.596 ± 0.091	0.973 ± 0.139	0.809 ± 0.111	0.973 ± 0.090	1673 ± 248
3	69	7.20 ± 1.28	0.859 ± 0.152	0.686 ± 0.104	0.600 ± 0.102	0.971 ± 0.139	0.805 ± 0.113	0.976 ± 0.098	1643 ± 274
4	74	19.21 ± 10.66	0.852 ± 0.124	0.702 ± 0.096	0.605 ± 0.086	0.979 ± 0.117	0.814 ± 0.101	0.967 ± 0.080	1661 ± 222
P-value (ANOVA)	–	< 0.001	0.568	0.689	0.917	0.987	0.869	0.803	0.922
P-value (test for trend)	–	< 0.001	0.821	0.375	0.485	0.774	0.492	0.389	0.866
Years since menopause ≥ 4 ($n=185$)									
1	51	1.45 ± 0.83	0.805 ± 0.120^{+}	0.651 ± 0.102	0.564 ± 0.095*	0.912 ± 0.146*	0.759 ± 0.121*	0.929 ± 0.082*	1531 ± 252
2	50	3.85 ± 0.62	0.777 ± 0.125	0.656 ± 0.103	0.574 ± 0.110	0.929 ± 0.158	0.770 ± 0.129	0.925 ± 0.088^{c}	1503 ± 212$^{+,++}$
3	45	6.97 ± 1.38	0.844 ± 0.116	0.681 ± 0.083	0.594 ± 0.073	0.958 ± 0.101	0.797 ± 0.085	0.941 ± 0.086	1606 ± 235
4	39	19.78 ± 13.30	0.828 ± 0.151	0.679 ± 0.106	0.609 ± 0.113	0.985 ± 0.152	0.817 ± 0.129	0.966 ± 0.093	1625 ± 241
P-value (ANOVA)	–	< 0.001	0.063	0.330	0.148	0.080	0.084	0.137	0.042
P-value (test for trend)	–	< 0.001	0.111	0.092	0.022	0.010	0.011	0.040	0.017

*$P \leq 0.05$ from t-test comparing mean BMD values between Q1 and Q4 intake groups
$^{+}$ $P < 0.05$ by multiple range test comparing with the 3rd quartile
$^{++}$ $P < 0.05$ by multiple range test comparing with the 4th quartile

mean number of years since menopause was 4.76 (standard deviation = 2.98). The daily mean intakes of soy protein and calcium for all women were 7.9 g, and 567 mg respectively. Table 2 shows the mean values of soy protein by intake quartiles among all women, as well as for those within or beyond 4 years since menopause. Seven of the study subjects did not consume soy products during the 12 months prior to the baseline interview. Women belonging to the highest soy protein intake quartile had a mean intake of 19.4 ± 11.6 g/day with a range from 9.6 to 76.9 g/day. Except for the spine, there was a general trend toward higher BMD/BMC values among the higher soy protein intake quartile groups. The trend was non-significant among the early menopausal women. Among the later menopausal women, we observed a statistically significant

Table 3 Mean baseline calcium intake (mg/day) and mean BMD/BMC values at various sites by calcium intake quartiles

