



Published in final edited form as:

Nutr Res. 2011 October ; 31(10): 766–775. doi:10.1016/j.nutres.2011.09.016.

Soy milk and Dairy Consumption are Independently Associated with Ultrasound Attenuation of the Heel bone among Postmenopausal Women: The Adventist Health Study-2 (AHS-2)

Vichuda Lousuebsakul Matthews^{1,2}, Synnove F Knutsen¹, W Lawrence Beeson¹, and Gary E Fraser¹

¹Dept. of Epidemiology & Biostatistics, School of Public Health, Loma Linda University

²Los Angeles County, Dept. of Health Services

Abstract

Soy milk has become a popular substitute for dairy milk with important health claims. We hypothesized that soy milk, based on its nutrient composition, was comparable to dairy products and, therefore, beneficial for bone health. To test this hypothesis, we examined the benefit of soy milk and dairy products intake on bone health using broadband ultrasound attenuation (BUA) of the calcaneus. Post-menopausal Caucasian women (n=337) who had completed a lifestyle and dietary questionnaire at enrollment into the AHS-2 had their calcaneal BUA measured two years later. The association between osteoporosis (defined as a T-score <-1.8) and some dietary factors (soy milk, dairy) and selected lifestyle factors was assessed using logistic regression. In a multivariable model adjusted for demographics, hormone use and other dietary factors, osteoporosis was positively associated with age (OR= 1.08, 95% CI: 1.06–1.12) and inversely associated with BMI (OR=0.91, 95% CI: 0.86–0.97) and current estrogen usage (OR=0.27, 95% CI: 0.13–0.56). Compared to women who did not drink soy milk, women drinking soy milk once a day or more had 56% lower odds of osteoporosis (OR= 0.44, 95% CI: 0.20–0.98 (p_{trend}=0.04). Women whose dairy intake was once a day or more had a 62% reduction in the likelihood of having osteoporosis (OR=0.38, 95% CI: 0.17–0.86)(p_{trend}=0.02) compared to women whose dairy intake was less than twice a week. Among individual dairy products, only cheese showed an independent and significant protection (OR=0.28, 95% CI: 0.12–0.66)(p_{trend}=0.004) for women eating cheese more than once per week vs. those who ate cheese less than once a week. We concluded that osteoporosis is inversely associated with soy milk intake to a similar degree as dairy intake after accounting for age, BMI, and estrogen usage.

Keywords

Soy milk consumption; Dairy products; Bone health; Heel bone; Broadband ultrasound attenuation

1. Introduction

Low bone mass or osteoporosis is one of the most common medical conditions in the United States, especially among postmenopausal women. An estimated 8 million women in the United States have osteoporosis, which increases their risk of having fractures and subsequently decreases their quality of life [1]. In recent years, broadband ultrasound attenuation (BUA) has been widely used to assess bone health. It is a cost-effective and

convenient screening method and has been found to have high correlation with bone mineral density (BMD) measured by dual energy x-ray absorptiometry (DXA) [2–5] and is also able to predict the risk of bone fracture to a similar degree as DXA [6–13].

Dairy consumption has been promoted by some as essential for maintaining good bone health. In spite of this, the consumption of dairy products has decreased over the last decades [14]. Instead, alternatives to dairy, especially soy based products, have become prevalent and are widely consumed as an alternative drink among those who avoid using milk products. Soy milk contains a non-steroidal phytoestrogen which has demonstrated a protective effect against age-related bone loss and other chronic diseases in some studies [15]. Soy phytoestrogen has also been marketed as an alternate form of hormonal replacement therapy to prevent postmenopausal syndrome or osteoporosis in the postmenopausal years [15]. Soy milk is also commonly fortified with nutrients such as calcium and vitamin D. A cup of calcium-fortified non-sweetened soy milk contains about 6 gm of protein, 6 gm of carbohydrate and 450 mg of calcium. However, very little is known about the effect on bone health of this shift from dairy to soy-based foods.

Among most human studies, the association between soy phytoestrogen and bone health have been examined using BMD, bone mineral content (BMC) or markers of bone turnover as endpoints. A majority of these studies have found a protective effect of phytoestrogen on bone loss [15]. In a meta-analysis reviewing 9 randomized controlled trials ranging from 3 to 12 months follow-up period, there was a significant decrease in bone resorption marker (urinary deoxypyridinoline) and a significant increase in bone formation marker (serum bone specific alkaline phosphatase) among subjects who consumed soy isoflavone compared to those who did not [16]. Similarly, the other meta-analysis study based on 10 studies with a follow-up period of at least one year revealed that an intake of isoflavones of > 80 mg/day showed a weak beneficial effect on BMD at the lumbar spine [17].

Based on several factors in soy milk, we hypothesized that it has comparable benefits to dairy products for bone health. Therefore, we tested this hypothesis by examining the benefits of consuming soy milk and dairy products on bone health using broadband ultrasound attenuation (BUA) of the calcaneus. Since BUA is considered a valid tool in assessing bone health [2–13], we examined the association between intake of both soy milk and dairy products on the risk of osteoporosis using calcaneal BUA measurement among Caucasian postmenopausal women. Several lifestyle factors (age, education, smoking, body mass index (BMI), estrogen use, etc) and other foods were adjusted for in the final multivariable model.

2. Methods and materials

2.1 Study population

Subjects were enrollees in the Adventist Health Study-2 (AHS-2), a large National Cancer Institute (NCI) funded cohort study investigating the relationship between lifestyle factors and several disease outcomes. The study has been described in detail elsewhere [18] and consists of Adventists throughout the United States and Canada who completed a comprehensive lifestyle and dietary questionnaire between 2002 and 2006. Among the AHS-2 population, 559 Caucasian women who were part of a calibration study [19] or a pilot church clinic study [20] attended clinics in local Adventist churches where their calcaneal broadband ultrasound attenuation was measured.

Among the two clinic populations, 421 Caucasian women were post-menopausal. A total of 84 subjects who reported doctor-diagnosed osteoporosis at baseline were excluded, leaving

337 eligible subjects for this study. This study was approved by the Loma Linda University Institutional Review Board (IRB).

