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RESEARCH ARTICLE

Effect of soy isoflavones on bone health among female university students: A pilot study

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ABSTRACT

Low bone mineral density (BMD) has become more common in young women. In postmenopausal women, soy isoflavones have been demonstrated to have a powerful influence on bone health when taken as a food supplement. It is unclear how soy isoflavones affect premenopausal women, though. This study looked at how soy isoflavones affected female university students' BMD. **T**hirty female students, aged 18 to 25, were introduced to a Quasi-experimental design, where only 28 could complete the study. Participants received soy isoflavone at 60 mg/day for 28 days. BMD was assessed using dual-energy x-ray absorptiometry (DEXA) before and after supplementation. In addition, twenty-four hours food recall and food frequency questioners (FFQ) were used to evaluate the dietary intake of participants. The prevalence of osteoporosis among participants was 18% osteoporosis, 61% osteopenia and 21% normal. After 4 weeks of intervention, a slight improvement in the BMD was recorded. This improvement was noticed in 33, 59, and 40% of normal, osteopenia, and osteoporosis participants, respectively. Soy isoflavone supplementation can be useful in decreasing the risk of osteopenia among premenopausal women. These promising results ensure the viability of conducting full-scale clinical trials to thoroughly look at the relationship between soy isoflavones and bone health among pre-menopausal women.

Keywords: Osteoporosis; Bone mineral density; Soy isoflavone; Saudi Arabia

INTRODUCTION

Osteoporosis causes bones to become weak and brittle, which results in back pain, height loss of height, and upper back curvature. The loss of bone mineral density (BMD) may also cause broken bones in the spine, hips, ribs, and wrists. Over the age of 35, one in three women and one in four males may have weakness in their bones. Another 30% of people struggle with osteopenia, a disorder marked by low BMD that increases the risk of osteoporosis. One in three women and 20% of men over the age of fifty may experience an osteoporosis-related fracture during their lifetime (IOF, 2022). Osteoporosis and the fragility fractures it causes are a serious global public health issue. Dietary habits and physiological factors that affect bone health and density, such as estrogen levels, are risk factors for osteoporosis (Khalid and Krum, 2016). In order to maintain the BMD, estrogen interacts with alpha and beta estrogen receptors (ERα and ERβ) and activates various mechanisms in osteoblasts, osteocytes, osteoclasts, immune cells, and other cells (Candelaria *et al.*, 2013). Studies showed a positive relationship between the level of estrogen and the BMD. Although estrogen deficiency primarily affects postmenopausal and older women, younger ones are at an increasing risk according to several studies which stated that the prevalence of low BMD has recently increased among young women (Alassaf *et al.*, 2016). Postmenopausal women are more susceptible to spine, hip, and wrist fractures due to the rapid bone loss that occurs following menopause (Khosla, 2010; Qureshi, 2011). The results of randomized controlled trials revealed that taking an estrogen and progestin combination for longer than five years may increase the risk of insidious breast cancer and atherosclerotic diseases, despite the benefits of hormone therapy in maintaining bone health and preventing bone fractures (Rossouw *et al.*, 2002).

The best way to determine BMD and the risk of fracture is the Dual X-ray Absorptiometry (DEXA) scan or bone densitometry test (Kanis *et al.*, 2019). The test can detect

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osteoporosis, estimate the likelihood of fractures, and gauge how well treatment is working. It assesses BMD in the wrist, hip, and/or spine, which are the most often affected areas by osteoporosis-related fractures (Venjakob *et al.,* 2021). The results of the BMD test are compared with that of an established norm of a healthy young adult and give a T-score. A score of 0 signifies that the BMD is equal to the norm, and variations between obtained BMD and that of the norm are quantified as standard deviations (SDs). More standard deviations below 0 means lower BMD and greater fracture risk. Based on DEXA scan results, an individual may be classified as normal (T-score of –1 to +1), low bone density/osteopenia (T-score of –1 to –2.5), or osteoporosis (T-score of <–2.5). The test further gives BMD value expressed in g/cm^2 of bone surface (IAEA, 2010; Alawi *et al*., 2021).

