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DIET, WEIGHT, CYTOKINES AND BONE HEALTH IN POSTMENOPAUSAL WOMEN

C.A. GUNN, J.L. WEBER, M.C. KRUGER

Institute of Food, Nutrition and Human Health, Massey University, Private Bag 11222, Palmerston North 4442, New Zealand. Corresponding author: C.A. Gunn, Institute of Food, Nutrition and Human Health, Massey University, Private Bag 11222, Palmerston North 4442, New Zealand, c.a.gunn@massey.ac.nz

Abstract: *Objectives:* To investigate diet and nutrition-related factors associated with bone loss in a group of postmenopausal (PM) women. Nutritional intake, inflammatory markers and body composition (weight, body mass index, fat/lean mass) were analysed for associations with bone mineral density (BMD). *Design:* A cross sectional study examining correlations between BMD (Duel-energy X ray absorptiometry; (DXA) and dietary intake (3-day diaries), body composition and plasma bone and inflammatory markers: C-terminal telopeptide of type I collagen (CTX) and procollagen type I N propeptide (P1NP), C- reactive protein (CRP), interleukin 6 and 10 (IL-6, IL-10), tumour necrosis factor (TNF) and osteoprotegerin (OPG). *Setting:* Community dwelling women from the Auckland, Hawke's Bay and Manawatu regions in New Zealand. *Participants:* 142 healthy, PM women aged 50-70 years. *Results:* OPG (per kilogram fat mass) was increased in women with osteoporosis ($p<0.001$) compared to groups classified with normal BMD and osteopenia. Protein, vitamin B12, zinc, potassium and dairy intake were all positively correlated with higher BMD while dairy and potassium intakes also inversely correlated with CTX. Body composition (weight, BMI and fat/lean mass) had strong positive associations with BMD. Multiple regression analysis showed body weight, potassium and dairy intake were predictors of increased BMD in PM women and explained 39% ($r^2=0.39$, $p< 0.003$) of variance. *Conclusion:* BMD was negatively correlated with OPG and positively with weight, dairy and potassium intake. This study highlights the importance of maintaining adequate body weight and emphasising dairy and potassium predominantly sourced from fruit/vegetables to reduce bone loss at midlife.

Key words: Bone mineral density (BMD), osteoprotegerin (OPG), fruit/vegetables, potassium, dairy, weight.

Introduction

Osteoporosis has emerged as a leading public health issue in New Zealand (1) and the rest of the world health (2, 3). Current estimates in 2013, are for over 100,000 osteoporosis related fractures in NZ, with females over 50 years of age accounting for two thirds (4). Bone loss accelerates at menopause therefore determining modifiable influences is imperative. Nutrition has a direct influence on bone (5-7), also the immune system and levels of inflammation (8-10) and the protective influence of weight including fat mass on bone density is central to bone health in PM women (11-13).

Diet and exercise may account for up to 25% of variation in bone mineral density (BMD) (14) with important nutritional contributions attributed to adequate protein (15-17) calcium and dairy intake (18, 19). Long chain polyunsaturated fatty acids contribute to increased bone mass and reduced resorptive activity (20, 21) while fruit/vegetable's contribution to bone health is due to their micronutrients (6, 22), phytochemicals (23-26) and bicarbonate precursors which lower the renal acid load (27, 28).

Osteoporosis and low bone mineral density (BMD) have traditionally been referred to as a disease of low body mass index (BMI) and body fat composition (11, 13) and in older adults, low body weight is an established risk factor (29). There is also, however, agreement that increasing body fat levels contribute to rising levels of adipokines and other inflammatory cytokines (12). Bone metabolism and immune regulation is now viewed as intertwined due to the numbers of immune cells (macrophages, T and B cells) increasing in obesity and influencing bone (30). Increased levels of inflammatory cytokines emanating from immune cells in fat tissue contribute to inflammatory bone loss therefore, obesity may also be a negative determinant of bone health (31, 32) with abdominal (29) or visceral fat particularly implicated (29, 33-35).