2.2 Food Frequency Questionnaire (FFQ)

Dietary information was collected as part of enrollment into the parent AHS-2 study using a comprehensive self-administered and validated FFQ [21] reporting on the subject's dietary intake during the last twelve months. Subjects were asked to report how frequently they consumed a food: "never," "1–3 times per month," "1 time per week," "2–4 times per week," "5–6 times per week," "1 per day," and "2 or more times per day." Frequency per month was calculated for each food (soy milk, etc) or food group. A "dairy consumption index" was developed combining the monthly intake of milk, yogurt, cheese, and other dairy products. Meat consumption was assessed using the combined monthly intake of beef, poultry, lamb, pork and fish. Women who reported eating meat less than once per month were classified as vegetarians. Monthly consumption was further categorized into "times per month" or "times per day" for statistical analysis. In addition, the FFQ contained questions on the use of calcium and vitamin D supplements.

2.3 Lifestyle questionnaire

At enrollment, in addition to the FFQ, participants completed a comprehensive questionnaire on exercise, use of hormones, medical history, smoking, anthropometrics, education, personal and household incomes as well as other demographic variables. The question on osteoporosis asked "Have you ever been told by a doctor that you had any of these conditions?" One of the response categories was "Osteoporosis (thinning of the bones)". Those who answered yes (n=84) to this question were excluded from our study population.

2.4 Bone Ultrasound Measurement

About two years after enrollment into the parent study, subjects were invited to attend a clinic either as part of the calibration study or as part of the church clinic pilot study. During the clinics, broadband ultrasound attenuation (BUA) was assessed at the site of the calcaneus of the dominant foot using a contact ultrasound bone analyzer (CUBA) system [22]. After entering the age and gender of the subject, the CUBA system produced a T-score which is the basis for the outcome measure in this study.

2.5 Statistical Analyses

According to the World Health Organization (WHO), a T-score of less than -1.8 of any peripheral ultrasound measurement is an indication of osteoporosis [9]. We used this cut-off to categorize subjects as osteoporotic. Chi-Square and independent t-tests were used to determine the association between osteoporosis (T-score less than -1.8) and selected predictor variables (Table 1). Analysis of covariance was used to determine the difference in BUA measurement by the categories of each of the predictor variables adjusting for age, BMI and education (Table 2). Unconditional logistic regression was used to determine the association between soy milk intake, dairy food intake, other food intakes, selected lifestyle factors and osteoporosis (T-score < -1.8). Odds ratios and 95%CI were calculated from unconditional logistic regression models, with osteoporosis as the dependent variable and the following as independent variables: age at enrollment (continuous), BMI (continuous), education (less than bachelor's degree, bachelor's degree, graduate degree), estrogen usage (current user vs. past user and non-user), smoking (ever smoker vs. never smoker), calcium supplement intake (never, used for less than 5 years, used for 5 years or longer), vitamin D supplement intake (never, used for less than 5 years, used for 5 years or longer), soy milk intake, dairy intake, vegetarian status (eating meat/fish less than once per month vs. meat/fish 1+ times/month) and other foods (fruits, salads, vegetables, legumes, nuts, meat

analogs, grains, cereals, soy foods). Any independent variable (as presented in the “Candidate Variable Model” section of Table 3) which changed the estimated odds ratio of the primary exposure variables (i.e. soy milk, dairy) by at least 10% remained in the final multiple logistic regression model. We further stratified the analysis by vegetarian status to examine the association of dairy and soy milk intakes on low T-score in both omnivores and vegetarians. All statistical analyses were performed using SAS 9.1 (SAS Institute, Cary, NC).

3. Results

3.1 Descriptive analysis

Based on the BUA measurement, a total of 100 (29.7%) of the women were osteoporotic using the definition of a T-score < -1.8 . The 30% prevalence rate is identical to the rate reported by Melton, et al. among postmenopausal Caucasian women using a -2.5 T-score of BMD measurement at hip, spine and forearm [23].

Compared to the non-cases, our cases were older (73.8 vs. 65.6 yrs), had lower BMI (25 vs. 27.1), were less likely to currently be using estrogen replacement therapy (12% vs. 32.5%) and were more likely to report their health status as Fair/Poor (19.2 vs. 8.7%) (Table 1). There was a significance difference ($p < .05$) between cases and non-cases due to age, BMI, estrogen usage, dairy consumption, self-reported health status and history of minor accident fractures. No significant difference was observed in educational level, smoking status, soy milk intake, physical activity levels, vegetarian status, soy food intake or use of calcium and vitamin D supplements (Table 1).

3.2 Analysis of Covariance

Comparing the mean bone attenuated ultrasound levels with demographic and dietary factors (Table 2), there was a significant reduction in BUA with increasing age, decreasing BMI and not using estrogen replacement therapy. Also those who reported the lowest frequency of intake of dairy products had the lowest BUA levels ($p \text{ trend} < 0.0001$). There were no significant differences in BUA measurement related to education level, smoking status, physical activity level, use of calcium supplement, use of vitamin D supplement, vegetarian status, soy milk consumption, self-reported health status, soy foods intake and history of minor accident fractures.

3.3 Logistic Regression Analyses

Three models were used for logistic regression (Table 3): a demographic model with only demographic variables: age, BMI and education. Candidate Variable Model: a model with one other candidate variable controlling for demographic variables (age, BMI, education). A final model was used where all candidate variables that changed the main exposure effect (dairy or soy milk) more than 10% were included in the demographic model at the same time. In the demographic model, the odds of osteoporosis was increased by 8% for each year increase in age and reduced by 7% for each unit increase in BMI. There was an inverse, but not statistically significant, association with educational level with lowest odds of osteoporosis among those with graduate degrees.