Recent research is trying to find alternative natural sources that have similar estrogen properties without deleterious complications (Zhang *et al*., 2008). Soybean is a rich source of phytoestrogens especially isoflavones. Genistein, daidzein, and glycitein are the main isoflavones in aglycone form (Brandi, 1997). When the level of endogenous estrogen is low, isoflavones work by binding to the ERβ in the brain, arteries, and bones (Zhang *et al*., 2008; Taylor *et al*., 2009). Soy intake showed potential beneficial effects on BMD and the threat of fractures (Ho *et al*., 2003; Huang *et al*., 2006). Isoflavones have been exceedingly used in over-the-counter dietary supplements indicated for BMD improvement and body fat regulation. They were further shown to decrease LDL cholesterol levels and inhibit cancer development and progression (Xiao, 2008). In a meta-analysis by Liu *et al*. (2009**)**, results from 10 nutrition trials illustrated slight enhancement at the spine in the participants who received elevated doses of isoflavones. Soy isoflavones' capacity to slow bone turnover by preventing bone resorption and promoting bone formation has been verified (Zheng *et al*., 2016; Barańska *et al*., 2022). According to Lauderdale *et al*. (1997), Asian women consume more soy and soy-based products than Caucasian women do, and as a result, had a decreased incidence of hip fractures in the elderly. In addition to promoting calcium absorption, soy isoflavones may potentially have uterotrophic effects. In contrast to estrogen, which inhibits osteoclasts from releasing calcium from bone, Lien *et al*. (2006) found that rats treated with soy isoflavones had increased bone ash and calcium levels. In ovariectomized rats, Kim and Lee (2005) discovered that soy isoflavone supplementation (80 μg/g) prevented osteoporotic bone loss as well as estrogen therapy. According to clinical studies, soy isoflavone dosages that were beneficial varied from 40 to 110 mg per day (Zheng *et al*., 2016). At dosages ranging from 84 to 126 mg/day, Ye *et al*. (2006) found a linear dose-dependent beneficial effect on bone loss. Japanese women receiving 61.8 mg/day of soy isoflavones for 4 weeks showed significant changes in bone metabolism by reducing bone resorption (Uesugi *et al*., 2002).

Therefore, the purpose of this study was to ascertain how soy isoflavone supplementation (60 mg/day) affected BMD levels in female university students.

METHODS AND PARTICIPANTS

Study design and subjects

Quasi experimental study design was used to conduct an intervention study on 30 female university students for 28 days. Participants were recruited from Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia. Participants who were using medications or suffering from diseases that may interfere with isoflavones or affect BMD were excluded from participation in the study. Celecoxib, theophylline, paclitaxel, midazolam, imatinib, carbamazepine, valproic acid, repaglinide, omeprazole, danofloxacin, tamoxifen, and raloxifene are some of the drugs in this list. Additionally excluded were those who used calcium and vitamin D supplements and had glucose-6-phosphate deficiency (G6PD). To participate in the study, participants had to sign a written informed consent. Two participants dropped out because they did not assess the BMD after intervention, and 28 students completed the study.

Biographical information and dietary intake

A complete and valid questionnaire, and food frequency questionnaire (FFQ) were used to gather the required data including health history, socioeconomic status, lifestyle, and dietary intake. Additionally, 24-hour food recall was utilized to evaluate the intake of calories, fat, protein, vitamin D, vitamin C, calcium, magnesium, phosphorus, potassium, and iron. Dietary intake was analyzed using *ESHA* Food Processor Nutrition Analysis Software Version 11.5 (ESHA Research, OR, USA).

Body mass index (BMI)

The participants' weight and height were recorded using an ADE M318800 mechanical scale (Hamburg, Germany) and an Aurora Blue TANITA inner scan 50 (BC-310-BL, Tanita Corporation of America Inc, IL), respectively, to determine their BMI in kg/m^2.

Dual x-ray absorptiometry (DEXA) scanning

Lean body mass, fat mass, and BMD were measured by DEXA scan (Discovery QDR Series, Hologic Inc., MA, USA) with Apex Software, Version 3.2, before and after intervention. The assay was done using the whole-body scan with a precision of 0.3% at a radiation dose of 5 micro-Sievert, and the BMD values were stated in g/cm^2 of bone surface examined.

Intervention

In 28 days of intervention, the participants were given 60 mg of soy isoflavone supplement (Puritans Pride, NBTY Inc., NY, USA) per day. The BMD was then measured using DEXA scan to determine the supplement's effects.

