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The Effect of Dairy Intake with Caloric Restriction on Bone Mineral Density and Lipids in Overweight/Obese 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 NM performed the statistical analysis and the first draft of the manuscript. Author TAD was the physician on site and screened all potential participants. Author ZCM was the principle investigator, designed the study and wrote the protocol. Authors DHF, WLB and NM managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Women in the post-menopausal stage of life are susceptible to a number of chronic health conditions related to obesity and osteoporosis. The objective of this study was to assess the association between lipids and bone mineral density (BMD) in overweight/obese postmenopausal women placed on a dairy calcium weight-reduction diet.

Methodology: A total of 56 overweight/obese postmenopausal women (mean age: 55.61±8.19; mean BMI: 32.95±6.12 kg/m^2; mean weight: 86.88±17.25 kg; and mean BMD level: 1.05±0.17 g/cm^2) were randomly assigned into a low dairy servings [DS-2] (800 mg/d of calcium or high diary servings [DS-4] (1400 mg/d of calcium) diet to evaluate differences in bone mineral density (BMD), body mass index (BMI) and lipid profiles (total cholesterol (TC), low-density lipoproteins cholesterol (LDL-C), high-density lipoproteins cholesterol (HDL-C), and triacylglycerol (TAG)) during a 3 month lifestyle education program.

Results: For the high calcium group, the change "Δ" in values at 3 months compared to baseline were: ΔBMD: 0.03 (p=0.31); ΔBMI: -0.69 (P=0.005); ΔLDL: -25.41 (p<0.001); ΔHDL: 3.49

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(p=0.365); ΔTC: -22.14 (p=0.004) and ΔTAG: -1.97 (p=0.998). In the low calcium group, the 3 month – baseline changes were: ΔBMD: -0.04 (p=0.69); ΔBMI: -0.74 (P=0.002); ΔLDL: -10.86 (p=0.314); ΔHDL: 3.99 (p=0.269); ΔTC: -5.96 (p=0.769) and ΔTAG: 4.53 (p=0.97). ΔBMD was correlated with ΔLDL and ΔTC: r=-0.27 (p=0.052) and r=-0.27 (p=0.054), respectively.

Conclusion: This study concludes that overweight/obese post-menopausal women who were placed on a dairy calcium weight-reduction diet during a 3 month educational program had lower in BMI, LDL, TC and higher HDL values.

Keywords: Diary calcium; postmenopausal; bone mineral density; lipid profiles.

ABBREVIATIONS

BMD : Bone Mineral Density
BMI : Body Mass Index
Ca : Calcium
DXA : Dual X-ray Absorptiometry
DS-2 : Dairy 2 Servings
DS-4 : Dairy 4 Servings
HDL : High Density Lipoproteins
LDL : Low Density Lipoproteins
NPQ : Nutrition Profile Questionnaire
TAG : Triacylglycerol
TC : Total Cholesterol

1. INTRODUCTION

Women in the post-menopausal stage of life are susceptible to a number of chronic health conditions related to obesity and osteoporosis as a result of estrogen deficiency triggered by the onset of menopause [1,2]. Women who lose a large part of the endogenous estrogen from the time of menopause to age 60 results in around 25% reduction of their bone mass leading to bone fractures [3]. An increased risk for obesity after the onset of menopause is linked to lipid abnormalities, such as elevated levels of plasma cholesterol, particularly LDL cholesterol and triglyceride concentrations. Dairy consumption has been hypothesized to increase weight loss, therefore reducing the risk of obesity, with several studies evaluating the effect of dairy calcium intake on adipose metabolism [4-13]. The thinning of bone tissue and loss of bone density over time as is seen in osteoporosis is the most common type of age-associated bone disease affecting women [2]. Interestingly, these diseases both share a genetic predisposition and a common precursor. Body weight is thought to be a positive predictor of bone mass, however, the pathophysiological relationship between obesity and bone remains controversial. Many studies have suggested a relationship between high levels of serum lipids and lower BMD when compared to people with normal lipid levels, although the results of this relationship is controversial [14-22]. In the present study, we examine the effect of caloric restriction with dairy calcium consumption on weight reduction and further effect on serum lipids and bone mineral density. Details of this lifestyle education program are detailed elsewhere [23-25].