In the candidate variable model, osteoporosis showed an inverse relationship with dairy consumption (OR=0.48 for the highest category of once a day or more vs. lowest category of less than twice a week). There was also a negative association with soy milk intake, calcium supplement intake and being a vegetarian, but these relationships did not reach statistical significance in this model. Osteoporosis was negatively associated with estrogen use (OR=0.25 for current users versus past/never users) and positively associated with self-

reported health status (OR=2.4 for Fair/Poor vs. Excellent/Good). Compared to never smokers, those who had ever smoked cigarettes were more likely to have osteoporosis (OR=1.53). The final multivariate model included the soy milk, dairy index and vegetarian status in addition to age, BMI, education and estrogen use. The relationship between osteoporosis and dairy and soy milk intake were both strengthened. Those drinking soy milk at least once a day or more (highest category) were 57% less likely to have osteoporosis than those who never used soy milk (OR=0.43 (95% CI: 0.21–0.89)). Likewise, those eating dairy products at least once a day or more were 62% less likely to have osteoporosis than those consuming dairy less than twice a week (OR=0.38 (95% CI: 0.17–0.86))(p_{trend}=0.02). Being a vegetarian continued to be associated with lower likelihood of osteoporosis (OR=0.65), but this relationship was not statistically significant.

We further stratified the analysis by vegetarian status and found a non-significant protective association of dairy and soy milk in both omnivores and vegetarians (data not shown). However, these associations were only showed a significant protective trend (p_{trend} = 0.02) among vegetarians who comprised two-thirds of the population while the sample size was too small among the omnivores.

Lastly, when the dairy product category was substituted with the individual dairy foods (cheese, milk, ice cream and yogurt) (Table 4), only cheese showed an independent and significant protection with those eating it more than once a week having 72% lower odds of osteoporosis compared to those who reported consuming cheese less than once a week. Both milk and yogurt/ice cream also showed an inverse association with risk of osteoporosis, but these did not reach statistical significance.

4. Discussion

We found that women who consumed about 1.3 cups of soy milk daily reduced the odds of having a low T-score by 57% compared to non-users even after adjusting for other important covariates including dairy consumption. This is almost identical to the 62% reduction in the odds of a low T-score among those consuming at least one serving of dairy compared to those who used these products less than twice a week. Our findings agree with several animal and human studies showing that calcium fortified soy milk are beneficial to bone loss prevention to a similar degree as cows' milk [24–28].

The similarity in physiologic characteristics between ovariectomized rats and postmenopausal women has brought supportive evidence from animal studies indicating the protective effect of soy milk on bone loss [24]. Compared to the control ovariectomized rats, soy fed ovariectomized rats have been found to have higher levels of trabecular bone volume, insulin-like growth factor I (IGF-I), serum estradiol levels, and serum osteocalcin [25–26]. In addition, there were lower levels of serum parathyroid hormone, trabecular area of resorption surface and bone resorption marker urinary deoxypyridinoline (DPD) among the soy milk fed rats compared to the control ovariectomized rats [25–26].

Few studies have directly compared soy milk to cow's milk. However, the effect of soy protein versus milk-based protein on bone health has been studied [24–28]. The bone formation biomarker - serum insulin like growth factor I (IGF-I), bone resorption biomarker - serum urinary deoxypyridinoline (DPD) and urinary calcium excretion were compared among postmenopausal women who were fed with either soy protein or milk-based protein. Soy protein was found to have more impact in increasing serum IGF-I, especially among women who were not on hormonal replacement therapy, compared to the milk-based protein group. The bone resorption marker DPD was significantly reduced among the soy protein group, but not so among the milk-based protein group. While there was a 33% increase in

urinary calcium excretion among the milk-based protein group, this effect was not observed among the soy protein group [27]. In another study, the bone sparing effects of soy milk was observed in a 2-year randomized, placebo-controlled trial comparing the changes in BMD and BMC among postmenopausal women who were either fed soy milk containing isoflavones or applying transdermal progesterone or having both treatments. In both the soy milk group and the transdermal progesterone application group, there was no significant loss in BMD or BMC from baseline measurements as compared to the control group without any treatment [28].

Supporting these studies is evidence from the Shanghai Women's Health Study which found a significant negative association between fracture incidence and quintiles of soy protein intake among a large prospective cohort study of 24,403 postmenopausal women [29]. The mechanisms behind a possible protective effect of soy is unclear. There is some suggestive evidence indicating that a higher consumption of soy is related to the capacity for equol production, a metabolite of soy isoflavone, that may have a favorable effect on bone loss [30–31]. Thus, women in our study, having on average higher soy consumption due to their often lifetime lacto-ovo-vegetarian diet, may have acquired the capacity to produce more equol. However, this needs further assessment.

The protective effect of soy milk observed in our study could also be enhanced due to our endpoint - calcaneal BUA. Calcaneal bone consists mainly of trabecular bone which has been found to be the first region to respond to any mechanical stimulus [32]. In one animal study, high doses of isoflavones improved microstructural properties of trabecular bone independent of BMD among ovariectomized rats compared to the control group [33]. Among postmenopausal women, the effect of physical activity has been found to be related to calcaneal BUA independently of BMD [34–35]. Heel bone is superior to femur or spine in reflecting the effect of physical activity [36]. It is possible that the BUA can better reflect the trabecular changes of the calcaneal bone due to dietary stimulus as well.

In our study, dairy products showed a similar protective effect on bone as did soy milk. However, of all the dairy products, only cheese showed a significant protective effect on low T-scores when evaluated separately. These findings are similar to earlier findings of a protective effect of cheese observed among peri- and postmenopausal women in our previous cohort of Adventists where intake of cheese 3 or more times per week reduced the risk of wrist fracture by 58% [37]. This could be due to the higher calcium and protein content per serving of cheese compared to milk [38].

The effects of age, BMI, hormonal replacement therapy, smoking and previous minor fractures are similar to that observed by others [39 – 48]. No significant associations between low T-score and use of calcium and vitamin D supplements were observed. The lack of association with calcium or vitamin D could be due to the several factors. The effect of calcium can only be observed when the total calcium intake is below the threshold point [49]. Also, serum 25 (OH)D is a better marker for vitamin D exposure [49]. Our findings of a protective effect of a vegetarian diet on low T-scores warrants further studies.

