Calcified Tissue International (2019) 105:573–581
https://doi.org/10.1007/s00223-019-00604-2

ORIGINAL RESEARCH

The Effect of Dried Beancurd on Bone Mineral Density in Postmenopausal Chinese Women: A 2-Year Randomized Controlled Trial

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Received: 21 May 2019 / Accepted: 21 August 2019 / Published online: 5 September 2019

Abstract
Soy foods contain several components such as isoflavones, calcium and protein that potentially modulate bone turnover and increase bone mineral density (BMD) in postmenopausal women. The study is to evaluate the effect of dried beancurd supplementation on skeletal health in postmenopausal Chinese women. Three hundred postmenopausal women aged 50–65 years were assigned into two groups, receiving 100 g dried beancurd or rice cake a day for 2 years. BMD at the lumbar spine and right proximal femur were measured with a dual-energy X-ray absorptiometry. The bone turnover biomarkers of serum alkaline phosphatase (ALP), bone Gla protein (BGP) and urinary N-telopeptide cross-links of collagen normalized for creatinine (NTX/CRT) were also determined. Serum isoflavone concentration was analyzed by high performance liquid chromatography. The 2-year dried beancurd supplementation generated a significant increase in lumbar spine BMD. An obvious decrease was found in urinary NTX/CRT, and a significant increase was detected in serum isoflavone concentration. The dried beancurd supplementation had no effect on changes of right proximal femur BMD and concentrations of serum ALP and BGP. Daily supplementation of dried beancurd could increase BMD of lumbar spine, but does not slow bone loss at right proximal femur in postmenopausal Chinese women.

Keywords Dried beancurd · Postmenopausal women · BMD · Calcium · Isoflavones

Introduction

Osteoporosis is one of the diseases associated with postmenopausal syndrome. The menopause-related estrogen deficiency accelerates the rate of bone reabsorption and decreases the rate of bone formation, causing a rapid loss of bone mass and leading to an increased susceptibility to bone fractures [1]. It is reported that the osteoporosis prevalence is 32.1% for the women over the age of 50 and reaches up to 51.6% over the age of 65 in China [2].

Hormone replacement therapy has been used to prevent and treat osteoporosis in postmenopausal women, which could decrease the risk of osteoporosis by about 50% at the onset of menopause [3]. However, potential risks of adverse effect such as stroke, breast cancer and endometrial cancer outweigh the benefits of hormone replacement therapy [4]. Soybean and soy products are traditional Chinese food, which are the significant source of dietary isoflavones, calcium and protein; the beneficial effects of which on bone mass of ovariectomized rat models have been observed [5]. Soy protein isolate is effective in preventing bone loss and soy isoflavones may stimulate duodenal calcium transport and increase intestinal calcium absorption [6]. The potential use of phytoestrogens and protein from soy foods to prevent the onset of osteoporosis has also been addressed on human subjects [7]. A positive association of protein/isoflavones

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00223-019-00604-2) contains supplementary material, which is available to authorized users.

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intake with hip BMD as well as total body bone mineral content (BMC) of postmenopausal women has been found in Ho et al.'s study [8]. Alekel et al.'s work also led to the conclusion that soy protein isolate containing 80 mg of isoflavones attenuated bone loss in the lumbar region of perimenopausal women [9].

However, some human intervention trials have failed to find beneficial effects of soy isoflavones and/or protein on bone [10, 11]. In a 1-year nutrition intervention study, Kenny et al. did not find the significant beneficial effect of soy protein and soy isoflavones alone and in combination on BMD in postmenopausal women [12]. Most of these studies with controversial results mainly featured isolated soy protein, and purified or concentrated soy isoflavones rather than whole soy foods. The limited data available on whole soy food consumption and bone status in postmenopausal women emanated from epidemiological studies conducted in China [13, 14]. Until now, few intervention researches have investigated the long-term bone-protective effects of whole soy food consumption. Therefore, more rigorous study design is required to establish an irrefutable causal link between traditional soy food and bone health. The present study is to investigate the effect of dried beancurd intake on the bone health of postmenopausal Chinese women, to testify whether the dried beancurd could delay or prevent the occurrence of osteoporosis in Chinese aged women.

