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Dairy Calcium Intake and Relationship to Bone Mineral Density (BMD), Bone Mineral Content (BMC) and Leptin in Post- Menopausal Women

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Authors' contributions

This work was carried out in collaboration between all authors. Author DHF did the data collection and manuscript revision. Authors WLB and RGN performed the statistical analysis and the first draft of the manuscript. Author TAD was the physician on site and screened all potential participants. Author ZRCM was the principle investigator, designed the study and wrote the protocol. Authors DHF and RGN managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Previous research has demonstrated that dairy calcium along with calorie restriction can contribute to weight loss while maintaining BMC and BMD. This study was a 3-month demonstration of a culturally sensitive program to evaluate the effects of dairy calcium.

Caloric intake was limited to 1400 kcal/d [≅ 92% of resting energy expenditure]. A total of 56 female subjects were randomized into two equal groups receiving either low dairy calcium ~800 mg/d or high dairy calcium ~1400 mg/d intake. The age and body mass index (BMI) at baseline for the low calcium group was 54.46±7.39 years, 32.5±6.6 kg/m^2 respectively; and the high calcium group was 56.75± 8.90 years, 33.5±5.8 kg/m^2 respectively. Differences after 3 months in weight, BMI, leptin, BMD and BMC were analyzed. Correlations were calculated between leptin and BMD (g/cm^2) or BMC (g) before and after intervention. After the intervention in the high calcium group there was an average reduction in weight -1.52±2.08 (kg), (P=0.001); BMI: -0.70±0.86 kg/m^2, (p<0.001); leptin: -1.18±5.10 ng/ml, (P =0.231) BMC: -0.009±1.41, (p=0.975) and BMD: 0.001±.017, (p=0.684). Despite a greater reduction in leptin levels in the low calcium group, changes in all parameters were not different from changes in the low calcium group with an

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average reduction in weight of -1.93±3.04 (kg), (p=0.002); BMI: -0.74±1.2 kg/m^2, (P=0.002); leptin: -2.58±8.38 ng/ml, (P=0.114), BMC: 0.038±1.38, (P=0.887) and BMD: <0.001±.022, (P=0.912). The decrease in leptin level was not correlated with BMD and BMC in both intervention groups (all P>0.05). We observed a significant treatment effect only for leptin where the low calcium group had a bigger reduction compared to the high calcium group. There was no significant correlation between the change in leptin, BMC and BMD.

Keywords: Dairy calcium; post-menopausal women; bone mineral content; bone mineral density.

ABBREVIATIONS

BMC : Bone Mineral Content
BMD : Bone Mineral Density
BMI : Body Mass Index
Ca : Calcium
CHO : Carbohydrate
CV : Coefficients of Variance
DXA : Dual X-ray Absorptiometry
DS-2 : Dairy 2 Servings
DS-4 : Dairy 4 Servings
Kcal : Kilocalories
Kg : Kilograms
NPQ : Nutrition Profile Questionnaire
PAQ : Physical Activity Questionnaire

1. INTRODUCTION

As women age, they tend to lose bone mineral density (BMD) and bone mineral content (BMC); furthermore, weight loss also adds to the burden of BMD and BMC loss [1-5]. Other investigators have studied calcium supplementation with other variables such as: age, sex, and weight at baseline and have suggested that "dairy calcium augmentation should be recommended as a strategic option in helping prevent early postmenopausal bone loss" [6]. Reid et al. [7] demonstrated that even a low increase in calcium intake less than 400 mg per day resulted in a reduction in bone loss among women in late menopause. A study by Wu et al. [8] that analyzed quantitatively the efficacy and factors associated with calcium intake on bone mineral density in post-menopausal women concluded that calcium can postpone lowering BMD levels and delays the onset time. The study concluded that dose-response and age were factors. However, the effects of higher calcium intake, 1000 mg or more per day, on postmenopausal women have yet to be studied [7]. We hypothesized that increasing dairy in one's diet will help obese and/or overweight postmenopausal women lose weight and contribute to BMC and BMD maintenance because dairy products contain both protein and more importantly calcium which is vital for bone health and facilitate appetite control [9]. This study was done because; "the effectiveness of calcium in retarding bone loss in postmenopausal women is unclear" [10]. We examined the relationship between dairy calcium intake and BMD, BMC and leptin levels in a dietary intervention study. The aim of this study was to examine the effect of two levels of dairy calcium intake on changes in BMD, BMC, and leptin (a hormone that regulates appetite control) levels during weight loss.