2. EXPERIMENTAL METHODS

2.1 Study Design

Fifty-six overweight or obese postmenopausal women (BMI ≥ 25 kg/m²), with a mean age of 55.61±8.19 yr., mean weight of 86.88±17.25 kg, and mean body mass index (BMI) of 32.95 ± 6.12 kg/m^2 were enrolled in a three-month prospective study evaluating the effects of dairy calcium diet paired with caloric restriction. The study by Cordero-MacIntyre et al. [26] 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 approximately1400 kcal/d. 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 [27]: [R.E.E. = 655.1 + (9.563*kg) + (1.85*cm) – (4.676*age)]. The study population was randomized using randomized tables [28] into two groups receiving either a low dairy calcium intake [~800 mg/d which is equivalent to 2 servings of dairy products a day (DS-2)]; or to high dairy calcium intake [~1400 mg/d which was equivalent to 4 servings of dairy products a day (DS-4)].

Body weight, height, body mass index (BMI), BMD and lipids [total cholesterol (TC), high density lipoproteins (HDL), low density lipoproteins (LDL) and triacylglycerol (TAG)] were measured both at baseline and at three months where subjects served as their own controls. Height, weight, waist and hip circumference measurements, fasting blood samples and whole-body dual X-ray absorptiometry (DXA), which was used to measure BMD, were taken at baseline before beginning the intervention and at 3 months after

starting the diet intervention. Differences after 3 months in weight, BMI, BMD and lipids were analyzed. BMD was assessed using Hologic Fan Beam DXA (QDR4500, software V8.26a). The precision of DXA in its primary role of measuring BMD is excellent with a coefficient of variation of ≅ 1%. The study was approved by the Loma Linda University Institutional Review Board (IRB # 5100135).

2.2 Subjects

The estimated sample size of 40 in each group based on a presumed type I error of 5% and a power of 80% was ascertained to be sufficient to detect a statistically significant 7% weight reduction in the high calcium group compared to the low calcium group. A total of 86 subjects were initially recruited but was hindered due to drop-outs because of personal issues and non-compliance to the dietary program. Therefore, the study ended with a sample size of 56. All participants were recruited via mail requests and flyers that were distributed in health clinics, grocery stores, beauty parlors, churches, and post offices located throughout Loma Linda and Riverside, CA. The research coordinator interviewed interested women and fully disclosed all aspects and expectations of our study. If after the interview subjects agreed to the terms and conditions of the study, they were required to sign an informed consent form. The subjects that met the inclusion 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.

After initial screening selected volunteers were asked to complete a full physical exam by a physician and report their medical history prior to starting the diet intervention. 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. Qualified subjects were instructed to discontinue use of any calcium supplements two weeks before the beginning of the study. After a complete screening of all volunteers, 56 obese and over-weight postmenopausal women were selected to participate in the study.

2.3 Diet

The overweight/obese postmenopausal women were placed on a dairy calcium diet to reduce body weight while maintaining BMD during a 3 month intervention. Twenty- eight subjects were randomized to the high calcium diet (DS-4) and 28 were randomized to the low calcium diet (DS-2). 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 of calcium requirements per day of this study per protocol.

All participants followed the dietary recommendations of the American Diabetes Association. The diet was 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 [29]. 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) [30], 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. 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 [26].

2.5 Anthropometry

Anthropometric measurements including height and weight were made according to the Anthropometric Standardization Reference Manual [31]. Body weight was measured on a balance scale (Detecto, Web City, MO). Each of the measurements was taken three times by trained research assistants and these were then averaged. For each participant, the measurements at baseline and at 3 months were carried out by the same research assistant.

2.6 Plasma Lipids

Blood was drawn at baseline, and 3 months. At each time point after a 14 hour fast and postprandial in the morning (7-9 a.m.), blood was collected into a Vacutainer brand by standard venipuncture procedures performed by certified phlebotomists. Blood to measure lipoproteins was collected in a Vacutainer tube with anticoagulant EDTA (purple top). Blood was centrifuged at 2000 g x 10 minutes. Serum and plasma was aliquoted and transferred into –80 C for storage until assayed. Total cholesterol was assessed by an enzymatic method using Boehringer Mannheim standards and kits [32]. Cholesterol was measured enzymatically in a series of coupled reactions that hydrolyze cholesteryl esters and oxidize the 3-OH group of cholesterol. HDL cholesterol was measured in the supernatant after precipitation of apo B-containing lipoproteins. LDL-C was calculated using Friedewald's equation (LDL-C = TC – (HDL-C -TAG/5) [33]. Triacylglycerol (TAG) was determined using kits from Wako (Richmond, VA), in triplicate, using the enzymatic method which adjusts for free glycerol [34].