Materials and Methods

Participants

We recruited the participants from villages located in north of Shandong Province primarily through local advertisements as well as fliers distributed in senior centers of villages. All eligible participants were recruited between December 2015 and January 2017. Participants were elderly women aged from 50 to 65 years with a body mass index (BMI) 22–28 kg/m^2, a follicle stimulating hormone (FSH) concentration > 40 IU/L and at least 12 months of amenorrhea. Exclusion criteria included abnormal screening mammogram; significant or pathological endometrial hyperplasia; a history of vertebral, hip or wrist fractures, cancer, or active liver, kidney, heart, parathyroid and thyroid diseases. Women were excluded from the study if they were taking hormonal replacement therapy; or phytoestrogen treatment within the previous 3 months, or fluoride, calcitonin, chronic systemic corticosteroid or any other treatment affecting BMD within the previous 6 months. Women were also excluded from the study if they had undergone oophorectomy and/or hysterectomy.

As reported in a study on elderly Chinese women [15], the variation of lumbar BMD of 0.03 g/m^2 is considered to be clinically meaningful. The sample size is calculated to be 112 per group assuming 80% power and a 5% significance level. Considering the study period is as long as 2 years, we anticipated a 25% dropout rate, recruiting no fewer than 150 participants in each group. Therefore, 300 elderly women aged 50–65 years were recruited for the study.

Study Design

All the participants were randomly assigned into two groups by using permuted block randomization method (block size = 4). A statistician who was not involved in the clinical procedures of our study conducted the allocation. The women in the control group were provided with 100 g rice cake a day containing 3.3 g protein, 31 mg calcium and no isoflavones. Those in the dried beancurd group were provided with 100 g dried beancurd a day containing 16.2 g protein, 308 mg calcium and 64.4 mg isoflavones.

All the participants were visited by the field staffs every 2 weeks. The field staffs carefully monitored their compliance with the protocol. To ensure compliance of the participants, a new food supply was distributed and unused study food or empty packages retrieved every 2 weeks to estimate compliance rates. The compliance rate was calculated as the percentage of total taken study food in the total dispensed study food. Participants were requested to suspend taking any nutritional supplements that would interfere with the outcomes.

Dried beancurd and rice cake were purchased from the manufacturer (Xuerun Food Ltd, Anhui, China) and vacuum packed. It could be cooked in many ways such as making salad, being fried with different vegetables or meats. Participants could choose their favorite cooking way depending on their own taste.

BMD Measurements

Lumbar spine (L2–L4) and right proximal femur BMD were measured by a dual-energy X-ray absorptiometry (DXA) at baseline and 6, 12, 18 and 24 months after randomization. The long-term stability of this instrument was determined by daily measurement of a spine phantom. The BMD of each participant was measured by the same certified technician using the same instrument throughout the entire study period. The intra- and inter-assay coefficients of variation were 3.7% and 4.2%. The percentage change of BMD relative to the baseline was determined for each women. The effect of treatment on bone is greater in those with lower bone mass [16], and hence, it is important to consider baseline BMD. Percentage change of BMD was calculated as the difference between BMD at each time point of intervention (6, 12, 18 and 24 months, respectively) and BMD at baseline, divided by BMD at baseline, and multiplied by 100.

Biochemical Marker Analysis

Blood samples were collected between 07:00 and 10:00 am after the participants fasted overnight. Serum alkaline phosphatase (ALP) and bone Gla protein (BGP) were measured by using *p*-nitrophenyl phosphate method and the radioimmunoassay kit (Incstar, Stillwater, MN), respectively. Serum isoflavone concentration was measured by using high performance liquid chromatography (HPLC) as described by Craft [17].

Urine samples were collected in polypropylene containers and kept cold (4 °C) until each participant's sample was processed. Urinary NTX was analyzed by using the Osteomark NTX Direct Response assay (Ostex International, Seattle, WA). The average intra-assay variability of each of these biomarkers was $< 5\%$.