The strengths of our investigation are its prospective design with a follow-up period of at least 2 years and the employment of our validated food frequency questionnaire [19, 21]. The food frequency questionnaire has shown a high correlation with dietary intake, including soy protein intake, estimated from 24-hour dietary recall and isoflavonoid urinary excretion [19]. The follow-up period of at least 2 years is ideal to test the effect of any clinical intervention on bone since the normal bone remodeling cycle takes approximately 6 to 12 months [50]. A limitation is that despite a high correlation between ultrasound measurement, bone mineral density and fracture risk, we cannot apply our results to overall

bone health as changes observed in bone density after feeding trials do not occur uniformly at all sites but vary even in the same individual [50]. Furthermore, subjects could have changed their dietary pattern during the 2-year period between the baseline questionnaire and the BUA measurement. However, we have found that subjects in this population rarely change their overall dietary pattern over time [51].

In conclusion, both soy milk and dairy intake are beneficial to bone health showing an independent protective association with a low T-score when evaluated together. While the protective effect of dairy products ranges from 14% – 83%, the protective effect of soy milk is similar from 11%–79%. Our findings on soy milk support the belief that soy isoflavones tend to reduce bone loss among subjects with low baseline bone density or those with low serum estrogen levels such as among postmenopausal women [52]. Based on our findings, postmenopausal women could benefit from drinking soy milk and/or having a regular intake of dairy products.

Acknowledgments

The Adventist Health Study-2 is supported by Grant # RO1-CA94594 from the National Cancer Institute. None of the authors had any conflict of interest related to this manuscript.

Abbreviations

BUA	broadband ultrasound attenuation
AHS-2	Adventist Health Study –2
BMI	body mass index
BMD	bone mineral density
BMC	bone mineral content
DXA	dual energy x-ray absorptiometry
NCI	National Cancer Institute
FFQ	Food Frequency Questionnaire
CUBA	Contact ultrasound bone analyzer
WHO	World Health Organization
OR	Odds ratios
IGF-I	Insulin-like growth factor-I
DPD	deoxyypyridinoline
Mg	milligram

References

1. Riggs BL, Melton LJ 3rd. The worldwide problem of osteoporosis: insights afforded by Epidemiology. *Bone*. 1995; 17:505S–11S. [PubMed: 8573428]
2. Jorgensen HL, Warming L, Bjarnason NH, Andersen PB, Hassager C. How does quantitative ultrasound compare to dual X-ray absorptiometry at various skeletal sites in relation to the WHO diagnosis categories? *Clin Physiol*. 2001; 21:51–59. [PubMed: 11168297]
3. Njeh CF, Boivin CM, Langton CM. The role of ultrasound in the assessment of osteoporosis: a review. *Osteoporos Int*. 1997; 7:7–22. [PubMed: 9102067]

4. Nicholson PH, Muller R, Cheng XG, Ruegsegger P, Van Der Perre G, Dequeker J, et al. Quantitative ultrasound and trabecular architecture in the human calcaneus. *J Bone Miner Res.* 2001; 16:1886–92. [PubMed: 11585354]
5. Gregg EW, Kriska AM, Salamone LM, Wolf RL, Roberts MM, Ferrell RE, et al. Correlates of quantitative ultrasound in the Women's Healthy Lifestyle Project. *Osteoporos Int.* 1999; 10:416–424. [PubMed: 10591840]
6. Frost ML, Blake GM, Fogelman I. Contact quantitative ultrasound: an evaluation of precision, fracture discrimination, age-related bone loss and applicability of the WHO criteria. *Osteoporos Int.* 1999; 10:441–449. [PubMed: 10663343]
7. Schott AM, Weill-Engerer S, Hans D, Duboeuf F, Delmas PD, Meunier PJ. Ultrasound discriminates patients with hip fracture equally well as dual energy X-ray absorptiometry and independently of bone mineral density. *J Bone Miner Res.* 1995; 10:243–249. [PubMed: 7754803]
8. Hans D, Srivastav SK, Singal C, Barkmann R, Njeh CF, Kantorovich E, et al. Does combining the results from multiple bone sites measured by a new quantitative ultrasound device improve discrimination of hip fracture? *J Bone Miner Res.* 1999; 14:644–651. [PubMed: 10234587]
9. Frost ML, Blake GM, Fogelman I. Can the WHO criteria for diagnosing osteoporosis be applied to calcaneal quantitative ultrasound? *Osteoporos Int.* 2000; 11:321–330. [PubMed: 10928222]
10. Frost ML, Blake GM, Fogelman I. Does the combination of quantitative ultrasound and dual-energy X-ray absorptiometry improve fracture discrimination? *Osteoporos Int.* 2000; 12:471–477. [PubMed: 11446563]
11. Hans D, Dargent-Molina P, Schott AM, Sebert JL, Cormier C, Kotzki PO, et al. Ultrasonographic heel measurements to predict hip fracture in elderly women: the EPIDOS prospective study. *Lancet.* 1996; 348:511–514. [PubMed: 8757153]
12. Miller PD, Siris ES, Barrett-Connor E, Faulkner KG, Wehren LE, Abbott TA, et al. Prediction of fracture risk in postmenopausal white women with peripheral bone densitometry: evidence from the National Osteoporosis Risk Assessment. *J Bone Miner Res.* 2002; 17:2222–30. [PubMed: 12469916]
13. Diez-Perez A, Gonzalez-Macias J, Marin F, Abizanda M, Alvarez R, Gimeno A, et al. Prediction of absolute risk of non-spinal fractures using clinical risk factors and heel quantitative ultrasound. *Osteoporos Int.* 2007; 18:629–639. [PubMed: 17235664]
14. Huth PJ, DiRienzo DB, Miller GD. Major scientific advances with dairy foods in nutrition and health. *J Dairy Sci.* 2006; 89:1207–21. [PubMed: 16537954]
15. Cassidy A, Albertazzi P, Lise Nielsen I, Hall W, Williamson G, Tetens I, et al. Critical review of health effects of soyabean phyto-oestrogens in post-menopausal women. *Proc Nutr Soc.* 2006; 65:76–92. [PubMed: 16441947]
16. Ma DF, Qin LQ, Wang PY, Katoh R. Soy isoflavone intake inhibits bone resorption and stimulates bone formation in menopausal women: meta-analysis of randomized controlled trials. *Eur J Clin Nutr.* 2008; 62:155–161. [PubMed: 17392695]
17. Liu J, Ho SC, Su YX, Chen WQ, Zhang CX, Chen YM. Effect of long-term intervention of soy isoflavones on bone mineral density in women: a meta-analysis of randomized controlled trials. *Bone.* 2009; 44:948–953. [PubMed: 19168161]
18. Butler TL, Fraser GE, Beeson WL, Knutsen SF, Herring RP, Chan J, et al. Cohort profile: The Adventist Health Study-2 (AHS-2). *Int J Epidemiol.* 2008; 37:260–65. [PubMed: 17726038]
19. Jaceldo-Siegl K, Fraser GE, Chan J, Franke A, Sabaté J. Validation of soy protein estimates from a food-frequency questionnaire with repeated 24-hour recalls and isoflavonoid excretion in overnight urine in a Western population with a wide range of soy intakes. *Am J Clin Nutr.* 2008; 87:1422–27. [PubMed: 18469267]
20. Chan J, Knutsen SF, Sabate J, Haddad E, Yan R, Fraser GE. Feasibility of running clinics to collect biological specimens in a nationwide cohort study--Adventist Health Study-2. *Ann Epidemiol.* 2007; 17:454–457. [PubMed: 17395486]
21. Knutsen SF, Fraser GE, Beeson WL, Lindsted KD, Shavlik DJ. Comparison of adipose tissue fatty acids with dietary fatty acids as measured by 24-hour recall and food frequency questionnaire in Black and White Adventists: the Adventist Health Study. *Ann Epidemiol.* 2003; 13:119–127. [PubMed: 12559671]