Ethics approval

The study protocol has been approved by the Institutional Review Board of Imam Abdulrahman Bin Faisal University in Dammam, Saudi Arabia (IRB –UGS-2018-03-273). The study was further registered on the ClinicalTrials.gov (Identifier: NCT04547010). All participants gave their informed consent.

Data analysis

The collected data were analyzed by IBM SPSS Statistics 25. Baseline characteristics (age, weight, height, fat mass, lean mass and physical activity) were presented as frequency tables and compared among the three BMD levels using Chi square test. Intake of nutrients (calcium, phosphorus, sodium, magnesium, iron, vitamin C and vitamin D) among the three groups (normal, osteopenia and osteoporosis) was expressed as mean ± standard deviation (SD). The significance was measured by Kruskal Wallis H test, where p value of <0.05 was considered as statistically significant. Graphical representation of results was reported as bar plot and histogram plot.

RESULTS AND DISCUSSION

Osteoporosis has recently become a widespread condition, and its prevalence is predicted to rise in the coming decades. The WHO classified osteoporosis as a T-score that is 25% lower than the typical 30-year-old, 2.5 standard deviations below the mean, or a T-score lower than -2.5 based on BMD evaluation. Osteopenia, however, is defined as a T-score between -1.0 and -2.5 standard deviations below normal, or 10-25% below an average healthy 30-year-old adult (Kanis, 1994). In the current study, DEXA has been used to measure BMD in the participants using spectral imaging. DEXA scan is the most widely used bone density measurement technology to diagnose and follow osteoporosis.

Table 1 lists the study participants' initial characteristics. The mean of BMD pre-supplementation was 1.09, 0.95 and 0.86 g/cm^2 for normal, osteopenia and osteoporosis participants, respectively. The mean bone mineral content (BMC) was 1.23±0.24 kg in osteoporosis group as compared to 1.82±0.15 kg in the normal group. The research revealed that overall prevalence of low BMD was 79% with 61% osteopenia and 18% osteoporosis. Other studies reported corresponding values of 52 and 4%, respectively, where 44% of the study group showed normal BMD (Alassaf *et al*., 2016). The calculated BMD values are consistent with those found by Manisali *et al*. (2003), who discovered that the BMD of the average reference group of females (20-39 years old) was 0.963±0.121 g/cm^2 in the lumbar spine and 0.891±0.119 g/cm^2 in the whole femoral area. They are further consistent with the findings of Jáuregui *et al*. (2021), who stated that for women between the ages of 20 and 29 and 30 and 39, respectively, the BMD values obtained in the lumbar spine were 1.150±0.11 and 1.169±0.10 g/cm^2. In the femoral neck, the equivalent values were 0.991±0.10 and 0.970±0.11 g/cm^2.

Several vitamins and minerals affect bone health including vitamin D, calcium, magnesium, phosphorus and potassium. Table 2 and Fig. 1 illustrate the dietary intake of study subjects, including the consumption of vitamin C, vitamin D, calcium, magnesium, iron, phosphorus, and potassium among different BMD groups. Intake of vitamin D is below the suggested dietary allowance (RDA) in all subjects, while only 17% of subjects met the RDA in calcium intake. Data also showed that only 13% and 5% of subjects met the RDA of vitamin C and phosphorus, respectively. None of subjects met RDA of both magnesium and potassium. All participants consumed lower vitamin D (0.11, 0.20 and 0.75 mcg/day in normal, osteopenia and osteoporosis subjects, respectively) than the RDA of 15 mcg/day (Fig. 1). Calcium intake was 476.71, 659.26 and 460.57 mg/day in normal, osteopenia and osteoporosis subjects, respectively. The corresponding values were 908.70, 756.14 and 1172.12 for potassium intake. Tayel *et al*. (2013) assessed the effect of nutrient intake on bone health among 300 female university students