While older New Zealand women (51-70 years) are known for consuming a better diet (more fruit/vegetables) than other groups (36, 37) there has been no study assessing the combined effects of dietary composition and renal acid load, weight (BMI, fat and lean mass) and levels of inflammatory cytokines with bone status in this group. The aim of the present study was therefore to explore the relationship between these factors and bone health in a group of healthy, postmenopausal (PM) New Zealand women.

Methods

Study population

Ethical approval was obtained from Massey University Human Research Ethics Committee (Southern A) Reference number 11/11. All participants were fully informed of the study requirements and gave written informed consent. The trial was registered with the Australian and New Zealand Clinical Trials Registry (ANZCTR) http://www.ANZCTR.org.au. Trial Registration: ACTRN 12611000763943.

The study commenced in August 2011, with 142 women

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between 50-70 years, who were at least 5 years postmenopausal and who had volunteered for a dietary intervention (The Scarborough Fair study). The study was conducted by Massey University, New Zealand and women were recruited from 3 regions: Auckland, Palmerston North and Hawke's Bay by advertisement in local papers. Exclusion criteria included any known significant health condition or regular use of medication which could affect bone or inflammation including HRT, NSAID's and proton pump inhibitors. Results presented here represent the baseline data obtained on bone turnover and inflammatory markers, dietary intake and bone mineral status determined by dual-energy X ray absorptiometry (DXA).

Plasma markers

The plasma markers included: bone markers of resorption, C-terminal telopeptide of type I collagen (CTX) and formation, procollagen type I N propeptide (P1NP) and inflammatory markers: C- reactive protein (CRP), interleukin 6 and 10 (IL-6, IL-10), tumour necrosis factor (TNF) and osteoprotegerin (OPG). In short, overnight fasted blood samples were taken between 8-10am, immediately centrifuged at 3000 rpm, separated and stored at -80ºC until bone markers were analysed at Canterbury Health Endocrine Laboratory (Roche Elecsys 2010.Roche Diagnostics) and inflammatory markers at Plant and Food Research Auckland (FlowCytomix kits (Bender Medsystems, Austria). Inflammatory markers are expressed per kilogram fat mass as production is correlated with increasing fat mass (12).

Dual-energy X ray absorptiometry (DXA)

DXA of lumbar spine (L1-L4) and hip (total and femoral neck) was performed using a Hologic QDR-Discovery A densitometer (Hologic Inc, Bedford, Mass., USA) giving measures of bone mineral content (BMC)(grams), BMD (grams/cm^2), T and Z scores, body fat and lean measures (android and gynoid). The machines were calibrated daily with in vivo reproducibility of coefficient of variation 0.45-0.54% for all measured sites.

DXA classification

The groups DXA classification was based on the W.H.O classification (38-40), however, in this study, the osteopenic group was further divided into mild or significant osteopenia according to T-score as below.

Normal: A value for BMD that is higher than 1 SD below the young adult female reference mean (T-score greater than or equal to – 1 SD).

Mild osteopenia: A value for BMD 1 SD or more below the young female adult mean (T-score < – 1 and > – 1.5 SD).

Significant osteopenia: A value for BMD 1.5 SD or more below the young female adult mean (T-score < – 1.5 and > – 2.5 SD).

Osteoporosis: A value for BMD 2.5 SD or more below the young female adult mean (T-score less than or equal to – 2.5 SD).

The division into mild and significant osteopenia in women in this age range (50-70 years) was based on the specialist radiologist's recommendations for referral. Women with normal BMD or mild osteopenia were not referred for follow-up whereas those with significant osteopenia and osteoporosis were.

Diet

Dietary intake data was assessed from 3 Day Diet Diaries (3DDDs). These were considered more reliable than a food frequency questionnaire for monitoring short term change in dietary habits as well as assessing potential renal acid load (PRAL). Participants received verbal and written instructions on how to complete and examples of standard portion sizes were listed. All food and beverages consumed over 2 weekdays and 1 weekend day were recorded along with information on brands and amounts along with recipes for homemade dishes. A registered nutritionist reviewed the diary with each participant, using prompting for specific food types and a food display demonstrating portion sizes of both raw and cooked food to ascertain quantities and servings. Data was entered into Foodworks (version 9, Xyris NZ) and compared with New Zealand dietary reference values (41). A random subset of 20 diaries were analysed to determine the main food sources of potassium. PRAL and net endogenous acid production (NEAP) was determined according to Remer and Manz (42).