Intake quartile	*n*	Intake (mg/day)	BMD (g/cm^2)						BMC (g)
			Spine	Femoral neck	Trochanter	Intertrochanteric	Total hip	Total body	Total body
All women (n = 454)									
1	113	277.6 ± 60.1	0.821 ± 0.135	0.670 ± 0.093**	0.579 ± 0.093**	0.943 ± 0.138**	0.781 ± 0.111**	0.953 ± 0.089	1592.0 ± 253.0
2	114	434.3 ± 42.8	0.843 ± 0.125	0.677 ± 0.101	0.591 ± 0.100	0.955 ± 0.129	0.793 ± 0.111	0.953 ± 0.086	1600.0 ± 225.0
3	114	606.1 ± 61.6	0.851 ± 0.138	0.698 ± 0.114	0.606 ± 0.100	0.980 ± 0.157	0.814 ± 0.127	0.967 ± 0.094	1649.0 ± 266.0
4	113	949.0 ± 178.6	0.836 ± 0.125	0.678 ± 0.089	0.594 ± 0.092	0.967 ± 0.123	0.801 ± 0.103	0.964 ± 0.089	1636.6 ± 243.0
P-value (ANOVA)	–	< 0.001	0.358	0.181	0.198	0.220	0.155	0.542	0.241
P-value (test for trend)	–	< 0.001	0.336	0.309	0.126	0.096	0.081	0.208	0.082
Years since menopause < 4 (n = 269)									
1	66	277.5 ± 59.2	0.838 ± 0.134	0.676 ± 0.092**	0.586 ± 0.095**	0.949 ± 0.137**	0.785 ± 0.110**	0.967 ± 0.082	1623.3 ± 229.4
2	59	437.3 ± 42.3	0.869 ± 0.125	0.695 ± 0.105	0.599 ± 0.101	0.979 ± 0.126	0.811 ± 0.108	0.977 ± 0.085	1665.8 ± 222.3
3	74	607.6 ± 62.9	0.875 ± 0.138	0.714 ± 0.111***	0.617 ± 0.093	0.998 ± 0.146	0.830 ± 0.117	0.982 ± 0.094	1696.3 ± 270.6
4	70	933.5 ± 185.6	0.842 ± 0.119	0.680 ± 0.085	0.593 ± 0.088	0.966 ± 0.116	0.799 ± 0.097	0.970 ± 0.090	1646.6 ± 252.5
P-value (ANOVA)	–	< 0.001	0.233	0.099	0.222	0.162	0.094	0.748	0.347
P-value (test for trend)	–	< 0.001	0.797	0.578	0.452	0.331	0.299	0.816	0.451
Years since menopause ≥4 (n = 185)									
1	47	277.7 ± 62.1	0.797 ± 0.135	0.662 ± 0.095	0.568 ± 0.090	0.935 ± 0.139	0.774 ± 0.114	0.933 ± 0.096	1547.4 ± 278.8
2	55	431.0 ± 43.4	0.815 ± 0.121	0.658 ± 0.094	0.583 ± 0.099	0.928 ± 0.128	0.774 ± 0.111	0.928 ± 0.080	1529.2 ± 208.0
3	40	603.3 ± 59.9	0.807 ± 0.129	0.668 ± 0.116	0.586 ± 0.110	0.945 ± 0.173	0.784 ± 0.140	0.938 ± 0.089	1560.4 ± 237.0
4	43	974.1 ± 165.7	0.827 ± 0.135	0.674 ± 0.095	0.597 ± 0.100	0.969 ± 0.135	0.804 ± 0.112	0.956 ± 0.088	1618.0 ± 226.9
P-value (ANOVA)	–	< 0.001	0.742	0.874	0.589	0.542	0.573	0.455	0.319
P-value (test for trend)	–	< 0.001	0.359	0.489	0.180	0.214	0.198	0.174	0.130

*$P \leq 0.01$ from *t*-test comparing mean BMD values between Q1 and Q4 intake groups
**$P < 0.05$ by multiple range test comparing with 3rd quartile
***$P < 0.05$ by multiple range test comparing with 4th quartile

trend and a dose-response relationship of higher BMD values at the hip sites and total body with increasing soy protein intake quartiles. For the latter group, differences in BMD values between the first and fourth intake quartiles was 2.9% for the spine; and ranged from 4% to 8% for the various hip sites, and total body. As calcium has a potential effect on bone mass, we also investigated the association between calcium intake quartiles and BMD/BMC values (Table 3). There was also a trend for higher BMD/BMC in the higher intake quartiles, but the tests for trend were non-significant.

Table 4 shows the final models based on the stepwise multiple regression analyses. Body weight and years since menopause were retained in the final models for all women, and for women within 4 years of menopause, but we observed little association between soy protein intake and BMD/BMC values. Among women belonging to the later menopausal group, besides soy protein that was forced into the model, the only other variable retained was body weight. Soy protein was significantly associated with trochanteric, intertrochanteric and total hip BMDs, as well as total body BMC, after taking into account the presence of body weight. The two variables explained about one-fifth to one-quarter of the variances of BMDs at the various hip sites; and about 30% of total body BMC. Soy protein alone explained about 3% of the hip BMD or total body BMC variances. We also observed similar trends and associations when soy isoflavones instead of soy protein was used in the analyses (data not shown).