Table 1: Baseline characteristics of study participants

	Normal (n=6)	Osteopenia (n=17)	Osteoporosis (n=5)	p-value
Age (yr)	21.67±1.97	20.40±1.18	19.71±1.50	0.11
Weight (kg)	67.37±13.25	61.81±14.48	48.64±6.94	0.03
Height (cm)	148.18±62.62	155±0.07	152±0.03	0.01
Fat mass (kg)	27.83±9.19	21.89±8.73	19.17±8.69	0.14
Lean mass (kg)	39.21±6.05	35.17±6.11	33.75±6.38	0.15
BMD-pre (g/cm^2)	1.09±0.05	0.95±0.03	0.86±0.02	<0.01
BMC-pre (kg)	1.82±0.15	1.40±0.19	1.23±0.24	<0.01
BMI (kg/m^2)	26.43±5.51	24.33±5.38	21.09±2.95	0.17

Yr: year; kg: kilogram; g: gram; cm: centimeter; m: meter. Values reported as Mean±SD (standard deviation).

Table 2: Dietary intake of participants

	Normal	Osteopenia	Osteoporosis	P-value
Protein (g)	61.32±27.86	55.00±22.56	48.21±25.67	0.66
Fat (g)	67.13±35.44	58.21±25.25	60.44±44.00	0.84
Calorie (Kcal)	1397.61±593.90	1736.72±659.03	1518.56±989.45	0.30
Na (mg)	1868.57±844.30	2537.86±2236.28	1865.30±1439.95	0.53
Ca (mg)	476.71±326.03	659.26±430.26	460.57±247.88	0.72
Mg (mg)	68.23±74.41	66.39±71.45	71.90±52.89	0.87
P (mg)	199.47±120.56	271.01±251.19	353.73±221.09	0.67
K (mg)	908.70±1005.05	756.14±709.27	1172.12±831.86	0.63
Iron (mg)	5.14±2.35	10.59±15.12	6.34±3.61	0.93
V-D (mcg)	0.75±0.90	0.20±0.57	0.11±0.22	0.21
V-C (mg)	30.38±26.06	38.78±32.00	19.12±18.47	0.46

Na: sodium; Ca: calcium; Mg: magnesium; P: phosphorus; K: potassium; V-D: vitamin D; V-C: vitamin C. Values reported as Mean±SD (standard deviation).