Dietary Assessment

A 24-h recall technique on three consecutive days was employed to assess their food consumption at baseline and post-intervention. The participants were asked to visit the temporary clinics located in their village for dietary assessment. For the participants who missed the interview, the dietitian performed the household survey. Dietary data were collected from participants during a face-to-face interview with a qualified dietitian. During the interviews, each participant was first asked what kinds of foods (including foods in three meals, snacks, beverages and supplements) she had consumed in the previous day, followed by a question on the amount consumed. Serving size and measurement tools were provided as references to estimate the amount of food. Nutrient intake was estimated based on the Chinese Food Component Database [18].

Statistical Analysis

Baseline and clinical characteristics were reported as mean ± SDs for normally distributed data, medians for data not normally distributed and number (percent) for categorical variables. Student's *t* test was performed to determine the difference of normally distributed data. Mann–Whitney U test was used to evaluate the difference of not-normally distributed data and χ^2-square test for categorical variables. For analyses of repeated measures of BMD, a mixed-effects model was employed. It is a generalized form of linear regression analysis that allows for repeated measures on each participant while accounting for the considerable variation across participants in overall average BMD and BMD change over time. The age and body weight were potential confounding variables for BMD [8], which were included as covariates in the analysis. All analyses were done by using SPSS version 18.0 (SPSS Inc, Chicago, IL). A P value of 0.05 or less was considered statistically significant.

Results

Study Subjects

The enrollment flowchart of participants is displayed in Fig. 1. Of the 612 postmenopausal women screened, 300 were randomly assigned to one of two intervention groups. One hundred and seventeen out of 150 participants in the dried beancurd group and 112 out of 150 participants in the control group completed the study. There were a total of 71 participants in two groups discontinuing the study because of withdrew consent, moving or violation of the protocol.

There were 23 participants with co-morbidities including coronary heart disease ($n = 11$), higher pressure ($n = 8$), skin diseases ($n = 3$) and retinopathy ($n = 1$). Ten participants with co-morbidities were in the control group and 13 in the dried beancurd group. Randomization resulted in baseline equivalence for women in two groups. There were no significant differences detected at baseline in age, age at menopause, body mass index (BMI), education and family income. Lumbar spine and right proximal femur BMD showed no significant differences between two groups (Table 1). Compliance with the study protocol was confirmed by left-over counts in that about 90% of the participants in two groups consumed $> 85\%$ of the supplemented food. The average consumption of dried beancurd was 88.79 ± 4.68 g per day.

Dietary nutrient intakes are presented in Table 2. There were no statistical differences between two groups at baseline for any of these nutrients. Due to the study supplements, the isoflavones and calcium levels significantly increased in the dried beancurd group as expected. Their protein intake also exhibited an increase, but the difference was not significant due to the great variance. No changes were found in caloric, carbohydrate and fat intakes in either group through the study.

For the women who received dried beancurd, a significant increase of BMD at lumbar spine was observed during the study (Fig. 2). However, no significant difference in BMD change at right proximal femur was detected between two intervention groups (Fig. 3). As indicated in Table 3, the 2-year supplementation generated a significant increase in serum isoflavone concentration and a decrease in bone resorption indicated by urinary NTX/CRT. However, no significant difference was observed in serum markers of bone formation, i.e., ALP and BGP between two groups.