Discussion

Animal studies have noted that the administration of soy protein containing phytoestrogens, and the administration of isoflavones—daidzein or genistein—prevent bone loss in ovariectomized rats [21, 22, 23, 24, 25]. Limited data are available on the relation between soy intake and bone health in humans. Table 5 presents a summary of the published studies. Short-term trial results have suggested a bone conserving effect of soy protein containing isoflavones on the spine BMD in perimenopausal and postmenopausal women [4, 5]. However, a 9-month randomized double-blind study on early postmenopausal women showed no effect of either 52 mg or 96 mg isoflavones (contained in soy protein) on the spine and hip BMD when compared with the control group consuming alcohol-washed soy protein [6]. Anderson et al. [26] also observed a lack of effect of soy isoflavones enriched soy protein isolate on BMD in young women. A recent 1-year randomized double-blind placebo-controlled study [27] on the effects of daily 54 mg of phytoestrogen genistein on BMD in postmenopausal women revealed a 3% increase in the spine and femoral neck BMD compared to a –1.6% and –0.65% decrease in the respective bone sites in the placebo group.

Soy intake is part of the regular diet of the Asian populations. Recent studies have revealed a positive association between habitual soy intake and bone health in premenopausal women [7, 8]. Two studies in

Table 4 Stepwise multiple regression analysis on association between soy protein intake quartiles and BMD/BMC. Independent variables: soy protein intake quartile (method = enter); body weight, years since menopause, calcium intake quartile, soy protein-calcium interaction, total protein intake, and energy intake (method = stepwise; *F*-to-enter = 0.05, *F*-to-remove = 0.1)

Dependent variables	All women ($n = 454$)				Women < 4 ($n = 269$)				Women ≥ 4 ($n = 185$)			
	β	SE of β	*P*-value	r^2	β	SE of β	*P*-value	r^2	β	SE of β	*P*-value	r^2
Spine BMD												
Soy protein intake quartile	0.0034	0.005	0.497	0.1	−0.0024	0.006	0.705	0.1	0.0110	0.008	0.172	1.0
Body weight	0.0057	0.001	< 0.001	15.8	0.0060	0.001	< 0.001	16.9	0.0055	0.001	< 0.001	14.7
Years since menopause	−0.0086	0.002	< 0.001	4.5	−0.0130	0.006	0.033	1.7	–	–	–	–
Total r^2	–	–	–	18.9	–	–	–	17.6	–	–	–	15.9
Femoral neck BMD												
Soy protein intake quartile	0.0048	0.004	0.200	0.4	0.0014	0.005	0.764	0.03	0.0094	0.006	0.123	1.3
Body weight	0.0048	0.0005	< 0.001	19.0	0.0051	0.001	< 0.001	20.9	0.0044	0.004	< 0.001	16.8
Years since menopause	−0.0057	0.001	< 0.001	3.5	−0.0092	0.005	0.042	1.6	–	–	–	–
Total r^2	–	–	–	21.6	–	–	–	21.5	–	–	–	18.1
Trochanter BMD												
Soy protein intake quartile	0.0056	0.004	0.119	0.5	0.0003	0.005	0.945	0.002	0.0133	0.006	0.025	2.7
Body weight	0.0050	0.0005	< 0.001	21.5	0.0051	0.001	< 0.001	23.2	0.0049	0.001	< 0.001	19.9
Years since menopause	−0.0041	0.001	0.002	2.1	−0.0102	0.004	0.017	2.1	–	–	–	–
Total r^2	–	–	–	23.2	–	–	–	24.0	–	–	–	22.2
Intertrochanteric BMD												
Soy protein intake quartile	0.0069	0.005	0.162	0.4	−0.0031	0.006	0.616	0.1	0.0211	0.008	0.012	3.4
Body weight	0.0077	0.001	< 0.001	25.8	0.0080	0.001	< 0.001	29.0	0.0074	0.001	< 0.001	22.8
Years since menopause	−0.0067	0.002	< 0.001	2.8	−0.0133	0.006	0.020	2.0	–	–	–	–
Total r^2	–	–	–	27.6	–	–	–	29.5	–	–	–	25.6
Total hip BMD												
Soy protein intake quartile	0.0070	0.004	0.087	0.7	−0.0001	0.005	0.981	0.0002	0.0171	0.007	0.013	3.3
Body weight	0.0065	0.001	< 0.001	26.7	0.0066	0.001	< 0.001	29.0	0.0064	0.001	< 0.001	24.5
Years since menopause	−0.0055	0.002	< 0.001	2.8	−0.0111	0.005	0.019	2.1	–	–	–	–
Total r^2	–	–	–	28.6	–	–	–	29.6	–	–	–	27.1
Total body BMD												
Soy protein intake quartile	0.0071	0.004	0.842	0.01	−0.0064	0.005	0.160	0.7	0.0108	0.006	0.058	2.0
Body weight	0.0026	0.0004	< 0.001	7.1	0.0026	0.001	< 0.001	7.4	0.0026	0.001	< 0.001	7.2
Years since menopause	−0.0074	0.001	< 0.001	6.4	−0.0125	0.004	0.004	3.2	–	–	–	–
Total r^2	–	–	–	12.3	–	–	–	9.8	–	–	–	9.4
Total body BMC												
Soy protein intake quartile	5.974	8.784	0.497	0.1	−11.837	11.487	0.304	0.4	31.065	13.639	0.024	2.8
Body weight	13.508	1.096	< 0.001	25.4	13.632	1.459	< 0.001	24.9	13.489	1.658	< 0.001	26.9
Years since menopause	−19.923	3.313	< 0.001	7.5	−30.764	10.766	0.005	3.0	–	–	–	–
Total r^2	–	–	–	29.3	–	–	–	25.9	–	–	–	29.2