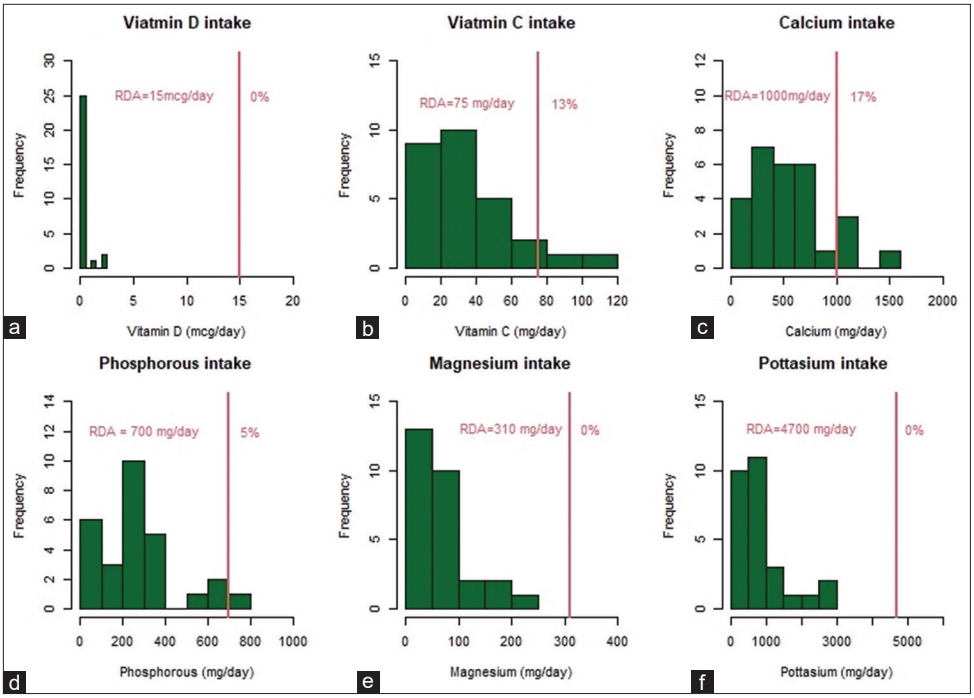

Fig 1. (a-f) Nutrient intake of vitamins and minerals

in Egypt. The study found that students' intakes of calcium, magnesium, potassium, and vitamin D were insufficient (8.49±4.51 mcg, 230.07±81.28 mg, 3.49±1.44 mg and 685.67±224.51 mg, respectively). Generally, calcium, vitamin D, and vitamin K influence bone metabolism and provide potential protection against osteoporosis. Therefore, insufficient consumption of these nutrients may lead to weak bones, especially for later life stages (Tayel *et al*., 2013). Another study was conducted in Saudi Arabia to evaluate the relationship between calcium and vitamin D intake and BMD among 257 premenopausal women (20 to 50 years old) divided into two groups: insufficient vitamin D intake (<400 IU/day) and sufficient vitamin D intake (>400 IU/day) (Zareef *et al*., 2018**)**. Sixty-five percent of women had vitamin D intake below estimated average requirements (EARs), while 61% had calcium intake below EAR. After adjusting for body mass index and energy intake, the authors additionally revealed a substantial favorable correlation between calcium intake and BMD. However, there were no appreciable differences in vitamin D intake between women with low and normal bone mass. The function of vitamin D is to support the mineralization of the skeleton and maintain adequate levels of calcium and phosphorus.

Bioactive vitamin D is created mostly by the effect of magnesium, and this process can help the body absorb calcium (Uwitonze and Razzaque, 2018). Our results showed that all the participants consume less than the recommended amount of magnesium according to RDA (NIH, 2022). The mean daily intake of magnesium was 68.23 mg in the normal group, 66.39 mg in osteopenia group, and 71.90 mg in osteoporosis group. These results agree with Kim *et al*. (2011) who evaluated the magnesium

intake and its relationship with bone quality among 19-25 years old women. The authors found that magnesium intake was unsatisfactory and below the recommended dietary intake according to RDA. According to an observational research of more than 73,000 women, those who ingested 400 mg of magnesium daily tended to have BMDs that were 2-3% greater than those who consumed only half of this amount (Orchard *et al*., 2014).

We found that the mean daily intake of potassium was 908.70, 756.14 and 1172.12 mg in the normal, osteopenia and osteoporosis groups, respectively. Like the intake of magnesium, all participants consume less than the recommended RDA of potassium (NIH, 2022). Epidemiologic studies indicate that the lower intake of dietary potassium leads to decreased BMD (Farrell *et al*., 2009), which confirm the findings of the current study. Magnesium and potassium affect calcium homeostasis directly and indirectly through several mechanisms (Weaver, 2013; Wu *et al*., 2014). Hayhoe *et al*. (2015) investigated the influence of magnesium and potassium intakes on BMD and fracture risk in adults of both genders. The authors reported a significant increase in BMD among individuals who consume both magnesium and potassium in adequate amounts. They had lower risk of hip fracture compared to those who consume lower amount of magnesium and potassium.

Overall, in this study, phosphorus intake did not meet reference daily intake (RDI). The mean intake was 199.47 mg in normal participants, followed by 271.01 mg in osteopenia and 353.73 mg in osteoporosis group. This finding contrasts with other those of Calvo & Uribarri (2013) who discovered that, with the exception of growing teenagers, all age and gender groups' average phosphorus intakes surpassed the EAR.

According to human research, the calcium-to-phosphorus ratio is crucial for healthy bones (Brot *et al*., 1999). The short-term effects of four dosages of phosphorus (0, 250, 750, or 1500 mg) on calcium and bone metabolism were examined in fourteen healthy women aged 20 to 28 years in a controlled dose-response research by Kemi *et al*. (2006). It was found serum ionized Ca concentration decrease due to phosphorus intake and the significant decline was only in response to the highest dose of phosphorus (1500 mg). The study also demonstrated that P affects parathyroid hormone (PTH), whose secretion dramatically rises in response to reduced Ca intake. Severely high phosphorus intake has a deleterious effect on bone metabolism by decreasing bone formation and increasing bone resorption.