2. METHODS

2.1 Study Design

This was a three-month prospective demonstration study on the effects of dairy calcium combined with a calorie restricted diet on overweight and obese postmenopausal women. The study by Cordero-MacIntyre et al. [11] was used to calculate the power and percentage of estimated weight loss in the current study.

The diet was restricted to an energy intake of 1400 kcal/d. The 1400 value was chosen to be an approximation. The estimate of 92% of the average resting energy expenditure (R.E.E.) for this study group was based on the Harris Benedict equation for women [12]: [R.E.E. = 655.1 + (9.563*kg) + (1.85*cm) – (4.676*age)]. The study population was randomized into two groups receiving either a low calcium intake (~800 mg/d which is equivalent to 1-2 servings of dairy products a day) or to high calcium intake (~1400 mg/d which was equivalent to 3-4 servings of dairy products a day). Body weight, leptin, BMC, BMD, height, weight, waist and hip circumference measurements, fasting blood samples were measured both at baseline and at three months where subjects served as their own controls. Dual X-ray absorptiometry (DXA) was used to measure BMC and BMD. Differences after 3 months in weight, BMI, leptin, BMD and BMC were analyzed. BMC and BMD were assessed using Hologic Fan Beam DXA (QDR4500, software V8.26a). The study and all the procedures were approved by the Loma Linda University Institutional Review Board (IRB).

2.2 Subjects

Eighty-six postmenopausal overweight and obese women were recruited through mail and flyers that were distributed in health clinics, grocery stores, beauty parlors, churches, and post offices in the cities of Loma Linda and Riverside, CA. The research coordinator interviewed interested women and fully disclosed all aspects and expectations of our study. If the interviewed subjects agreed to the terms and conditions of the study they were required to sign an informed consent form. The subjects that met the criteria were invited to participate in the study. Prior to starting the study potential subjects went through initial screening both by telephone and in person by a physician in group meetings where investigators administered a questionnaire that would collect basic information on their past physical activity, diet, and medical history.

The inclusion criteria were as follows: Age (37-75) and either surgically or naturally postmenopausal) sex (female); BMI (>25 kg/m^2), stable diabetes status (known to have diabetes type II for no more than five years); stable medication for at least 3 months and dietary habits (omnivores and lacto-ovo-vegetarians).

Exclusion criteria included: Vegans; < 2 servings/day of dairy products, participation in any weight loss program, history of active alcohol and drug abuse, impaired mental condition, current glucocorticoid therapy use, history of hypersensitivity to dairy products present in the research diet, clinically relevant cardiovascular disease, pacemaker, hepatic, neurologic, endocrine; or other major systemic disease.

Two weeks prior to the dietary intervention subjects were instructed to discontinue any use of calcium supplements if any were being taken. Also, study subjects were given a detailed explanation of the study and any possible risks were disclosed through the informed consent process.

A meeting was held for all recruited subjects in which the intervention program was again explained in full detail. Subjects were consented and then evaluated by the study physician. After a complete screening of all volunteers, 56 obese and over-weight postmenopausal women were selected to participate in the study.

2.3 Diet

A total of fifty-six obese postmenopausal women were placed on a dairy calcium diet to reduce body weight while maintaining BMC and BMD during a 3 month intervention. Twenty- eight subjects were randomized to the high calcium diet (4 dairy servings (DS-4) per day) and 28 were randomized to the low calcium diet (2 dairy servings (DS-2) per day). Participants were randomized using a randomization table [13]. Starting randomly in the table each subject was assigned to either group (DS-4) or group (DS-2) depending on whether the random number was even or odd. Participants in the DS-4 group (~1400 mg/day of Ca) were instructed to consume the equivalent of four 8 oz. servings of plain yogurt and the DS-2 group (~800 mg/day of Ca) to consume an equivalent of two 8 oz. servings (each 350 mg of calcium) plain yogurt daily. The participants were allowed to have any non/low fat dairy products as long as they maintained the calcium requirements per day of this study per protocol.