postmenopausal Japanese women [10, 11] reported a significant positive association between soy protein or isoflavones intake and spinal BMD. Another study in older Chinese postmenopausal women (mean age 63 years and about 13 years postmenopausal) also reported a positive association between phytoestrogens intake and spine and hip bone mass [9]. These cross-sectional studies were conducted in postmenopausal women with a mean age in the 60s [9, 11], or with a wide age range up to 80 years old [10], and therefore older than our study population. Our study results showed no association between soy intake and bone mass in women within the first four years since menopause, but we did observe a dose-response association for the hip and total body BMD in women belonging to later menopausal years.

We observed that, based on stepwise multiple regression analysis, years since menopause was a significant determinant of bone mass in early postmenopausal, but not in later menopausal women. Studies have revealed that bone loss in the spine, femoral neck and total body diminishes about 3–4 years after the last menses [17]. Estrogen deprivation is largely related to spinal bone loss, while both age and estrogen decline account for bone loss in the femoral neck and total body [17]. The moderate dosage of soy protein or isoflavones derived from the habitual intake of soy in our population could be suboptimal to counteract the drastic effect of estrogen decline on bone loss during the initial postmenopausal years. However, habitual soy intake seems to exert a protective effect on bone mass after those dramatic years. In the later menopausal women, we also observed a positive effect of soy on the spine BMD, though the association did not reach a level of statistical significance.

Animal experimental results suggest that an effective optimal dosage of isoflavones need to be consumed before any measurable effect on bone can be observed [2, 28]. In our study, women belonging to the highest quartile of soy protein intake had a mean intake of about 20 g per day, which contributes to roughly 40 mg soy isoflavones. The amount is lower than the apparent effective doses of 80 to 90 mg soy isoflavones needed to

Table 5 Summary of human studies on soy intake and bone mineral density. *SPI+* soy protein isolate containing isoflavones, *SPI–* soy protein isolate not containing isoflavones, *(+)* positive effect, *(–)* no effect, *ysm* years since menopause