2.7 Statistical Analysis

Weight, height, body mass index (BMI), bone mineral density (BMD), and serum lipids (total cholesterol [TC], low-density lipoproteins [LDL], and high-density lipoproteins [HDL], and triacylglycerol [TAG]) were measured both at baseline and at three months. Repeated measures analysis of variance was conducted using a generalized least squares approach. Since there are only two measures per person (i.e. baseline and 3 months), we have assumed that the correlation structure among repeated measures is compound symmetry. Statistical models include: group, time as categorical, and (group x time) interaction. To examine if changes in each outcome were different between the two groups, a p-value for the (group x time) interaction is reported. The Tukey-Kramer post-hoc procedure was used to investigate if there was a significant change over time in each group. Spearman's rank correlations were used to assess the relationship between BMD, BMI and lipid profile (HDL, LDL, TAG, TC). Pearson product moment correlations did not differ appreciably from the Spearman's rank correlations. Multiple linear regression analysis was also performed to examine the strength of these relationships after adjusting for potential

confounders such as age and treatment group. A two-tailed P<0.05 was considered to indicate a significant difference. BMC and BMD were measured using Hologic Fan Beam DXA. All analyzes were done using both SPSS (IBM SPSS Inc., Ver 25, Armonk, NY) and SAS (Ver 9.4, SAS Institute, Inc., Cary, NC).

3. RESULTS

Demographic characteristics of the randomly selected 56 participants at baseline are summarized in Table 1 which shows the categorical distribution of the subjects' age, race, education level, occupation, hormone replacement therapy, and dietary preferences. The subjects served as their own control which maximizes control of unmeasured confounders since they are the same at baseline and at 3 months.

The physical characteristics at baseline (Table 2) are also shown as categorical distributions with a given mean and standard deviation per treatment (DS-4 and DS-2) and are as follows: BMD [g/cm^2] of 1.06±0.18 vs. 1.05±0.17; BMI [kg/m^2] of 33.36±5.75 vs. 32.54±6.55; LDL cholesterol [mg/dl] of 129.2±53.34 vs. 103.8±35.92 (p<0.05); HDL cholesterol [mg/dl] of 58.1±15.72 vs. 60.80±15.92;TAG [mg/dl] of 166.4±75.35 (p<0.05) vs. 122.9±64.07 (p<0.05); and TC [mg/dl] of 218.6±55.32 (p<0.05) vs. 189.2±35.79 (p<0.05). The complete lipoprotein profiles (HDL, LDL, TAG, TC) are based on ATPIII classification provided by the National Heart, Lung, and Blood Institute in accordance with the US Department of Health and National Institutes of Health [35]. Mean baseline measurements do not significantly differ between treatment groups for most of the variables, however, TAG, LDL and TC show a significant deviation of means between treatments.

For within group comparisons of 3 month values minus baseline (Δ) where subjects served as their own control (Table 3), the DS-4 group had significant results for: ΔBMI: -0.69 $\pm$ 0.86, (p<0.001), ΔLDL: -25.41 $\pm$ 33.00, (p=0.001), and ΔTC: -22.14 $\pm$ 39.80, p=0.007. There were no significant changes in BMD, HDL, or TAG. Similarly, the DS-2 group had significant ΔBMI: -0.74 $\pm$ 1.17, (p=0.002) and ΔLDL: -10.86 $\pm$ 27.19 (p=0.044). There were no significant changes in BMD, HDL, TC or TAG.