All participants followed the dietary recommendations of the American Diabetes Association. The diet comprised of a daily intake of less than 30% of total calories from fat, about 50% to 55% total calories of carbohydrates, and about 15% to 20% of total calories from protein [14]. All subjects had complete support and guidance to be able to learn, apply and maintain their new diet in their daily life. Classes were held once a month during the intervention and subjects were encouraged to meet and support each other. The classes gave instruction on new eating, nutrition and habits, exercise regime, and stress management techniques. In addition, these classes offered subjects personal guidance from research assistants to answer any questions and/or concerns and to aid them in maintaining their new diet.

Dietary intake was measured by Nutrition Profile Questionnaires (NPQ), (Health Awareness Series, Wellsource Inc. of Clackamas, OR) [15], once at baseline and again at 3 months. Three 24-hr recalls were collected from the participants by trained individuals during the intervention (2 weekdays and one weekend during the 3 months). Calcium intake was assessed using the NPQ food frequency questionnaire that was administrated once at baseline and again at 3 months. Subjects also noted their daily dairy intake on forms provided, which included the serving size, type of dairy, flavor and amount. A booklet with most of the dairy products with amounts of calcium, calories, and fat was given to each volunteer to help them make correct choices and estimate their daily calcium intake with different dairy products. The coefficients of variance (CV) for the macronutrients and calcium consumed for all participants were % Energy Carbohydrates: 0. 171, % Energy Fat: 0.277, % Energy Protein: 0.231, and % Dairy Calcium: 0.621.

Physical Activity Questionnaires (PAQ), (Health Awareness Series, Wellsource Inc. of Clackamas, OR) [16], which volunteers were trained to fill out at the first interview, were completed once a month. The PAQ included questions regarding the type, frequency and intensity of the different forms of exercise. Examples of calorie restricted menus with DS-4 versus DS-2 were handed out to the participants on the meeting day when the registered dietitian explained how to reduce calories and include dairy.

2.4 DXA Protocol and Quality Control of DXA Data

Whole body and regional DXA scans were made with a QDR 4500-A densitometer (Hologic Inc., Waltham, MA). Certified technicians using standard subject positioning and data acquisition protocols carried out whole body scans. The scan time was approximately three minutes, and radiation exposure was 1.5 mrems (0.00015 sieverts). Follow-up scans were performed at 3 months using the standard protocol. The same trained technicians, using software version 8.24a, performed the analysis of the scans at baseline and again at three months [17].

2.5 Anthropometry

Anthropometric measurements including height, weight, and circumferences (waist, hip) were made to further characterize body shape and composition and muscle and fat distribution. All anthropometric measurements were made according to the Anthropometric Standardization Reference Manual [18]. Each of the measurements was taken three times by trained research assistants and these were then averaged. Trained individuals were assigned to carry out each of the anthropometric measurements. For each participant, the measurements at baseline and at 3 months were carried out by the same research assistant. The CVs for body composition measurements were as follows: weight= 0.207; BMI= 0.195; waist circumference= 0.154; trunk fat= 0.367; and total body fat= 0.305.

2.6 Plasma Leptin

Plasma leptin was measured using a competitive-binding radioimmunoassay (Linco Research Inc., St. Louis, MO). The mean leptin values (BMI ranges 18-25) in lean women is reported as 7.4 ± 3.7 µg/L.

2.7 Statistical Analyses

All data were entered into a computer master file and analyzed using SPSS (SPSS, Inc. Chicago, IL) for Windows. Double entry of data and cross-checking was carried out to assure error-free data. After 3 months intervention the differences in weight, BMI, leptin, BMD and BMC were analyzed and leptin changes were correlated (using Pierson's correlation method) with BMD (g/cm^2) and BMC (g) before and after intervention. Paired t-test were used to test changes within each group from baseline to 3 months where subjects served as their own controls. Independent t-test to test the difference in treatment effects (i.e. high and low calcium groups) during the intervention. Additionally, we calculated paired differences by subtracting variables at baseline from 3 months.

3. RESULTS AND DISCUSSION

We analyzed the general demographics as shown in Table 1 which included: race, education, and occupation. For race, 67.9% of the study population for both high and low calcium groups was white. In regards to education, 46.4% of the low calcium group and 35.7% of the high calcium group had a college degree or advanced degree and 42.9% of the low calcium group (DS-2) and 46.4% of the high calcium group (DS-4) had occupations in health care and medical services.