Researcher	Populations	Study design total sample size (*n*)	Age (years), mean ± SD, range (in brackets)	Menopausal status	Type and dosage	Duration	Effect and BMD site
Potter et al. [4]	Caucasian	RCT on 66 subjects in three arms	61.2 ± 10.3 (39–83)	Postmenopausal (12–13) ysm	SPI+ (56 mg/90 mg isoflavones) compared with whey	6 months	(+) at L1–L4 BMD in SPI+ 90 mg group, NS at other sites
Gallagher et al. [6]	Caucasian	RCT on 65 subjects in three arms	55	Early postmenopausal	Soy protein with / 52 mg/96 mg isoflavones compared with alcohol-washed soy protein	9 months	(–)
Alekel et al. [5]	Caucasian	RCT on 69 subjects in three arms	50 (42–62)	Perimenopausal	SPI+ (80.4 mg), SPI– and compared with whey	6 months	(+) on (L1–L4) BMD/BMC change in SPI+80 mg group (other sites not mentioned)
Anderson et al. [26]	Caucasian	RCT on 28 subjects in two arms	24 ± 1.1 (21–25)	Premenopausal	SPI+ (90 mg) compared with SPI– with alcohol extraction	1 year	(–)
Morabito et al. [27]	Caucasian	RCT on 90 subjects in three arms	52 ± 3 (47–57)	Postmenopausal	Phytoestrogen genistein 54 mg/day compared with placebo	1 year	(+) on spine BMD and femoral neck BMD
Horiuchi et al. [11]	Japanese	Cross-sectional (*n* = 85)	66.9 ± 7.4 (52–83)	Postmenopausal	Habitual intake of soy protein 12.6 ± 5.4 g/day (2.8–32.9)*	–	(+) with (L2–L4) BMD (other sites not mentioned)
Somekawa et al. [10]	Japanese	Cross-sectional (*n* = 478)	(44–80)	Postmenopausal	Habitual soy product isoflavones intake with mean isoflavones of 54.3 mg/day ± 1.0	–	(+) on (L2–L4) BMD in early postmenopausal < 5 ysm, (+) in late postmenopausal > 5 ysm (other sites not mentioned)
Ho et al. (present study)	Chinese	Cross-sectioned (*n* = 454)	55.1 ± 3.57 (48–62)	Postmenopausal < 4 ysm (5–12)* ysm	Habitual intake of soy protein from soy products 7.9 ± 9.1 g/day (0–76.9 g/day)*	–	(–) in women < 4 ysm, (+) with trochanters, intertrochanter and total hip BMD, and total body BMC in women (5–12) ysm
Mei et al. [9]	Chinese	Cross-sectional (*n* = 293 for premenopausal) (*n* = 357 for postmenopausal)	All: (19–86) Pre: 37.5 ± 9.4 Post: 63 ± 8.3	Premenopausal Postmenopausal 13.6 ± 8.2 ysm	Phytoestrogens intake 21.9 ± 37.5 mg/day	– – –	(–) in premenopausal (+) (L2-L4) BMD in postmenopausal women (+) total hip t-score in postmenopausal women
Tsuchida et al. [8]	Japanese	Cross-sectional (*n* = 995)	45 ± 3 (40–49)	Premenopausal	Habitual soy protein intake	–	(+) with 2nd metacarpal BMD
Ho et al. [7]	Chinese	3-year longitudinal (*n* = 132)	35.3 ± 3 (30–40)	Premenopausal	Habitual soy protein/isoflavone intake from soy foods (0–48.3 mg/d)*	–	(+) with spine (L2–L4) BMD change

[a]Numbers in brackets indicate range of intake

maintain spinal BMD in trials in perimenopausal and postmenopausal Caucasian women [4, 5]. The discrepancies in the apparent beneficial dosages reported in the different studies could also be due to the differences in age and physiological characteristics, including menopausal stages, and the amount and duration of soy intake. As trabecular bone is markedly affected during estrogen decline, the moderate level of soy intake in this population may be inadequate to exert a protective effect at the spine comprising mainly of trabecular bone. It is potentially feasible that a higher soy intake would produce a beneficial effect on the spine BMD, as well as on other bone sites in women immediately postmenopausal. We observed a beneficial effect of habitual soy intake on bone at both the hip and total body consisting of both trabecular and cortical bone. The beneficial dosages of soy isoflavones on bone health may thus vary with age and stages of menopause, and possibly according to the different levels of adaptation to soy intake. However, direct comparisons of dosages between observational studies and trials may be inappropriate as the food frequency questionnaire method is meant for the estimation of relative rather than absolute soy intake. Further studies would be required to elucidate the optimal dosage of soy on bone health in different population groups.

The mechanisms of the effect of soy on bone health are still unknown. Researchers have proposed several mechanisms through which soy might exert an effect on bone mass. The bone sparing effect of soy could be due to its estrogen-like effect of isoflavones in the inhibition of bone resorption or stimulation of bone formation [21, 29], or through its effect on estrogen-receptors in bone cells [30]. As reported by Arjmandi et al. [31], soy could also have an anabolic effect on bone through the enhancement of insulin-like growth factor-I (IGF-1) synthesis, and IGF-I has been shown to have an effect on bone mineral accretion [32].

Other mechanisms could be related to the effect of soy in the enhancement of calcium absorption and the reduction of urinary calcium excretion [2]. The calcium conserving effect of soy protein would be of special importance in Asian populations with relatively low dietary calcium intake and calcium density [19, 33]. However, because of colinearity, we were not able to tease out the effects of soy calcium interaction in our study. The most commonly consumed soy product, tofu, also has high calcium content. Our on-going prospective crossover trials would investigate the effects of soy isoflavones on calcium metabolism.