Table 1. Demographics of study participants

Variable	DS-2 group (%) n=28		DS-4 group (%) n=28		*p-value*
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
Services (Secretary, Operator, Hair Stylist)	8	(28.6)	7	(25.0)	
Other (Retired, Homemaker)	5	(17.9)	5	(17.9)	
Hormone Replacement Therapy [1]					
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)	

[1] *Over the counter herbal hormone replacement therapy; DS-4 = Dairy Supplement of 4 servings/day ~1400 mg/d; DS-2 = Dairy Supplement of 2 servings/day ~800 mg/d*

Table 2. Baseline characteristics of postmenopausal women

Variable	DS-2 (n=28) %	DS-4 (n=28) %
BMD (g/cm^2)		
0.662 - 0.946	25.0	25.0
0.950 – 1.040	25.0	25.0
1.041 – 1.169	25.0	25.0
1.170 – 1.488	25.0	25.0
BMI (kg/m^2)		
20 - 29	39.3	35.7
30 - 39	39.3	42.9
≥ 40	21.4	21.4
LDL Cholesterol (mg/dl)		
< 100	50.0	21.4
100 - 129	32.1	42.9
130 - 159	10.7	17.9
160 – 189	3.6	3.6
≥ 190	3.6	10.7
HDL Cholesterol (mg/dl)		
< 40	3.6	10.7
40 – 59	46.4	50.0
≥ 60	50.0	39.3
Total Cholesterol (mg/dl)		
< 200	64.3	46.4
200-239	28.6	35.7
≥ 240	7.1	17.9
Triacylglycerol (mg/dl)		
< 150	71.4	53.6
150 – 199	14.3	14.3
200 – 239	14.3	32.1
240 - 435	3.6	17.9

** P<0.05*

Table 3. Bone Mineral Density (BMD), Body Mass Index (BMI), low and high density cholesterol, total cholesterol and triacylglycerol for the difference between pre- and post-treatment for DS-4 (n=28) and DS-2 (n=28) groups

Subjects	BMD (g/cm^2)[1]	BMI (kg/m^2)[1]	LDL[1]	HDL[1]	Total Chol[1,4]	TAG[1,4]
DS-4[2]						
Baseline	1.06 ± 0.17	33.52 ± 5.79	129.21 ± 53.34	58.53 ± 15.87	218.64 ± 55.32	166.40 ± 77.35
3 Months	1.09 ± 0.25	32.82 ± 5.67	103.80 ± 42.02	62.02 ± 12.67	196.49 ± 45.10	164.43 ± 73.51
3 Mth - Baseline	0.03 ± 0.16	-0.69 ± 0.86	-25.41 ± 33.00	3.49 ± 10.61	-22.14 ± 39.80	-1.97 ± 55.54
p-value[6]	0.778	0.005	<0.001	0.365	0.004	0.998
DS-2[3]						
Baseline	1.05 ± 0.17	32.54 ± 6.55	103.82 ± 35.92	60.80 ± 15.92	189.19 ± 35.80	122.87 ± 64.07
3 Months	1.01 ± 0.25	31.79 ± 6.86	92.96 ± 30.11	64.79 ± 12.48	183.23 ± 32.24	127.40 ± 89.40
3 Mth - Baseline	-0.04 ± 0.19	-0.74 ± 1.17	-10.86 ± 27.19	3.99 ± 12.31	-5.96 ± 23.44	4.53 ± 56.37
p-value[6]	0.692	0.002	0.314	0.269	0.769	0.973
Treatment effect[5]						
p-value[6]	0.154	0.855	0.105	0.907	0.069	0.665

[1]Mean ± Standard Deviation; [2]DS-4: Dairy servings ≅ 1400 mg/day; [3]DS-2: Dairy servings ≅ 800 mg/day
[4]Chol = Cholesterol, TAG= Triacylglycerol; [5]Treatment effect = the addition of 2 extra servings of dairy calcium;
[6]p-values based on repeated measures ANOVA including the Tukey-Kramer Post-hoc procedure

No significant baseline to 3 month changes was observed when evaluating the treatment effect (Table 3). Variations in mean changes in comparing the DS-4 vs. DS-2 groups were as

follows: ΔBMD: showed a slight increase in DS-4 (mean = 0.03) and a slight decrease in the DS-2 (mean = -0.04), p=0.154; the changes in ΔBMI were similar for the DS-4 and DS-2 groups: -0.69 vs. -0.74 (p=0.855), respectively. The mean decreases in ΔLDL and ΔTC were larger for the DS-4 group as compared to the DS-2 group: -25.41 vs. -10.86, (p=0.105) and -22.14 vs. -5.96, (p=0.069), respectively. In addition, while the DS-4 group exhibited a slight decrease in ΔTAG with a mean of -1.97, the DS-2 group showed an increase with a mean of 4.53 (p=0.66564). The increase in ΔHDL was similar for both groups: 3.49 for DS-4 and 3.99 for DS-2 (p=0.907).