Additionally, we analyzed a detailed breakdown of baseline characteristics for high and low calcium cohorts. There was no significant difference in the distribution of most of the baseline variables (except weight) when comparing high and low calcium groups.

Table 2 compares mean values for weight, BMI and leptin levels both at baseline and 3 months after the intervention between groups with a high and low calcium intake. Both high and low calcium groups experienced reductions for all these variables. There were no significant differences comparing the high with the low calcium groups (i.e. the treatment effect) in the changes in weight, BMI, BMC and BMD as shown by the independent sample T-test for low vs. high calcium intake in Table 2.

As demonstrated in Table 3 after intervention the participants in both the high calcium group and in the low calcium group saw a statistically significant weight loss. We also saw a statistically significant loss in BMI in both high and low calcium groups.

There was no significant correlation between leptin, BMC and BMD. However as seen in Table 4 there was a slight positive correlation between BMI and BMD for both high and low calcium groups; the correlations are ($r=0.42; p=.048$) and ($r=0.53; p=0.005$) respectively.

We did a sensitivity analysis by looking at only those subjects who appeared to be poor responders (i.e. they either had a small weight gain or their weight did not change in the 3 months follow-up). For the DS-2 group (n=7), the changes (3 months – baseline) in BMD and BMC were: 0.007 (95% CI: -0.020, 0.033) and 0.477 (95% CI: -1.044, 1.998) respectively. Similarly for the DS-4 group (n=7), the changes in BMD and BMC were: 0.002 (95% CI: -0.014, 0.018) and -0.343 (95% CI: -1.735, 1.049) respectively.

Zemel [19] describes the mechanisms of the anti-obesity effect of dietary calcium. Increasing the level of calcium decreases 1,25 dihydroxy vitamin D or calcitrol and inhibits the lypolysis pathway which causes a decrease in adiposity. This triggers the initiation of fatty acid synthesis (FAS) transcription which increases de novo lipogenesis and creates new fatty acids which are excreted in the feces. Increasing calcium inhibiting mitochondrial uncoupling protein 2 (UCP2) genes in humans increases FAS [19]. This causes the core temperature to rise, thus releasing energy in the form of adenosine triphosphate (ATP). The exact mechanism for UCP2 action is not clear. Increasing calcium inhibits lipogenesis and decreases adiposity or fat and a decreasing calcium intake stimulates lipogenesis, increases calcitrol, and increases adiposity [19]. This mechanism explains the science behind how dairy calcium specifically assists in weight loss and why it should be used for weight management especially in obese populations.

Dairy calcium has been identified as a weight loss aid. Diets high in dairy have helped in reducing total body fat [20] as demonstrated in the mechanism of the anti-obesity effect of dairy calcium. Other's have shown that dairy calcium increases amounts of fat and calories that is expelled from the body [20-23]. Bendsen et al. [22] have stated the rate of excretion for "saturated, monounsaturated and poly-unsaturated fats increase significantly with high calcium intakes". This trend might be due to high amounts of calcium that create calcium soap and binding of bile acid in the intestine. Furthermore, dairy calcium has shown greater success in weight loss than supplemental calcium. This outcome may be attributable to the bioactive mechanisms in dairy that increase metabolism and fat loss [23]. In addition, according to Zemel et al. [19], "high calcium diets reduced lipogenesis by 51% and stimulated lipolysis three- to fivefold, resulting in 26% to 39% reductions in body weight and adipose tissue mass" in a six week study done on mice. The studies mentioned above [19-23] document that dairy calcium aids in weight loss.

The present study shows that an increase in dairy calcium in the diet in combination with a calorie restriction (i.e. 1400 kcal/day) has helped these overweight and obese women lose weight, reduce BMI, and leptin levels while simultaneously maintaining BMD and BMC. These statistically significant positive outcomes are due to the effects of dairy calcium incorporated in the women's diet. Bone loss can be prevalent in postmenopausal women because of the decrease in estrogen levels [24]. The loss of bone mineral is usually insidious causing a delay in diagnosis [25,26]. To date, calcium intake is a universal method to reduce bone loss with age, although the daily dosage is still being debated [9,27]. The main reason may be the differences in dietary calcium intake or racial differences in calcium requirement [8]. Also, that the recommendations of western countries were divided between high dosage and low dosage, although they have similar ethnic and dietary characteristic [8]. These disputes are mainly from a lesser or unclear understanding in the "dose-response and time course of calcium intake" [28].