PRAL (mEq/d) = 0.49 protein (g/d) + 0.037 × phosphorous (mg/d) - 0.021 × potassium (mg/d) – 0.026 × magnesium (mg/day) - 0.013 × calcium (mg/day).

NEAP = Potential Renal Acid Load (PRAL) + Organic Acid (OA)

Anthropometric data

Body weight was measured to the nearest 0.5 kg with subjects in light clothing without shoes using digital scales (UWE Gilbarco, NZ). Body height was measured without shoes to the nearest 0.5 cm using a stadiometer (SECA 213). BMI was calculated as weight (kg)/height (m^2). Hip and waist circumference were measured by trained nutrition staff using standardised procedures and equipment (Tape measure, Douglas Pharmaceuticals Limited, Auckland).

Statistical analyses

The Statistical Package for Social Sciences version 20.0 (SPSS Inc. Chicago, IL, USA) was used for all analysis with a significance level of 0.05 (2 tailed). Data was checked for distribution and if normally distributed was expressed as means and standard deviations (SD), or if not normally distributed, as both means (SD) and medians (interquartile range) for comparison purposes. Log transformations of non-normally distributed data were used for correlations and models. Pearson and partial correlations were used to determine associations between nutritional indices (servings, macro and micronutrients

and PRAL/NEAP), weight, BMI and soft tissue indices, inflammatory and bone markers and BMD. Potential differences among groups were evaluated with one way analysis of variance. Regression analyses were used to determine the influence of single predictor variables (nutrients, inflammatory markers and body composition variables (fat mass, lean mass, body fat percentage, android and gynoid fat percentages) on BMD (spine, hip and femoral neck of hip). We used a multiple regression model to assess the simultaneous effects of soft tissue indices, inflammatory markers and nutrients shown in the Pearson's correlation to have a significant effect on bone mineral density. Coefficients of variation were used to check the validity of the regression model. Nutrients were energy adjusted and multicollinearity avoided by not including variables such as weight and fat/lean mass together or nutrients protein and vitamin B12 in the model. Variance inflation factors with values above 2 were used to indicate problems with the model.

Results

The majority of the 142 women recruited were European (98%) with 2% Maori or Pacific Islander. This is not representative of the population (68% European, 15% Maori, 7% Pacific Islander) but reflects the difficulty in recruiting older indigenous women without a significant health issue. Mean BMI was 25.7, age 60.4 years and 11 years since menopause (YSM). Nearly half the women had normal BMD (<1 SD below normal) (36%) or were mildly osteopenic (1.0-1.49 SD below normal) (15%) while the other half were either significantly osteopenic (1.5-2.49 SD below normal) (37%) or osteoporotic (≥2.5 SD below normal) (12%). The older women in this study had higher bone resorption and lower bone formation markers but no significant differences were seen in bone density or body composition between the two age groups 50-60 years and 61-70 years. 50% of women had normal BMI, 27% were overweight and 23% were classified as obese (Table 1).

Reported dietary intake was similar between all groups of women (Table 2).

The women's diets met or exceeded New Zealand estimated average requirement (EAR) or adequate intake (AI) for most nutrients except calcium. Sodium intake exceeded the suggested dietary target (SDT) while potassium, folate and fibre intake didn't reach the SDT. All groups met the 5-plus/day recommendations servings of fruit/vegetables and had adequate meat/protein intake (1.9 servings/day), however intakes of breads/cereals (4.4 servings) and dairy (1.6 servings) were lower than recommended (43). A significant difference was noted between the groups for dairy consumption with group 1 (normal BMD) having the highest consumption (1.9 serves). No significant differences were seen in estimated

Table 1
Bone markers and bone mineral density of postmenopausal study women (n=142)