3.1 Correlation of Bone Mineral Density and Serum Lipid Parameters

Although ΔBMD did not correlate with ΔBMI, ΔHDL, and ΔTAG, a negative correlation was observed for ΔBMD with ΔLDL (p =0.052; r = -0.274) and TC (p =0.054; r = -0.271). ΔLDL showed a significant positive correlation with ΔTC (p< 0.001; r = 0.842), but not with any other parameter (Table 4).

The multivariate associations between 3-month change in BMD and baseline measurements of BMI and lipid parameters were evaluated with a linear regression model adjusting for age and treatment effect (Table 5). Our analysis revealed no significant association between ΔBMD and ΔBMI (r^2=0.04, p=0.585), and ΔBMD and serum lipids. The results show the regression coefficients and percent change in regression coefficients to evaluate treatment effect (i.e. DS-4 vs. DS-2). Although, BMI and serum lipids are not significant predictors of ΔBMD with less than 1% change in regression coefficients and low r^2 values, the highest percent change was observed for TAG at a 5.71% increase (r^2=0.0414, p=0.163).

Table 4. Spearman's rank correlation coefficients (p-value) for the Relation between BMD[1], BMI[1], and lipid parameters[1]

Variable	ΔBMD [g/cm^2]	ΔBMI [kg/m^2]	ΔHDL [mg/dL]	ΔLDL [mg/dL]	ΔTC [mg/dL]	ΔTAG [mg/dL]
ΔBMD [g/cm^2]	1.000	0.129 (0.370)	0.119 (0.402)	-0.274 (0.052)	-0.271 (0.054)	0.016 (0.912)
ΔBMI [kg/m^2]		1.000	-0.142 (0.311)	-0.097 (0.482)	-0.044 (0.749)	0.119 (0.391)
ΔHDL [mg/dL]			1.000	-235 (0.087)	0.078 (0.577)	-0.003 (0.981)
ΔLDL [mg/dL]				1.000	0.842** (<0.001)	-0.219 (0.108)
ΔTC [mg/dL]					1.000	0.144 (0.295)
ΔTAG [mg/dL]						1.000

*[1]Δ=3months-baseline; ** p<0.001*
BMD=Bone mineral density; BMI=Body mass index; HDL=High density lipoprotein; LDL=Low density lipoprotein; TC=Total cholesterol; TAG=Triglyceride

Table 5. Percent change in regression coefficients for treatment effect with ΔBMD as the dependent variable

Model	Regression coefficient for treatment	Percent change regression coefficient for treatment effect[7]	p-value for Treatment	p-value for added covariable
Reduced Model (RM)[1]	0.700	N/A	0.160	N/A
RM+BMI[2]	0.700	0% change	0.164	0.863
RM+TC[3]	0.070	0% change	0.181	0.991
RM+HDL[4]	0.070	0% change	0.163	0.882
RM+LDL[5]	0.069	-1.43% decrease	0.180	0.971
RM+TAG[6]	0.074	5.71% increase	0.153	0.745

[1] Reduced Model (RM): ΔBMD = β(age)+β(treatment), where Δ=3months-baseline;
[2-6]At Baseline; [7]Treatment: DS-4 versus DS-2
Note: None of the regression coefficients were significant