The lifestyle characteristic of the three groups of BMD subjects, including the physical activity and the consumption of fast foods and beverages are shown in Table 3. The highest rate of physical activity was recorded in the normal group (50%), followed by that of osteopenia (26.7%), and osteoporosis subject (14.3%). The consumption of fast food was higher in osteopenia group (80%), compared to 66.7% among the normal group. Among osteoporosis group, this rate was 100%, however. Furthermore, the consumption of soft drinks was the highest within the osteoporosis group, whereas the normal group had the highest consumption of coffee and tea (66 and 33%, respectively).

In the present study, 50% of normal BMD subjects reported that they practice physical activity, compared to 27% and 14% among osteopenia and osteoporosis groups, respectively. This indicates that the practice of physical activity is drastically reduced by the decreased BMD. The same finding was obtained by Suominen (1993), who found a connection between BMC and BMD and long-term, habitual activity. Strength and power-trained athletes have been found to have the greatest BMD values, whereas endurance sports like long-distance running and swimming had less impact on peak bone density. According to our findings, there was no discernible link between physical activity and BMC or BMD ($p=0.52$).

In a cross-sectional study, BMD and BMC were compared at three level of physical activity in premenopausal women; classified in three categories (sedentary, maintenance and sport) each contains 16 participants (Salamat *et al*., 2013). The BMC was 2295±260 g, 2431±321 g and 2730±375 g in the three groups, respectively. There was a significant positive relation between BMC and physical activity in sedentary and maintenance groups ($p<0.01$ and $p<0.05$, respectively).

Several studies suggested that the consumption of soft drinks affects bone health. Tucker *et al*. (2006) found a connection between cola consumption and low BMD in 1413 women and 1125 men. Their findings also indicated a link between weight and the chance of having low BMD. These results are consistent with those of a different

Table 3: Lifestyle characteristic of the study participants

	Normal	Osteopenia	Osteoporosis	P-value
Physical activity practices				
Yes	3 (50%)	4 (26.7%)	1 (14.3%)	0.52
Sometimes	3 (50%)	7 (46.7%)	4 (57.1%)	
No	0 (0%)	4 (26.7%)	2 (28.6%)	
Fast food consumption				
Yes	4 (66.7%)	12 (80.0%)	7 (100%)	0.28
No	2 (33.3%)	3 (20%)	0 (0%)	
Beverage's consumption				
Soft drink	0 (0%)	2 (13%)	1 (14.3%)	0.05
Coffee	4 (66%)	5 (33%)	3 (42.9%)	
Tea	2 (33%)	0 (0%)	0 (0%)	
Other	0 (0%)	8 (53%)	3 (42.9%)	

investigation of the relationship between weight, BMI, and BMD (Saraví and Sayegh, 2013), where both body weight and BMI were positively related with BMC and BMD.

The influence of soy isoflavone on BMD is illustrated in Table 4. Following the WHO definition of osteopenia and osteoporosis according to the BMD assessment, participants were classified into three groups; normal (T-score: –1.0 to +1.0), osteopenia (T-score: –1.0 to –2.5), and osteoporosis (T-score: <–2.5). The number of participants was 6 (21 %), 17 (61 %) and 5 (18 %) in the three groups, respectively. Fig. 2 shows sample DEXA results of three participants each from each group (normal, osteopenia and osteoporosis). Soy isoflavone supplementation caused a slight decrease in the T-scores and BMD values (g/cm^2) in all participants. The rate of improvement in BMD upon soy isoflavone supplementation varied significantly among the three groups. It was 33%, 59%, and 40% among normal, oseopenia, and osteoporosis groups, respectively.