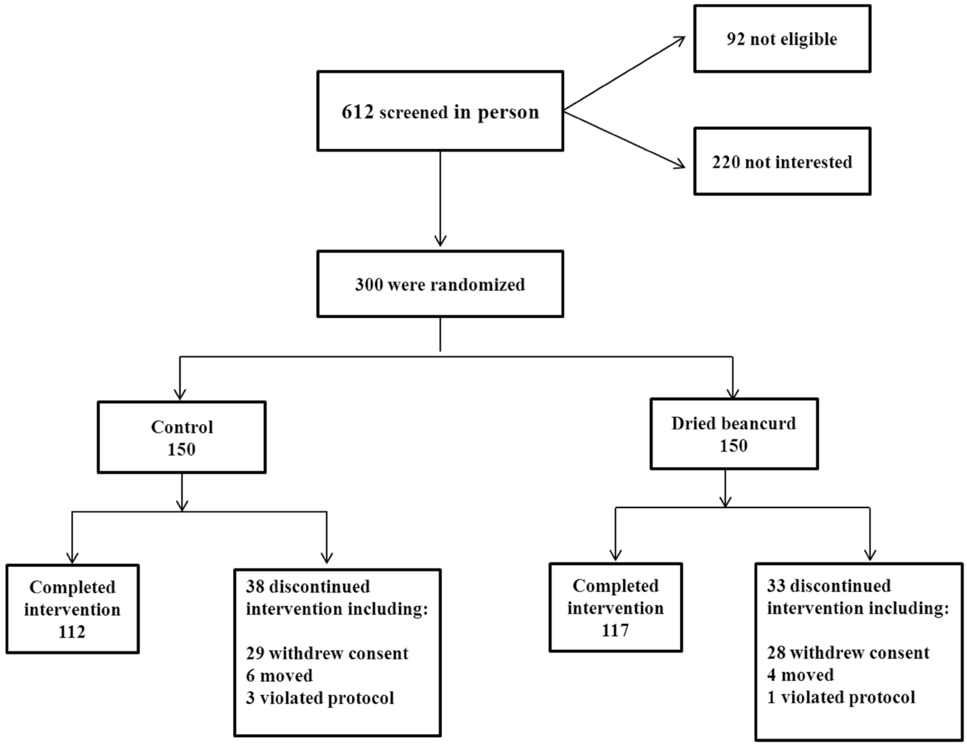

Fig. 1 Flow diagram of the study in each group. At the beginning of the study, there were 150 participants in each group. At the end, 112 participants in the control group and 117 in the dried beancurd group completed the study

Adverse Events

Throughout the intervention, we documented 12 adverse events: seven in the control group and five in the dried beancurd group. The adverse events included new-onset cardiac symptoms ($n=2$), increases in blood pressure ($n=4$), transient ischemic attack ($n=1$), respiratory infections ($n=4$) and head injury ($n=1$) (Online Resource 1). The overall incidence rate of adverse events was not significantly different between two groups.

Discussion

The primary purpose of this intervention study was to elucidate the potential bone benefit of incorporating whole soy food into the diets of postmenopausal women. After 2-year intervention, significant improvement in BMD at lumbar spine and marker of bone resorption was observed in the women consuming dried beancurd. The excellent compliance of participants with the dried beancurd was confirmed by assessing the serum isoflavone concentration at 24 months.

There is tremendous interest in the effect of soy food on BMD or markers of bone turnover in postmenopausal women. Some cross-sectional studies suggested that high consumption of soy products was associated with increased bone mass or decreased bone resorption or fractures [19–23]. Somekawa et al.'s study conducted in 478 postmenopausal Japanese women showed high intake of soy products was related to increased bone mass in postmenopausal women [19]. Significant increases in the lumbar spine and Ward's triangle BMD were also found in Chinese postmenopausal women with habitually high intake of dietary isoflavone [20]. Kritz-Silverstein found that the usual, unsupplemented dietary isoflavone consumption may be protective against bone loss in postmenopausal women through a reduction in bone resorption [21]. However, a few studies reported no significant effects or effects too small to be of clinical relevance [24–28]. A double blind, 15-month study by Gallagher did not show any significant positive effect of soy protein isolate with varying concentrations of isoflavones on BMD in early postmenopausal women [27]. Another double blind, 9-month study showed that soy protein did not favorably affect BMD in postmenopausal women [28]. Throughout these human studies, most of them mainly focused on some component of soy food, soy protein or isoflavones, not the whole soy food. Only some epidemiological studies provided limited data on whole soy food consumption and bone status in postmenopausal women [13, 14]. In a large perspective cohort study of 24,403 menopausal women in Shanghai, the incidence of bone fracture over 4.5 years was found to be inversely related to soy food consumption [13]. Another prospective cohort study of 63,257 Chinese men and women living in Singapore exhibited dietary soy was associated with reduced risk of osteoporotic hip fracture for women but not for men [14]. However, these studies are not intervention trials and cannot establish causality.