A study by Riedt et al. [29] showed that when overweight postmenopausal women on caloric restriction and a daily dose of 1g of Ca/day still lost bone. Although increasing the intake to 1.7g Ca/day during caloric restriction diminishes the bone loss extent to that of normal age- related loss.

Studies such as the ones done by Jacobsen, Bendson, and Zemel [19-23,30] have demonstrated similar outcomes and have even established the association of higher intake of dairy calcium with increased weight loss. One dairy study in particular found that diets with obese subjects that had three or more servings of dairy products per day had significant reductions in adipose tissue mass without any calorie restrictions and even faster weight and body fat loss with a restricted calorie diet compared with diets with little or no dairy products [23]. Another study also supported the hypothesis that dairy calcium helps obese adults lose weight especially with calorie restriction [20]. These studies had similar out comes and support the findings we had in our dairy diet intervention. This study and the studies done by Zemel [19,20,23,30] show a positive correlation with increase dairy calcium intake and weight loss; in particular when on a restricted calorie diet.

Although there are various dairy studies [19-23,30] that claim that there is a positive relationship between calcium intake and weight loss, there are still some studies, like the one done by Venti et al. [31], that conclude that the association between dairy calcium intake and weight management in obese and overweight populations remain unclear. A study by Vergnaud [32] suggests that weight loss with dairy calcium is dependent on sex, baseline weight, and type of dairy consumed. In the same study, there was a negative association between dairy intake and anthropometric changes seen in overweight men. This fact may imply that factors other than dairy product components, such as calorie restriction, may explain weight loss associations seen in other dairy studies [32]; however, these components have not yet been adequately studied. Despite our efforts to demonstrate that there is a positive correlation between an increase dairy calcium intake and decrease in weight, there are some studies that oppose this theory and believe that there may be confounders such as sex, baseline weight, and type of dairy consumed that account for weight loss seen when consuming dairy products [30]. However other studies, like the ones done by Venti et al. [30], have not observed this clear inverse relationship between dairy calcium intake and weight loss.

This study showed that caloric restriction and calcium intake in obese postmenopausal women showed a positive correlation and the maintenance of BMD and BMC in this population. Although with increasing the intake to 1.7g Ca/day during caloric restriction diminishes the bone loss extent to that of normal age- related loss [29].

Table 1. Demographics of study participants

Variable	DS-2[a] Group (%) n=28	DS-4[a] Group (%) n=28	P-value[1]
Age (yrs)			
<50	5 (17.9)	5 (17.9)	
50-59	19 (67.9)	14 (50.0)	0.26
60+	4 (14.3)	9 (32.1)	
Race			
White	19 (67.9)	19 (67.9)	
Hispanic	4 (14.3)	4 (14.3)	0.92
Black	3 (10.7)	4 (14.3)	
All others	2 (7.1)	1 (3.6)	
Education			
High School or less	4 (14.3)	4 (14.3)	
Some College/Trade School	11 (39.3)	14 (50.0)	0.69
Bachelors, Masters, Doctorate	13 (46.4)	10 (35.7)	
Occupation			
Trade/Finance	3 (10.7)	3 (10.7)	
Health Care/Medical	12 (42.9)	13 (46.4)	0.99

Variable	DS-2[a] Group (%) n=28	DS-4[a] Group (%) n=28	P-value[1]
Services (Secretary, Operator, Hair Stylist)	8 (28.6)	7 (25.0)	
Other (Retired, Homemaker)	5 (17.9)	5 (17.9)	
Hormone Replacement Therapy *			
Yes	19 (67.9)	18 (64.3)	0.78
No	9 (32.1)	10 (35.7)	
Diet			
Lacto-ovo-vegetarian	3 (10.7)	5 (17.9)	0.45
Non-vegetarian	25 (89.3)	23 (82.1)	