22. Langton CM, Ali AV, Riggs CM, Evans GP, Bonfield W. A Contact method for the assessment of ultrasonic velocity and broadband attenuation in cortical and cancellous bone. *Clin Physiol Meas.* 1990; 11:243–249.
23. Melton LJ 3rd, Chrischilles EA, Cooper C, Lane AW, Riggs BL. How many women have osteoporosis? *J Bone Miner Res.* 2005; 20:886–892. [PubMed: 15931736]
24. Kalu DN. The ovariectomized rat model of postmenopausal bone loss. *Bone Miner.* 1991; 15:175–191. [PubMed: 1773131]
25. Taguchi H, Chen H, Yano R, Shoumura S. Comparative effects of milk and soy milk on bone loss in adult ovariectomized osteoporosis rat. *Okajimas Folia Anat Jpn.* 2006; 83:53–59. [PubMed: 16944838]
26. Jeon BJ, Ahn J, Kwak HS. Effect of isoflavone-enriched milk on bone mass in ovariectomized rats. *J Med Food.* 2009; 12:1260–67. [PubMed: 20041779]
27. Arjmandi BH, Khalil DA, Smith BJ, Lucas EA, Juma S, Payton ME, et al. 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.* 2003; 88:1048–54. [PubMed: 12629084]
28. Lydeking-Olsen E, Beck-Jensen JE, Setchell KD, Holm-Jensen T. Soy milk or progesterone for prevention of bone loss—a 2 year randomized, placebo-controlled trial. *Eur J Nutr.* 2004; 43:246–257. [PubMed: 15309425]
29. Zhang X, Shu XO, Li H, Yang G, Li Q, Gao YT, et al. Prospective cohort study of soy food consumption and risk of bone fracture among postmenopausal women. *Arch Intern Med.* 2005; 165:1890–95. [PubMed: 16157834]
30. Vatanparast H, Chilibeck PD. Does the effect of soy phytoestrogens on bone in postmenopausal women depend on the equol-producing phenotype? *Nutr Rev.* 2007; 65:294–299. [PubMed: 17605306]
31. Magee PJ, Rowland IR. Phyto-oestrogens, their mechanism of action: current evidence for a role in breast and prostate cancer. *Br J Nutr.* 2004; 91:513–531. [PubMed: 15035679]
32. Riggs BL, Wahner HW, Seeman E, Offord KP, Dunn WL, Mazess RB, et al. Changes in bone mineral density of the proximal femur and spine with aging. Differences between the postmenopausal and senile osteoporosis syndromes. *J Clin Invest.* 1982; 70:716–723. [PubMed: 7119111]
33. Devareddy L, Khalil DA, Smith BJ, Lucas EA, Soung do Y, Marlow DD, et al. Soy moderately improves microstructural properties without affecting bone mass in an ovariectomized rat model of osteoporosis. *Bone.* 2006; 38:686–693. [PubMed: 16406762]
34. Blanchet C, Giguère Y, Prud'homme D, Turcot-Lemay L, Dumont M, Leduc G, et al. Leisure physical activity is associated with quantitative ultrasound measurements independently of bone mineral density in postmenopausal women. *Calcif Tissue Int.* 2003; 73:339–349. [PubMed: 12874703]
35. Brooke-Wavell K, Jones PR, Hardman AE, Tsuritan, Yamada Y. Commencing, continuing and stopping brisk walking: effects on bone mineral density, quantitative ultrasound of bone and markers of bone metabolism in postmenopausal women. *Osteoporos Int.* 2001; 12:581–587. [PubMed: 11527057]
36. Heinonen A, Kannus P, Sievänen H, Pasanen M, Oja P, Vuori I. Good maintenance of high-impact activity- induced bone gain by voluntary, unsupervised exercises: An 8-month follow-up of a randomized controlled trial. *J Bone Miner Res.* 1999; 14:125–128. [PubMed: 9893074]
37. Thorpe DL, Knutsen SF, Beeson LW, Rajaram S, Fraser GE. Effects of meat consumption and vegetarian diet on risk of wrist fracture over 25 years in a cohort of peri- and postmenopausal women. *Public Health Nutr.* 2007; 9:1–9.
38. Weinsier RL, Krumdieck CL. Dairy foods and bone health: examination of the evidence. *Am J Clin Nutr.* 2000; 72:681–689. [PubMed: 10966884]
39. Grady D, Herrington D, Bittner V, Blumenthal R, Davidson M, Hlatky M, et al. Cardiovascular disease outcomes during 6.8 years of hormone therapy: Heart and Estrogen/progestin Replacement Study follow-up (HERS II). *JAMA.* 2002; 288:49–57. [PubMed: 12090862]