In conclusion, we observed a modest but an independent effect of soy protein intake in the maintenance of hip BMD, even after taking into account the other important predicting factor—body weight. Our study suggested that habitual soy protein intake of about 20 g/day was associated with significantly higher hip BMD and total body BMD and BMC in menopausal women after the initial period of rapid bone loss. The cross-sectional association has limitations in that no causal inference can be drawn. However, the study was conducted in a population-based sample with reasonable variability of habitual soy intake [34]. The findings add to the existing evidence that soy intake may be beneficial for bone conservation in postmenopausal women. Further studies, both longitudinal and longer-term randomized control trials, are needed to elucidate the components of soy protein, optimal dosages and the period of life that soy is most effective in bone mass maintenance.

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References

1. Schwartz AV, Kelsey JL, Maggi S, Tuttleman M, Ho SC, Jonsson PV, Poor G, Sisson de Castro JA, Xu, L, Matkin CC, Nelson LM, Heyse SP (1999) International variation in the incidence of hip fractures: cross-national project on osteoporosis for the World Health Organization Program for research on aging. Osteoporos Int 9:242–253
2. Anderson JJB, Ambrose WW, Garner SC, Ohue T (1997) The effects of phytoestrogens on bone. Nutr Res 17:1617–1631
3. Arjmandi BH, Alekel L, Hollis BW, Amin D, Stacewicz-Sapuntzakis M, Guo P, Kukreja SC (1996) Dietary soybean protein prevents bone loss in an ovariectomized rat model of osteoporosis. J Nutr 126:161–167
4. Potter SM, Baum JA, Teng H, Stillman RJ, Erdman JW Jr (1998) Soy protein and isoflavones: their effects on blood lipids and bone density in postmenopausal women. Am J Clin Nutr 68:1375S–1379S
5. Alekel DL, Germain AS, Peterson CT, Hanson KB, Stewart JW, Toda T (2000) Isoflavone-rich soy protein isolate attenuates bone loss in the lumbar spine of perimenopausal women. Am J Clin Nutr 72:844–852
6. Gallagher JC, Rafferty K, Haynatzka V, Wilson M (1999) Effect of soy protein on bone metabolism. Presented at Third international symposium on the role of soy in preventing and teaching chronic disease, Oct 31–Nov 3, Washington D.C.
7. Ho SC, Chan SG, Yi Q, Wong E, Leung PC (2001) Soy intake and the maintenance of peak bone mass in Hong Kong Chinese Women. J Bone Miner Res 16:1363–1369
8. Tsuchida K, Mizushima S, Toba M, Soda K (1999) Dietary soybeans intake and bone mineral density among 995 middle-aged women in Yokohama. J Epidemiol 9:14–19
9. Mei JJM, Yeung SSC, Kung AWC (2001) High dietary phytoestrogen intake is associated with higher bone mineral density in postmenopausal but not premenopausal women. J Clin Endocrinol Metab 86:5217–5221
10. Somekawa Y, Chiquchi M, Ishibashi T, Aso T (2001) Soy intake related to menopausal symptoms, serum lipids, and bone mineral density in postmenopausal Japanese women. Obstet Gynecol 97:109–115
11. Horiuchi T, Onouchi T, Takahashi M, Ito H, Orimo H (2000) Effect of soy protein on bone metabolism in postmenopausal Japanese women. Osteoporos Int 11:721–724
12. US Department of Health, Education and Welfare & Food & Agriculture Organization of the United Nations (1972) Food composition table for use in East Asia
13. Franke AA, Custer LJ, Cerna CM, Narala KK (1994) Quantitation of phytoestrogens in legumes by HPLC. J Agric Food Chem 42:1905–1913
14. Reinli K, Block G (1996) Phytoestrogen content of foods—a compendium of literature values. Nutr Cancer 26:123–148
15. Wang HJ, Murphy PA (1994) Isoflavone content in commercial soybean foods. J Agric Food Chem 42:1666–1673
16. Anderson JJW (1997) Isoflavone concentration in soyfoods. Presented at the American Dietetic Association 80th Annual Meeting and Exhibition, October 27–30, Boston, Massachusetts