** Herbal over the counter hormone replacement therapy.*
[1] P-values were created using the chi-square statistics
[a] DS-2 (2 dairy servings/day); DS-4 (4 dairy servings/day)

Table 2. DS-2 vs. DS-4 intake and treatment effect

Subjects	Weight (kg)	BMI (kg/m^2)	Leptin (ng/ml)	BMC (g)	BMD (g/cm^2)
DS-4[a] (n=28)					
Baseline	87.7±16.0	33.5±5.8	32.5±9.9	60.3±13.3	1.07±.17
3 Months	86.2±15.6	32.8±5.7	31.3±9.6	60.3±12.9	1.07±.18
P-value[1]	(0.001)	(<0.001)	(0.231)	(0.975)	(0.684)
DS-2[a] (n=28)					
Baseline	86.4± 18.9	32.5±6.6	27.8±9.9	58.0±12.2	1.05±.17
3 Months	84.4±19.6	31.8± 6.9	25.2±11.1	58.0±12.0	1.05±.17
P-value[1]	(0.002)	(0.002)	(0.114)	(0.887)	(0.912)
Treatment effects (n=56)	-0.41±.70	-0.05±.28	-1.4±1.85	0.05±.40	0.00±.01
P-value[2]	(0.56)	(0.86)	(0.45)	(0.90)	(0.86)

[a] DS-2 (2 dairy servings/day); DS-4 (4 servings servings/day)
[1] P-value based on paired samples T-test
[2] P-value based on independent T-test
Mean ± SD

Table 3. Paired differences between baseline and three months of the dairy diet intervention study, for DS-4 and DS-2 groups

	Paired differences[a]	Mean± SD	95% CI		P-value
DS-4[b] (n=28)	Weight (kg)	-1.52±2.08	-2.34,	-0.695	0.001
	BMI (kg/cm^2)	-0.695±.858	-1.03,	-0.355	<0.001
	Leptin (ng/ml)	-1.18±5.10	-3.16,	0.797	0.231
	Total Estimated BMC (g)	-0.009±1.41	-0.606,	0.587	0.975
	Total BMD (g/cm^2)	0.001±.017	-0.006,	0.009	0.684
DS-2[b]	Weight (kg)	-1.93±3.04	-3.11,	-0.749	0.002
	BMI (kg/cm^2)	-0.744±1.17	-1.20,	-0.290	0.002
	Leptin (ng/ml)	-2.58±8.38	-5.83,	0.666	0.114
	Total Estimated BMC (g)	0.038±1.38	-0.506,	0.582	0.887
	Total BMD (g/cm^2)	<0.001±.022	-0.008,	0.009	0.912

[a] Paired differences = variable at 3 months -variable at baseline
[b] DS-2 (2 dairy servings/day); DS-4 (4 dairy servings/day)

Table 4. Pearson's correlation between baseline and 3 months (P-value) between BMI, weight, BMC, and BMD in postmenopausal women in the dairy study for high and low calcium groups

		BMC (g)	BMD (g/ cm^2)
DS-4[a] (n=28)	BMI (kg/m²)	0.386 (0.069)	0.416* (0.048)
	Weight (kg)	0.415* (0.049)	0.423* (0.044)
	Leptin (ng/ml)	0.259 (0.221)	0.186 (0.385)
DS-2[a] (n=28)	BMI (kg/m²)	0.224 (0.262)	0.526** (0.005)
	Weight (kg)	0.402* (0.038)	0.492* (0.009)
	Leptin (ng/ml)	-0.011 (0.957)	0.224 (0.261)

*** P<0.01 level (2-tailed)*
** P<0.05 level (2-tailed)*
[a] DS-2 (2 dairy servings/day); DS-4 (4 dairy servings/day)

4. CONCLUSION

After the intervention, there was significant reduction in weight, BMI, and leptin in both intervention groups and a non-significant increase in BMC and BMD. There was no correlation between leptin, BMC and BMD. We should take note that this study had a limited sample size and short follow-up period. Nonetheless, based on these findings, we would suggest, that since postmenopausal women have age related bone loss, in addition to a restricted calorie diet (i.e. 1400 kcal/day), the inclusion of increased ≥ 4 servings of low fat dairy to one's diet for weight management, weight loss and to maintain BMD and BMC in this population.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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