Table 1 General characteristics of study participants at baseline

	Control ($n=112$)	Dried beancurd ($n=117$)	P value[a]
Age (years)	59.8 ± 6.4	61.7 ± 5.2	0.21
Height (cm)	155.2 ± 5.9	158.1 ± 6.3	0.56
Weight (kg)			
Baseline	60.8 ± 2.7	61.3 ± 3.9	0.49
Post-intervention	61.3 ± 3.2	62.0 ± 4.3	0.15
BMI (kg/m^2)	25.3 ± 2.8	24.5 ± 3.0	0.85
Age of menarche (years)	13.7 ± 3.2	13.2 ± 2.1	0.73
Age of menopause (years)	48.1 ± 6.1	46.8 ± 5.3	0.65
BMD (g/cm^2)			
Lumbar spine	0.810 ± 0.109	0.805 ± 0.098	0.37
Right proximal femur	0.672 ± 0.081	0.667 ± 0.092	0.18
Family type			
Farmer	73 (65.2%)	86 (73.5%)	0.23
Worker	3 (2.7%)	4 (3.4%)	0.72
Teacher	2 (1.8%)	2 (1.7%)	0.17
Housewife	34 (30.4%)	25 (21.4%)	0.80
Education			
Illiterate	69 (61.6%)	71 (60.7%)	0.11
Primary school	40 (35.7%)	41 (35.0%)	0.69
Middle school	2 (1.8%)	5 (4.3%)	0.42
High school or higher	1 (0.9%)	0 (0)	0.31
Family income per month			
< 1000 Yuan	18 (16.1%)	17 (14.5%)	0.12
1000–2000 Yuan	32 (28.6%)	36 (30.8%)	0.53
2000–3000 Yuan	57 (50.9%)	61 (52.1%)	0.26
> 3000 Yuan	5 (4.5%)	3 (2.6%)	0.49

Values are *n* (%) unless otherwise indicated

Mean ± SD (all such values)

BMI body mass index, *BMD* bone mineral density

[a] *P* value indicates difference between the dried beancurd and control groups

Soy, as part of regular diet in China, is not only a rich dietary source of isoflavones, but also calcium and protein. Tofu is one kind of soy products. During its processing, $CaSO_4$ is added into the soymilk as the coagulant, giving rise to the calcium content to 157 mg/100 g and increasing its bioavailability [29]. In our study, dried beancurd provided to the participants was a reprocessed food from tofu with less water and higher calcium, isoflavones and protein. The usual diet of the study participants was mainly based on plant foods; their average dietary calcium content was as low as 361.3 mg, only 36.1% of recommended nutrient intake (RNI) [30] with poor bioavailability, which may deteriorate the bone status of menopausal women. Furthermore, there were only 11 women among 300 participants who took calcium supplements with amount of 200–400 mg per day. The percentage of women with calcium supplements was as low as 3.7%, much lower than Wang et al.'s study [31]. Since our study was conducted in rural areas, the lack of nutrition knowledge and poor economic condition may contribute to the lower percentage. Seven out of 11 women suspended the supplements in the study, three in the control group and four in the dried beancurd group. The other four participants were excluded from the study because of violation of the protocol. On the average, 88.79 g of dried beancurd was consumed by the participants in the study, which provided additional 273.5 mg calcium and increased their calcium intake to 621.4 mg, which may contribute to its benefit of bone-protective effect as Messina suggested in his study [32]. The average daily intake of isoflavones in our study was 57.2 mg, lower than that in other studies reported a beneficial effect. However, a study among Chinese women conducted by Ho et al. [8] showed that habitual soy isoflavone intake of 40 mg had a positive effect on the spine BMD for Chinese postmenopausal women over 3 years. And Lydeking-Olsen et al.'s finding also suggested that soy food with isoflavones in the 50–90 mg range could reduce the bone loss of spine by providing an endocrine effect [33]. In addition to calcium and isoflavones, 14.4 g protein was also

Table 2 Daily total energy and macronutrient intakes at baseline and post-intervention in postmenopausal women of two groups