40. Teede HJ. Hormone therapy--weighing up the evidence. *Aust Fam Physician*. 2004; 33:875–880. [PubMed: 15584324]
41. Adami S, Giannini S, Giorgino R, Isaia G, Maggi S, Sinigaglia L, et al. The effect of age, weight, and lifestyle factors on calcaneal quantitative ultrasound: the ESOPPO study. *Osteoporos Int*. 2003; 14:198–207. [PubMed: 12730794]
42. Waugh EJ, Lam MA, Hawker GA, McGowan J, Papaioannou A, Cheung AM, et al. Risk factors for low bone mass in healthy 40–60 year old women: A systematic review of the literature. *Osteoporos Int*. 2008; 20:1–21. [PubMed: 18523710]
43. Welch A, Camus J, Dalzell N, Oakes S, Reeve J, Khaw KT. Broadband ultrasound attenuation (BUA) of the heel bone and its correlates in men and women in the EPIC-Norfolk cohort: a cross-sectional population-based study. *Osteoporos Int*. 2004; 15:217–225. [PubMed: 14745486]
44. Reid IR. Relationships among body mass, its components, and bone. *Bone*. 2002; 31:547–555. [PubMed: 12477567]
45. Villareal DT, Apovian CM, Kushner RF, Klein S. American Society for Nutrition; NAASO, The Obesity Society. Obesity in older adults: technical review and position statement of the American Society for Nutrition and NAASO, The Obesity Society. *Am J Clin Nutr*. 2005; 82:923–934. [PubMed: 16280421]
46. De Laet C, Kanis JA, Odén A, Johanson H, Johnell O, Delmas P, et al. Body mass index as a predictor of fracture risk: a meta-analysis. *Osteoporos Int*. 2005; 16:1330–38. [PubMed: 15928804]
47. Wong PK, Christie JJ, Wark JD. The effects of smoking on bone health. *Clin Sci (Lond)*. 2007; 113:233–241. [PubMed: 17663660]
48. Löfman O, Hallberg I, Berglund K, Wahlström O, Kartous L, Rosenqvist AM, et al. Women with low-energy fracture should be investigated for osteoporosis. *Acta Orthop*. 2007; 78:813–821. [PubMed: 18236189]
49. Heaney RP. Bone health. *Am J Clin Nutr*. 2007; 85:300S–303S. [PubMed: 17209214]
50. Heaney RP. Calcium, dairy products and osteoporosis. *J Am Coll Nutr*. 2000; 19:83S–99S. [PubMed: 10759135]
51. Tantamango YM, Knutsen SF, Beeson WL, Fraser G, Sabate J. Foods and food groups associated with the incidence of colorectal polyps: the Adventist Health Study. *Nutr Cancer*. 2011; 63:565–72. [PubMed: 21547850]
52. Weaver CM, Cheong JM. Soy isoflavones and bone health: the relationship is still unclear. *J Nutr*. 2005; 135:1243–47. [PubMed: 15867312]

Table 1

Food intakes and other lifestyle characteristics among osteoporotic cases (T-score <-1.8) and non-cases among 337 Caucasian Postmenopausal Women.

	Non-Cases T-score >= -1.8 (n=237)	Cases T-Score < - 1.8 (n=100)	Chi-Square Test (P Value)
Age (years), mean (SD)			<.0001
≤ 54 years	15.6% (n=37)	3.0% (n=3)	
55–64 years	35.9% (n=85)	22.0% (n=22)	
65–74 years	32.1% (n=76)	25.0% (n=25)	
≥ 75 years	16.5% (n=39)	50.0% (n=50)	
BMI (kg m ⁻²), mean (SD)			<.004
≤ 20	4.6% (n=11)	16.0% (n=16)	
21–24	30.8% (n=73)	31.0% (n=31)	
25–29	35.0% (n=83)	31.0% (n=31)	
≥ 30	29.5% (n=70)	22.0% (n=22)	
Education Level:			0.41
No Bachelor's degree	62.5% (n=148)	67.0% (n=67)	
Bachelor's degree	18.6% (n=44)	20.0% (n=20)	
Graduate degree	19.0% (n=45)	13.0% (n=13)	
Estrogen Usage:			<.0001
Past/never Users	67.5% (n=160)	88.0% (n=88)	
Current Users	32.5% (n=77)	12.0% (n=12)	
Smoking Status:			0.99
Never smokers	92.0% (n=218)	92.0% (n=92)	
Ever smokers	8.0% (n=19)	8.0% (n=8)	
Soy milk intake:			0.36
Never (0/month)	46.8% (n=111)	43.0% (n=43)	
Once a month or more (1–29 per month)	24.5% (n=58)	32.0% (n=32)	
Once a day or more (30+ per month)	28.7% (n=68)	25.0% (n=25)	
Dairy Consumption:			0.0005
Less than twice a week (0–7/month)	34.2% (n=81)	56.0% (n=56)	
Twice a week or more (8–29/month)	30.0% (n=71)	25.0% (n=25)	
Once a day or more (30+/month)	35.8% (n=85)	19.0% (n=19)	
Cheese Consumption:			<0.0001
Less than once a week (1–3/month)	48.1% (n=114)	75.0% (n=75)	
Once a week (4/month)	16.9% (n=40)	15.0% (n=15)	
More than one times per week (5+/month)	35.0% (n=83)	10.0% (n=10)	
Milk Consumption:			0.19
Less than once a week (1–3/month)	57.8% (n=137)	68.0% (n=68)	
Less than one times per day (4–29/month)	25.7% (n=61)	21.0% (n=21)	
Once a day or more (30+/month)	16.5% (n=39)	11.0% (n=11)	
Yogurt/Ice Cream Consumption:			0.5
None (0/month)	52.7% (n=125)	57.0% (n=57)	