4. DISCUSSION

In our previous papers [23-25] more data were analyzed for the effects of dairy calcium on weight loss for overweight/obese postmenopausal women. For this paper we looked at the effects of dairy calcium weight-reduction diet on BMD, BMI and lipid profile, in addition to investigating whether BMI and serum lipids are reliable predictors of BMD in a sub-population of postmenopausal overweight/obese women. Although the synergistic effect of dairy calcium intake and energy restriction contributed to the overall reduction in weight, BMI, and lipid profile, markedly greater mean reduction in serum lipids, particularly LDL and TAG observed in the DS-4 treatment clearly supports the beneficial role for high doses of dietary calcium. These data are supported by a similar study evaluating dietary calcium modulation of adiposity with transgenic mice placed on high or low calcium intake coupled with high fat/sucrose diet. Results showed that the high calcium diets reduced lipogenesis by 51% and stimulated lipolysis causing a 26-39% reduction in body weight and adipose tissue mass [5]. In addition, the magnitude of these effects is attributable to the influence of dairy sources of calcium than that of calcium carbonate supplements. The effect of dairy products versus supplemental calcium can be supported by clinical studies and epidemiological data that have showed at least three daily servings of dairy products result in significant decline in body fat mass and weight in obese individuals in the presence or absence of secondary caloric restriction compared to low dairy diets [36].

We did not find statistically significant correlation between ΔBMD, ΔBMI and serum lipids (ΔTC, ΔLDL, ΔHDL, ΔTAG). However, we did observe a negative correlation between ΔBMD and ΔHDL and ΔBMD and ΔTC. Although several studies have suggested a relationship between serum lipids and BMD in postmenopausal women, studies have had many conflicting results. For example, the Hertfordshire Cohort Study, attempted to replicate a larger U.S. study relating lipid profile to BMD in a UK population and found BMD to be strongly related to serum triglyceride and HDL levels. However, these relationships weakened when adjusting for body fat percentage [37]. The inverse relationship between BMD with TC and LDL, with a good portion of our subjects in the borderline to high TC category, support epidemiological studies that have suggested that osteoporosis or decreased bone mass in women may be associated with higher levels of plasma lipids [18,38,39]. For example, a study of postmenopausal women aged 50-75 years by Tanko et al., showed that the greatest decreases in BMD were observed in those with higher cholesterol levels [15]. Another small study seeking to find a relationship between HDL and the presence of postmenopausal osteoporosis found a similar weak inverse relationship with significantly higher levels of HDL in osteoporotic patients than in the controls [38]. Although the mechanism to confirm this relationship needs further study, a possible explanation can be that of estrogen deprivation causing an elevated lipid profile in postmenopausal women. In contrast, a similar study evaluating the relationship between lipid profiles and BMD in a large (n=2,661) postmenopausal population of Korean women, showed a positive correlation between HDL and BMD after adjustment, indicating the positive effect of an anti-atherogenic lipid profile on bone formation [39]. The only significant positive correlation was observed between ΔLDL and ΔTC ($r= 0.842$, $p<0.001$). These results can be supported by a larger study by Makovey et al., where a sample of 273 postmenopausal women (156 on hormone replacement therapy (HRT) and 117 no HRT) showed that TC and HDL were also negatively associated with BMD, and included a significant interaction between TC/LDL [40]. When a multiple regression analysis was performed with the change in BMD as dependent variable and BMI and lipid parameters as independent variables, we could not identify a significant association between serum lipids and BMD. After adjusting for treatment effect and age, TC and all other variables showed a less than 1% influence on BMD. These results are similar to a recent study of 107 postmenopausal women aged 45-79, who had been postmenopausal for more than 12 months and had not used any medications which could affect the lipid profile or bone metabolism for at least 6 months before the their clinical evaluation [41]. The only model displaying the highest increase in percent change regression coefficient for treatment was ΔBMD= Age+Treatment+TAG, with TAG causing a 5.71% increase in BMD (or only a 0.004 increase in the regression coefficient). However, TAG was a highly non-significant addition to the regression model so could easily be associated with a chance observation.