	Control ($n=112$)		Dried beancurd ($n=117$)		P^a	P^b	P^c
	Mean	Median	Mean	Median			
Energy (kcal)							
Baseline	1601.8	1386.3 (1197.8, 1932.2)	1512.5	1277.2 (1089.6, 1863.1)	0.43		
Post-intervention	1620.5	1505.7 (1257.4, 1967.7)	1717.3	1523.5 (1290.9, 2120.3)	0.32	0.59	0.31
Protein (g)							
Baseline	43.8	39.5 (30.6, 53.7)	43.1	38.7 (25.2, 51.5)	0.56		
Post-intervention	45.2	42.7(32.0, 59.6)	54.9	50.6(39.2, 71.9)	0.10	0.18	0.13
Carbohydrate (g)							
Baseline	284.0	221.6 (163.2, 382.1)	263.6	197.6 (123.8, 366.9)	0.62		
Post-intervention	276.9	208.5 (136.2, 379.8)	298.6	230.6 (183.1, 407.2)	0.28	0.38	0.27
Fat (g)							
Baseline	32.4	28.7 (22.3, 32.5)	31.9	27.3 (19.5, 30.4)	0.78		
Post-intervention	36.9	31.8 (25.6, 37.2)	33.7	29.6 (21.0, 33.9)	0.37	0.56	0.67
Calcium (mg)							
Dietary calcium							
Baseline	389.6	309.7 (172.3, 493.5)	361.3	287.9 (159.8, 456.2)	0.52		
Post-intervention	426.4	342.1 (236.5, 517.5)	621.4	539.2 (413.9, 792.6)	0.02	0.68	<0.01
Supplemental calcium							
Baseline[d]	266.7 (3)	200–400	287.5 (4)	200–400			
Post-intervention	0		0				
Isoflavones (mg)							
Baseline	31.3	25.1 (12.3, 40.6)	29.8	23.6 (11.7, 34.8)	0.73		
Post-intervention	28.7	22.9 (10.1. 39.2)	76.5	58.9 (48.6, 76.2)	<0.01	0.53	<0.01

Median; upper, lower quartile values in parentheses (all such values)

[a]*P* value indicates the difference between two groups at baseline and post-intervention

[b]*P* value indicates the difference from baseline to post-intervention of the control group

[c]*P* value indicates the difference from baseline to post-intervention of the dried beancurd group

[d]The baseline supplemental calcium intake was expressed as mean (*n*) and range

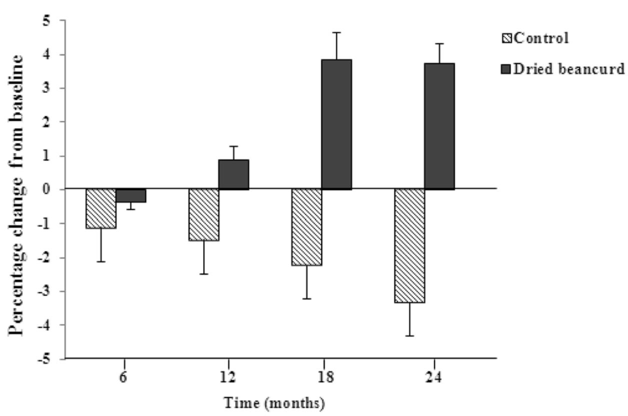

Fig. 2 Mean percentage change in BMD of lumbar spine at each time point (6, 12, 18 and 24 months) for two intervention groups: control, $n=112$; dried beancurd, $n=117$. Significant differences were detected both between treatment ($P<0.001$) and time ($P=0.027$) by the test using a mix-effects model with age and body weight as covariates

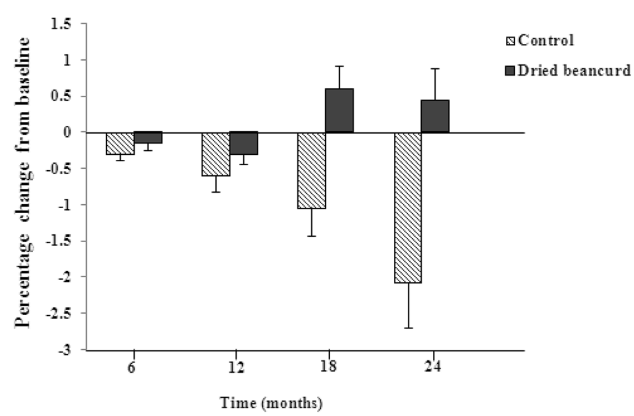

Fig. 3 Mean percentage change in BMD of right proximal femur at each time point (6, 12, 18 and 24 months) for two intervention groups: control, $n=112$; dried beancurd, $n=117$. No significant differences were detected between treatment ($P=0.82$) and time ($P=0.63$) by the test using a mix-effects model with age and body weight as covariates