	Non-Cases T-score ≥ -1.8 (n=237)	Cases T-Score < -1.8 (n=100)	Chi-Square Test (P Value)
Once a week or less (1–4/month)	35.0% (n=83)	35.0% (n=35)	
More than once a week (5+/month)	12.2% (n=29)	8.0% (n=8)	
Self-reported health Status ¹ :			.007
Excellent/Good	91.3% (n=210)	80.8% (n=80)	
Fair/Poor	8.7% (n=20)	19.2% (n=19)	
History of minor Accident Fractures:			.02
No	82.3% (n=195)	71.0% (n=71)	
Yes	17.7% (n=42)	29.0% (n=29)	
Physical Activity Level ¹ :			0.81
Level 1 (None)	21.5% (n=50)	20.4% (n=20)	
Level 2 (1–104 min/wk of Walk/Run/	21.9% (n=51)	22.5% (n=22)	
Level 3 (105–174 min/wk of Walk/Run/	32.6% (n=76)	28.6% (n=28)	
Level 4 (≥ 175 min/wk of Walk/Run/	24.0% (n=56)	28.6% (n=28)	
Vegetarian Status:			0.36
Vegetarians (< 1 x/month)	60.8% (n=144)	66.0% (n=66)	
Meat Consumers (≥ 1 /month)	39.2% (n=93)	34.0% (n=34)	
Soyfood (Tofu, Soy cheese) intake:			0.178
Never (0/month, mean =0)	29.9% (n=70)	20.2% (n=20)	
Less than twice a week (1–7 per month)	36.7% (n=86)	39.4% (n=39)	
Twice a week or more (8+ per month)	33.3% (n=78)	40.4% (n=40)	
Calcium Supplement:			0.65
Never Users	39.2% (n=93)	41.0% (n=41)	
Use for less than 5 years	27.9% (n=66)	31.0% (n=31)	
Use for 5 years or more	32.9% (n=78)	28.0% (n=28)	
Vitamin D Supplement:			0.18
Never Users	84.4% (n=200)	79.0% (n=79)	
Use for less than 5 years	7.6% (n=18)	14.0% (n=14)	
Use for 5 years or more	8.0% (n=19)	7.0% (n=7)	

SD – Standard deviation; BMI – body mass index.

¹ Less than 337 subjects due to missing values

Table 2

Least Square Mean (LS Mean) BUA Measurement Among 337 Caucasian Postmenopausal Women by Demographic, dietary and other Lifestyle Factors

	BUA ¹ (dB/Mhz)	R Square of the Model (%)	p ²
Age (years), LS mean (SE), ³		23.2%	<.0001
≤ 54 years	80.4 (2.8)		
55–64 years	74.6 (1.6)		
65–74 years	70.7 (1.6)		
≥ 75 years	58.3 (1.6)		
BMI (kg/m ²) ³		26.9%	<.0001
≤ 20	56.2 (3.0)		
21–24	67.9 (1.5)		
25–29	70.1 (1.5)		
≥ 30	72.9 (1.7)		
Education Level ³		25.3%	0.09
No Bachelor's degree	67.9 (1.1)		
Bachelor's degree	69.5 (2.0)		
Graduate degree	73.1 (2.1)		
Estrogen Usage ³		30.8%	<.0001
Past/Never Users	66.5 (1.0)		
Current Users	76.3 (1.6)		
Smoking Status ³		25.4%	0.44
Never smokers	69.3 (0.9)		
Ever smokers	66.7 (3.2)		
Soy milk intake ³		25.9%	0.25
Never (0/month)	69.7 (1.3)		
Once a month or more (1–29 per month)	66.7 (1.7)		
Once a day or more (30+ per month)	70.3 (1.7)		
Dairy Consumption ³		28.2%	<.0001
Less than twice a week (0–7/month)	65.2 (1.38)		
Twice a week or more (8–29/month)	70.8 (1.60)		
Once a day or more (30+/month)	72.6 (1.58)		
Self-reported Health Status ³		25.6%	0.05
Excellent/Good	69.7 (0.9)		
Fair/Poor	64.2 (2.6)		
History of Minor Accident Fractures ³		25.9%	0.1
No	69.8 (1.0)		
Yes	66.2 (1.9)		
Physical Activity Level (Walk/Run/Jog) ³		26.3%	0.64
Level 1 (None)	69.3 (2.0)		
Level 2 (1–104 min/wk)	67.2 (1.9)		

	BUA ¹ (dB/Mhz)	R Square of the Model (%)	P ²
Level 3 (105–174 min/wk)	69.4 (1.6)		
Level 4 (≥ 175 min/wk)	70.4 (1.8)		
Vegetarian Status ³		25.3%	0.63
Vegetarians (<1x/month)	68.7 (1.1)		
Meat Eaters (1+ per month)	69.6 (1.5)		
Soyfood (Tofu, Soy cheese) intake ³		24.6	0.92
Never (0/month)	69.8 (1.7)		
Less than twice a week (1–7 per month)	69.1 (1.4)		
Twice a week or more (8+ per month)	68.8 (1.5)		
Calcium Supplement ³			
Never Users	69.4 (1.4)	25.8%	0.31
Use for less than 5 years	67.1 (1.6)		
Use for 5 years or more	70.5 (1.6)		
Vitamin D Supplement ³		25.7%	0.41
Never Users	69.5 (0.9)		
Use for less than 5 years	65.6 (2.8)		
Use for 5 years or more	68.5 (3.2)		

SE – Standard Error; BMI – body mass index.

¹ Mean BUA (69.1 dB/Mhz); Standard Deviation (18.3 dB/Mhz); Coefficient Variation (26.5)

² P value from analysis of covariance testing the null hypothesis of no association between BUA and the exposure variable

³ Adjusted, as appropriate, for age, BMI, and education

Table 3

Demographic Model, Candidate Variable Model and Final Model Logistic Regression Examining Associations between Selected Variables and osteoporosis (T-score < -1.8)¹ adjusted for Selected Characteristics among 337 Postmenopausal Caucasian Women