Table 4: Effect of soy isoflavone supplementation on BMD

	Pre-Supplement		Post-Supplement		Difference in BMD
	T-Score	BMD (g/cm^2)	T-Score	BMD (g/cm^2)	
			Normal*		
1	– 0.20	1.09	– 0.20	1.09	0.00
2	– 0.60	1.05	– 0.55	1.05	0.00
3	– 0.40	1.17	– 0.40	1.17	0.00
4	– 0.80	1.03	– 0.80	1.03	0.00
5	– 0.20	1.08	– 0.20	1.12	0.04
6	– 0.10	1.10	– 0.00	1.12	0.02
			Osteopenia*		
1	– 2.50	0.89	– 2.20	0.91	0.02
2	– 2.00	0.92	– 1.50	0.97	0.05
3	– 1.80	0.95	– 1.60	0.97	0.02
4	– 2.40	0.89	– 2.40	0.89	0.00
5	– 1.50	0.97	– 1.50	0.97	0.00
6	– 1.40	0.99	– 1.30	0.99	0.00
7	– 1.70	0.95	-0.80	1.03	0.08
8	– 2.50	0.88	– 1.90	0.94	0.06
9	– 2.20	0.91	– 1.90	0.94	0.03
10	– 1.40	0.98	– 1.40	0.98	0.00
11	– 1.50	0.97	– 1.00	1.00	0.03
12	– 2.00	0.93	– 2.00	0.93	0.00
13	– 2.10	0.92	– 2.00	0.92	0.00
14	– 1.70	0.96	– 1.65	0.96	0.00
15	– 1.10	1.00	– 1.10	1.04	0.04
16	– 1.50	0.98	– 1.00	1.00	0.02
17	– 2.00	0.93	– 1.80	0.95	0.02
			Osteoporosis*		
1	– 2.60	0.88	– 2.60	0.88	0.00
2	– 2.60	0.87	– 2.55	0.89	0.02
3	– 3.10	0.83	– 3.00	0.83	0.00
4	– 3.20	0.83	– 3.20	0.83	0.00
5	– 2.80	0.86	– 2.60	0.87	0.01

* Normal (T-score: –1.0 to +1.0); Osteopenia (T-score: –1.0 to –2.5); Osteoporosis (T-score: <–2.5).

The results showed that daily consumption of 60 mg soy isoflavone as supplement slightly increased BMD in 55% of the participants (14 out of 28). The highest number of participants showing BMD improvement was recorded in the osteopenia group, where 10 out of 17 participants (59%) had better values after the intervention. The corresponding numbers were 2 out of 6 (33%), and 2 out of 5 (40%) in the normal and osteoporosis groups, respectively. The rate of improvement in BMD was 1.82–3.70%, 2.05–8.42% and 1.16–2.30% in normal, osteopenia and osteoporosis groups, respectively. These results indicate the the positive effect of isoflavones supplements. Weaver *et al.* (2005) reported that due to their estrogenic effect, isoflavones might have an effective role in the situations where estrogen is deficient or inadequate. Another study conducted on young female university students who had decreased bone mass (mean BMI of 18.2 kg/m^2) found that 90 mg/day isoflavone supplementation over three menstrual cycles enhanced their BMD (Baek and Sung, 2003). In a meta-analysis of the consequence of long-term intervention of soy isoflavones on BMD, Liu *et al.* (2009) reported that the mean does of 87 mg of soy isoflavones

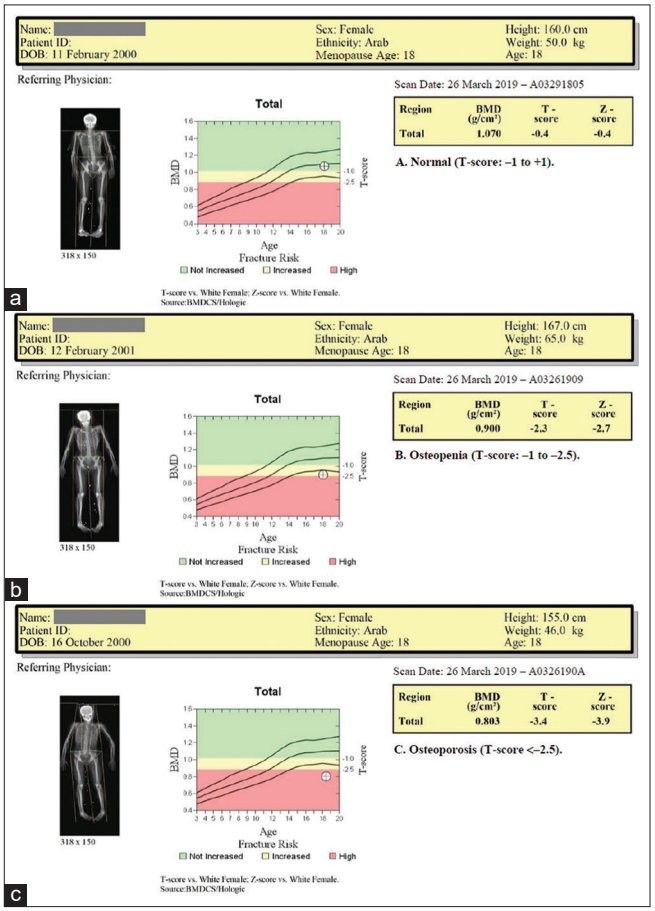

Fig 2. (a-c) Dual-energy x-ray absorptiometry (DEXA) results of three participants from different BMD groups (normal, osteopenia, and osteoporosis).

for a period of one year had a slight effect on BMD in postmenopausal women.