4.1 Role of Intracellular Ca2+, Adipocytes and Osteoblasts

The first observed "anti-obesity" effect of dietary calcium was during a year-long clinical trial conducted by Zemel et al., (2000) which investigated the antihypertensive effect of dietary calcium in obese African Americans, where an increase in daily calcium intake resulted in an overall decrease of body fat. Intracellular Ca2+ plays a vital role in obesity and insulin resistance, and is stimulated in various cells by the recombinant agouti protein, an obesity gene product [4]. Agouti and/or Ca^{2+} channel activation also stimulates fatty acid synthase, which inhibits lipolysis in energy storing cells called adipocytes [4]. Furthermore, increases in circulating calcitrophic hormones [1,25-(OH)2-D and/or parathyroid hormone], taking place during low calcium diets, have been shown to stimulate intracellular and adipocyte Ca^{2+} influx, thereby increasing lipid storage [42]. Thus, further investigations led researchers to propose that increasing dietary calcium would suppress calcitrophic hormones and reduced intracellular adipocyte Ca^{2+} influx and lipid storage [5-13]. Another possible mode of effect by dietary calcium is via the gastrointestinal tract, where studies in humans have shown that large amounts of calcium supplementation in the normal diet resulted in increased fecal excretion of fat and saturated fatty acids [43,44]. For example, a study by Murata, et al., demonstrated that a combination of chocolate enriched with calcium and fatty acids in the digestive tract inhibited the absorption of the saturated fatty acids and resulted in higher fecal fat excretion than did in the control group without calcium supplementation [43]. However, these results do not evaluate the effect of high-fat diets, which may interfere with intestinal calcium absorption and consequent effect of calcium on fatty acid fecal excretion. These data, demonstrating the influence of dietary manipulation on adipose tissue energy storage, lend further support to the proposed association between fat and bone mass given that adipocytes and osteoblasts (bone forming cells) are derived from common bone marrow stromal (skeletal) stem cells (MSC). In fact, the majority of conditions associated with bone loss, such as aging and osteoporosis, are accompanied by an increase in bone marrow adiposity due to the shifting of balance between osteoblast and adipocyte differentiation in the MSCs [45]. Although the mechanism in which obesity may affect bone metabolism is not yet fully understood, it's possible that obesity increases adipocyte differentiation while decreasing osteoblast and consequent bone formation. Another link between adipose tissue and bone density involves circulating pro-inflammatory cytokines present in obesity, which promote osteoclast (bone resorption cells) activity and bone resorption by activating receptor of NF-kB ligand [RANKL] and osteoprotegerin [OPG] [46]. Adipocytes can secrete pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6 that trigger osteoclast activity via regulation of RANKL/OPG pathway [46,47]. Finally, although previously mentioned studies with high calcium intake showed an inverse relationship with fat mass, including fatty acid excretion, a high fat diet may inhibit intestinal calcium absorption by forming insoluble calcium soaps, thereby decreasing healthy bone formation [47]. The relationship between serum lipids, which eventually convert into adipose tissue, and bone metabolism need further exploration, but the accumulation of data suggests an inverse relationship between fat and bone mass.

The mevalonate pathway has also been shown to have an effect on bone health. The pathways key role is in multiple cellular processes by synthesizing sterols such as cholesterol and non-sterols [48]. Many studies have shown its effect on bone metabolism because it stimulates osteoblast differentiation and the inhibition of osteoclast development [49-52].

The main limitation of this study was the sample size because of the high rate of subject drop outs. Withdrawal rates of this magnitude are not unusual in studies that require a change in diet composition and quantity. We initiated the study with 86 subjects, but 30 subjects withdrew from the study citing inability to follow the prescribed diet for their group assignment or for varied personal reasons. The demographics of this withdrawal group were not different in general from the subjects completing the study: (age distribution ($p=0.41$); race/ethnicity ($p=0.70$); education ($p=0.50$); occupation ($p=0.06$) [dropouts tended to be more in the "services" or "other" categories]; HRT ($p=0.95$); diet ($p=0.11$); BMI ($p=0.12$). However, with this limitation, our findings in this overweight/obese post-menopausal population of women suggest an association between bone mineral density and lipid profile.

The relationship between dairy calcium intake on lipid profiles, BMD and weight loss is

controversial, therefore, more research is needed in this area. This study explores the effect of dairy intake on bone mineral density and lipids in overweight/obese post-menopausal women.

5. CONCLUSION

Following the dairy calcium diet along with caloric restriction there was significant reduction in BMI, LDL, TC (only in the DS-4 group), with an additional beneficial increase (albeit non-significant) in HDL. These results demonstrate the presence of an additional effect of the dairy calcium diet on weight management.

Although we observed a positive correlation between ΔLDL and ΔTC, and a negative correlation for ΔBMD with ΔLDL and ΔTC, we conclude that our data suggest an association between bone mineral density and lipid profile in postmenopausal overweight and obese women. Further research and analysis using larger sample sizes and longer follow-up periods are needed to clarify the relationship between an atherogenic lipid profile and bone mineral density.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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