	Demographic Model ²		Candidate Variable Model ³		Final Model ⁴	
	OR	95%CI			OR	95%CI
Age (<i>Continuous</i>)	1.08*	(1.05–1.11)			1.08*	(1.06–1.12)
BMI (<i>Continuous</i>)	0.93*	(0.88–0.98)			0.91*	(0.86–0.97)
Education Level:						
No Bachelor's degree	1.0	Referent			1.0	Referent
Bachelor's degree	1.02	(0.53–1.99)			1.06	(0.53–2.14)
Graduate degree	0.78	(0.38–1.63)			0.84	(0.38–1.86)
Soy milk intake:						
Never (0/month)			1.0	Referent	1.0	Referent
Once a month or more (1–29 per month)			1.29	(0.69–2.42)	0.90	(0.44–1.82)
Once a day or more (30+ per month)			0.64	(0.34–1.22)	0.43*	(0.21–0.89)
					<i>Trend</i>	<i>P = 0.04</i>
Dairy Consumption:						
Less than twice a week (0–7/month)			1.0	Referent	1.0	Referent
Twice a week or more (8–29/month)			0.65	(0.35–1.20)	0.55	(0.28–1.11)
Once a day or more (30+ /month)			0.48*	(0.25–0.92)	0.38*	(0.17–0.86)
					<i>Trend</i>	<i>P = 0.02</i>
Estrogen Usage:						
Past/never Users			1.0	Referent	1.0	Referent
Current Users			0.25	(0.12–0.52)	0.27	(0.13–0.56)
Smoking Status:						
Never smokers			1.0	Referent		
Ever smokers			1.53	(0.58–4.04)		
Self-reported Health Status:						
Excellent/Good			1.0	Referent		
Fair/Poor			2.40*	(1.11–5.17)		
History of Minor Accident Fractures:						

	Demographic Model ²	Candidate Variable Model ³	Final Model ⁴
No	1.0	Referent	
Yes	1.59	(0.87–2.89)	
Physical Activity Level:			
Level 1 (None)	1.0	Referent	
Level 2 (1–104 min/wk of Walk/Run/Jog)	0.82	(0.37–1.82)	
Level 3 (105–174 min/wk of Walk/Run/Jog)	0.69	(0.33–1.45)	
Level 4 (\geq 175 min/wk of Walk/Run/Jog)	0.99	(0.46–2.11)	
Vegetarian Status:			
Meat Consumers	1.0	Referent	1.0 Referent
Vegetarians (meat intake < 1x/month)	0.79	(0.45–1.39)	0.65 (0.34–1.23)
Soyfood (Tofu, Soy cheese) intake:			
Never (0/month)	1.0	Referent	
Less than twice a week (1–7 per month)	1.26	(0.64–2.47)	
Twice a week or more (8+ per month)	1.17	(0.59–2.30)	
Calcium Supplement:			
Never Users	1.0	Referent	
Use for less than 5 years	1.24	(0.66–2.30)	
Use for 5 years or more	0.91	(0.49–1.70)	
Vitamin D Supplement:			
Never Users	1.0	Referent	
Use for less than 5 years	2.03	(0.90–4.59)	
Use for 5 years or more	1.66	(0.62–4.45)	

¹ T-score ≥ -1.8 (value=0, n=237); T-score < -1.8 (value=1, n=100)

² Demographic Model: Osteoporotic T-score = age + BMI + Education

³ Candidate Variable Model: Osteoporotic T-score = age + BMI + Education + one exposure variable

⁴ Final Model (included only the variables that changed the effect of dairy or soy milk by at least 10%):

Osteoporotic T-score = age + BMI + education + estrogen use + dairy consumption + vegetarian status + soy milk intake

* P < 0.05

Table 4

Additional Multivariate Logistic Regression Model Examining the Associations between Individual dairy foods and soy milk intake and osteoporosis (T-score < -1.8)¹ adjusted for Selected Characteristics among 337 Postmenopausal Caucasian Women

	Demographic Model ²		Candidate Variable Model ³		Final Model ⁴	
	OR	95%CI			OR	95%CI
Age (<i>Continuous</i>)	1.08*	(1.05–1.11)			1.08*	(1.05–1.11)
BMI (<i>Continuous</i>)	0.93*	(0.88–0.98)			0.91*	(0.86–0.97)
Education Level:						
No Bachelor's degree	1.0	Referent	1.0	Referent	1.0	Referent
Bachelor's degree	1.02	(0.53–1.99)	1.29	(0.69–2.42)	0.96	(0.46–2.03)
Graduate degree	0.78	(0.38–1.63)	0.64	(0.34–1.22)	0.44*	(0.2–0.98)
Soy milk Intake:						
Never (0/month)			1.0	Referent	1.0	Referent
Once a month or more (1–29 per month)			1.29	(0.69–2.42)	0.96	(0.46–2.03)
Once a day or more (30+ per month)			0.64	(0.34–1.22)	0.44*	(0.2–0.98)
						<i>Trend p = 0.04</i>
Cheese Consumption:						
Less than once a week (1–3/month)			1.0	Referent	1.0	Referent
Once a week (4/month)			0.78	(0.38–1.59)	0.75	(0.34–1.66)
More than one times per week			0.30*	(0.1–0.65)	0.28*	(0.12–0.66)
						<i>Trend p = 0.004</i>
Milk Consumption:						
Less than once a week (1–3/month)			1.0	Referent	1.0	Referent
Less than one times per day (4–29/month)			1.06	(0.56–2.0)	1.01	(0.44–2.31)
Once a day or more (30+/month)			0.63	(0.29–1.39)	0.53	(0.20–1.44)
Yogurt/Ice Cream Consumption:						
None (0/month)			1.0	Referent	1.0	Referent
Once a week or less (1–4/month)			1.01	(0.58–1.75)	1.30	(0.69–2.44)
More than once a week (5+/month)			0.83	(0.33–2.0)	0.88	(0.32–2.44)
Vegetarian Status:						
Meat Consumers			1.0	Referent	1.0	Referent

	Demographic Model ²	Candidate Variable Model ³	Final Model ⁴
Vegetarians (meat intake < 1x/month)	0.79	(0.45–1.39)	0.64 (0.33–1.26)
Estrogen Usage:			
Past/never Users	1.0	Referent	1.0 Referent
Current Users	0.25*	(0.12–0.52)	1.09 (0.54–2.19)

¹ T-score ≥ -1.8 (value=0, n=237); T-score < -1.8 (value=1, n=100)

² Demographic Model: Osteoporotic T-score = age + BMI + education

³ Candidate Variable Model: Osteoporotic T-score = age + BMI + education + one exposure variable

⁴ Final Model (substituted dairy intake with cheese, milk, yogurt/ice cream):

Osteoporotic T-score = age + BMI + education + estrogen use + cheese consumption + milk consumption + yogurt/ice cream consumption + vegetarian status + soy milk intake

* P < 0.05