Our results demonstrated that participants with osteopenia were more responsive to the isoflavone supplement than both normal and osteoporosis subjects. This finding suggests that soy isoflavone supplementation, especially during the osteopenia phase, might have a beneficial effect in improving the symptoms, keeping the bone health, preventing the worsening of patients' status and delaying or preventing them from proceeding to osteoporosis; the last stage of this disease. The findings of the present study conclude that soy isoflavone supplementation (60 mg/day) for 4 weeks has slightly enhanced BMD in female university students, and it was more useful for osteopenia than osteoporosis and normal subjects. Dietary intake of vitamin D, vitamin C, calcium, phosphorus, magnesium, and potassium did not meet the RDA. Additional research is required to establish the linkage between soy isoflavone supplement, along with adequate intake of specific food groups, and BMD among premenopausal females.

There are several strengths in this study including combined assessment of physical activity, body composition and dietary pattern for young women to obtain better understanding of their nutritional status and lifestyle in relation to bone health. Studies on the effect of dietary supplementation on bone health often require a long time to bring up a significant outcome. Changes in bone structure and health occur on the long-term process and often require a lifestyle orientation and dietary pattern manipulation, beginning in childhood and continuing into old age (OSG, 2004**)**. Despite the short duration (4 weeks) and the small sample size (n=28) which might have not been enough to generalize the results, this small-scale pilot study could successfully answer the research question and ensure the feasibility of conducting full-scale studies and/or randomized controlled trials (RCTs) to comprehensively investigate the association between soy isoflavones and bone health among young pre-menopausal women. The positive effect obtained in such a short period of treatment is a good indicator for the beneficial effects of soy isoflavones for bone health if used in longer term investigations. These promising findings urge further larger-scale studies or clinical trials to comprehensively investigate the link between such supplementation and the bone health, and elucidate the mechanisms involved.

The study, however, has some limitations including the use of short and easy to administer food frequency questionnaire, which might have caused a lack of comprehensiveness. Inclusion of most consumed food was a concern in designing the questionnaire. Moreover, using a survey to assess physical activity level might have resulted in inaccurate data compared to more objective methods such as accelerometer.

Numerous medications, including celecoxib, theophylline, paclitaxel, midazolam, imatinib, carbamazepine, valproic acid, repaglinide, omeprazole, and danofloxacin, may interact when soy isoflavone intake is excessively high over a prolonged period of time (Soyata *et al*., 2021). Isoflavones might also interfere with tamoxifen, used in breast cancer treatment, and the drug raloxifene, used to prevent and treat osteoporosis. Patients taking these medications should consult their physician/pharmacist before using isoflavone supplements. The minerals zinc and iron may also interfere with the absorption of isoflavones and could theoretically lower their levels in the body.

CONCLUSION

Soy isoflavone supplementation (60 mg/day) for 4 weeks has slightly enhanced BMD in female university students, and it was more useful for osteopenia than osteoporosis and normal subjects. Dietary intake of vitamin D, vitamin C, calcium, phosphorus, magnesium, and potassium did not meet the RDA. Further research is required to establish the linkage between soy isoflavone supplement, along with adequate intake of specific food groups, and bone mineral density among premenopausal females.

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Conflict of interest

The authors declare no conflict of interest.

Availability of data and material

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

NA, LA-A, ZA-S, EA-K and ZA-F supported the proposal writing, data collection and manuscript writing. SA-O was responsible for organizing and coordinating the execution of the study. OA supported the proposal writing and

collaborated with the revision of the final version of the article. TS supported the statistical analysis and writing of the article. AG supervised the entire project and participated in developing the final draft of the manuscript. RK wrote the final draft of the manuscript after thoroughly revising the entire piece. The final paper was read and approved by all authors.

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