Table 3 Mean serum and urinary marker values at baseline and post-intervention in postmenopausal women of two groups

	Control ($n = 112$)	Dried beancurd ($n = 117$)	P value
Serum ALP (mmol/L)			
Baseline	71.19 ± 13.51	71.93 ± 15.20	0.67[a]
Post-intervention	75.23 ± 11.34	70.61 ± 12.53	0.32[b]
Serum BGP(μg/L)			
Baseline	7.62 ± 1.31	7.78 ± 1.43	0.56[a]
Post-intervention	7.89 ± 1.37	7.15 ± 1.27	0.46[b]
Urinary NTX/CRT (nmoL/mmoL)			
Baseline	62.31 ± 5.73	64.65 ± 9.85	0.29[a]
Post-intervention	65.89 ± 7.52	58.12 ± 8.37	0.02[b]
Serum isoflavones (nmol/L)			
Baseline	102.70 (81.56, 124.51)	89.28 (68.73, 118.69)	0.22[a]
Post-intervention	95.59 (75.38, 121.52)	576.92 (432.76, 768.27)	< 0.01[b]

Means ± SD (all such values)

[a]Groupwise comparison at baseline

[b]Groupwise comparison at post-intervention

ALP alkaline phosphatase, *BGP* osteocalcin/bone Gla protein, *NTX/CRT* N-telopeptide cross-links of collagen normalized for creatinine

provided to the participants. In their usual diet, the protein intake was only 43 g/day, 60.56% of RNI. Lower dietary protein intake possibly influenced bone metabolism and was significantly related to greater BMD loss of elderly people [34–36]. Some studies have claimed the need for the presence of soy protein for isoflavones to exert their protective effect on BMD [37, 38], which suggested that soy foods providing 15 g protein/day could have a general sparing effect of postmenopausal calcium loss [33]. Taken together, the calcium, isoflavones and protein provided with dried beancurd may play a combined and synergetic effect to reduce the boss loss of postmenopausal women in this study, which may contribute to the increase of BMD at lumbar spine.

There were some strengths of our study. The randomized controlled trial was as long as 2 years, and sample size was 229 postmenopausal women, which had sufficient power to detect a measurable response to intervention. Whole soy food was employed instead of soy isoflavones or protein isolate, to evaluate its effect on bone health of postmenopausal women. In addition to BMD, biomarkers of bone turnovers were also used to investigate the efficacy of soy food on skeletal health.

The limitations of this study included that we experienced a 23.7% dropout rate, similar to other long-term trials [12]. Because of restriction of funding, the BMD at femoral neck was not investigated. The femoral neck typically shows less variance and is easier to detect change over time due to the lesser amounts of arthritis. The lack of data on femoral neck reduced the power to estimate the effect of dried beancurd. More cautious monitoring in this regard is warranted in future studies. And we could not evaluate the potential adverse effects of soy-derived isoflavones on breast tissue density, endometrial thickness and thyroid function. The dried beancurd as a natural food should be safe and benign at the amount we provided [39].

Conclusion

In conclusion, a significant effect of dried beancurd supplementation was observed in increase of lumbar spine BMD of postmenopausal Chinese women. Our study suggested that daily consumption of dried beancurd could prevent bone loss and improve bone health of the lumbar spine, which may offer a natural and economical intervention for prevention of postmenopausal osteoporosis.

Acknowledgements We appreciate the support of all the participants involved in the study. We are grateful to the Nestlé Foundation for the funding that made the entire work possible.

Author Contributions Author LL designed the study and prepared the first draft of the paper. She is guarantor. Authors MS, JS, HK and WZ contributed to the human study and experimental work. Author HW was responsible for statistical analysis of the data. All authors revised the paper critically for intellectual content and approved the final version. All authors agreed to be accountable for the work and to ensure that any questions relating to the accuracy and integrity of the paper were investigated and properly resolved.

Funding This study was funded by Nestlé Foundation.

Compliance with Ethical Standards

Conflict of interest The authors declare no conflict of interest.

Human and Animal Rights and Informed Consent The study was conducted in accordance with the ethical standards of Institutional Review Committee of Public Health School of Xiamen University and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all participants included in the study.

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