17. Recker R, Lappe J, Davies K, Heaney R (2000) Characterization of perimenopausal bone loss: a prospective study. J Bone Miner Res 15:1965–1973
18. Ho SC, Hsu S, Leung PC, Chan C, Swaminathan R (1993) Anthropometric measurements and bone mineral density among young Chinese adult women aged 21–40. Ann Epidemiol 3:256–263
19. Ho SC, Leung PC, Swaminathan R, Chan C, Chan SG, Lindsay R (1994) Determinants of bone mass in Chinese women aged 21–40. II Pattern of dietary calcium intake and association with bone mineral density. Osteoporos Int 4:167–175
20. Ho SC, Eric Wong, Chan SG, Lau J, Chan C, Leung PC (1997) Determinants of peak bone mass in Chinese women aged 21–40 years. III. Physical activity and bone mineral density. J Bone Miner Res 12:1262–1271
21. Ishida H, Uesugi T, Hirai K, Toda T, Nukaya H, Yokotsuka K, Tsugi K (1998) Preventive effects of the plant isoflavones, daidzin and genistin, on bone loss in ovariectomized rats fed a calcium-deficient diet. Biol Pharm Bull 21:62–66
22. Anderson JJ, Ambrose WW, Garner SC (1998) Biophasic effects of genistein on bone tissue in the ovarietomized, lactating rat model. Proc Soc Exp Med 217:345–350
23. Blair H, Jordan SE, Peterson TG, Barnes S (1996) Variable effect of tyrosine kinase inhibitors on avian osteoclastic activity and reduction of bone loss in ovariectomized rats. J Cell Biochem 61:629–637
24. Arjmandi BH, Birnbaum R, Goyal NV, Getlinger MJ, Juma S, Alekel L, Hasler CM, Drum ML, Hollis BW, Kukreja SC (1998) Bone-sparing effect of soy protein in ovarian hormone-deficient rats is related to its isoflavone content. Am J Clin Nutr 68:1364S–1368S
25. Arjmandi BH, Getlinger MJ, Goyal NV, Alekel L, Hasler CM, Juma S, Drum ML, Hollis BW, Kukreja SC (1998) Role of soy protein with normal or reduced isoflavones content in reversing bone loss induced by ovarian hormone deficiency in rats. Am J Clin Nutr 68:1358S–1363S
26. Anderson JJ, Chen X, Boass A, Symons M, Kohlmeier M, Renner JB, Garner SC (2002) Soy isoflavones: no effects on bone mineral content and bone mineral density in healthy, menstruating young adult women after one year. J Am Coll Nutr 21:388–393
27. Morabito N, Crisafulli A, Vergara C, Gaudio A, Lasco A, Frisina N, D'Anna R, Corrado F, Antonia Pizzoleo M, Cincotta M, Altavilla D, Ientile R, Squadrito F (2002) Effects of genistein and hormone-replacement therapy on bone loss in early postmenopausal women: a randomized double-blind placebo-controlled study. J Bone Miner Res 17:1904–1912
28. Anderson JJB, Anthony, Messina M, Garner SC (1999) Effects of phyto-oestrogens on tissues. Nutr Res Rev 12:75–116
29. Fanti P, Monier-Faugere MC, Geng Z, Schmidt J, Morris PE, Cohen D, Malluche HH (1998) The phytoestrogen genistein reduces bone loss in short-term ovariectomized rats. Osteoporos Int 8:274–281
30. Anderson JJB, Miller CP (1998) Lower lifetime estrogen exposure among vegetarians as a possible risk factor for osteoporosis: a hypothesis. Veg Nutr 2:4–12
31. Arjmandi BH, Khalil DA, Smith BJ, Lucas EA, Juma S, Payton ME, Wild RA (2003) Soy protein has a greater effect on bone in postmenopausal women not on hormone replacement therapy, as evidenced by reducing bone resorption and urinary calcium excretion. J Clin Endocrinol Metab 88:1048–1054
32. LandinWihelmsen K, Nilsson A, Bosaeus I, Bengtsson B-A (2003) Growth hormone increases bone mineral content in postmenopausal osteoporosis: a randomized, placebo-contolled trial. J Bone Miner Res 18:393–405
33. Ho SC, Donnan S P B, Sham A (1988) Dietary intake among Chinese elderly in Hong Kong. J Hum Nutr Dietet 1:205–215
34. Ho SC, Woo J, Leung SSF, Sham A, Lam TH, Janus ED (2000) Intake of soy products is associated with better plasma lipid profiles in the Hong Kong Chinese population. J Nutr 130:2590–2593