	Groups				
	1 Normal BMD (n=51)	**2 Mild Osteopenia (n=21)**	**3 Significant Osteopenia (n=53)**	**4 Osteoporotic (n=17)**	**P <**
Age (yrs)	60 (5)	63(4)	60(4)	61(4)	0.04
CTX base ug/L	0.34(0.11)	0.39(0.23)	0.40(0.15)	0.51(0.16)	0.001
P1NP base ug/L	41.7(14)	46.9(24)	48.4(17)	54.6(16)	0.046
BMI	27.9(5.6)	27.7(3.4)	24.1(4.1)	22.0(3.1)	0.001
BMI (18.5-24.9)	17(24%)	4(6%)	33(48%)	15(22%)	-
BMI (25-29.9)	15(36%)	11(26%)	15(36%)	1(2%)	-
BMI (30-34.9)	14(58%)	5(21%)	4(17%)	1(4%)	-
BMI >35	5(71%)	1(14%)	1(14%)	0(0%)	-
YSM	11(5)	13(4)	9(4)	12(5)	-
Spine BMC(g/cm)	60(11)	54(15)	51(9)	45(11)	0.007
Spine BMD(g/cm^2)	1.1(1)	1.0(0.1)	0.9(0.1)	0.8(0.07)	0.001
Spine T-score	0.25(0.85)	-0.23(0.9)	-1.4(1.1)	-2.55(0.6)	0.001
Hip BMD (g/cm^2)	0.96(0.1)	0.88(0.1)	0.84(0.1)	0.72(0.06)	0.001
Hip BMC (g/cm)	33(4)	30(4)	28(5)	25(3)	0.001
Hip T-score	0.11(.67)	-0.48(.67)	-0.85(1.0)	-1.87(.5)	0.001
FN Hip BMD(g/cm^2)	0.85(1.)	0.74(.1)	0.69(.1)	0.59(.1)	0.001
FN Hip BMC(g/cm)	4.2(.6)	3.7(.4)	3.4(.6)	2.96(.5)	0.001
FN Hip T-score	-0.05(.8)	-1.0(.6)	-1.4(.7)	-2.3(.6)	0.001

Values are means and standard deviations (SD) except for BMI which also are number of participants in each BMI category and percentage (%) of women within each BMI classification in each category of BMD. P values determined from ANOVA.CTX= C terminal telopeptide of type 1 collagen, P1NP= procollagen type 1N propeptide. BMI= body mass index (weight (kg)/height m^2), YSM=years since menopause, FN= Femoral neck of hip, BMD=bone mineral density, BMC=bone mineral content; DEXA groups 1-4 are based on W.H.O classifications of T scores (33):Normal T score <1.0 SD below normal, osteopenia T score 1.0-2.49 SD below normal and osteoporosis T score ≤-2.5 SD below normal. Osteopenia is separated into mild (T-score ≥1-1.49 SD below normal and significant osteopenia (T score ≥1.5 ≤ 2.49 SD below normal).

Table 2
Dietary intake data of postmenopausal study women (n=142)

	Groups						
	Total	**1**	**2**	**3**	**4**	**NZ reference values**	
	(n=142)	**Normal BMD (n=51)**	**Mild Osteopenia (n=21)**	**Significant Osteopenia (n=53)**	**Osteoporosis (n=17)**	**EAR/AI**	**RDI/SDT**
Alcohol(units/week)	7(8)	6(9)	8(10)	7(7)	7(9)	≤14units/week	
Energy (kj)	7595(1730)	7550 (2099)	8220(1380)	7389(1642)	7602(1631)	N/A	
Protein (g)	79(20)	79(21)	83(18)	77(22)	76(21)	37	46
Fat (g)	70(23)	71(27)	78(22)	66(23)	74(21)	N/A	
Fibre (g)	26(9)	24(8)	30(9)	26(8)	26(12)	/25	/28
Vitamin C (mg)	195(199)	207(247)	203(156)	185(175)	196(173)	30	45/190
Vitamin E (mg)	12(11)	11(5)	12(5)	14(16)	13(12)	N/A	7/14
Vitamin B12 (ug)	5(6)	5(3)	4(4)	5(6)	5(6)	2.0	2.4
Folate (ug)	360(212)	314(134)	442(209)	347(148)	459(437)	320	400/600
Vitamin A equiv	1350(1397)	1328(713)	1081(575)	1395(1746)	1809(2295)	500	700/1220
Carotene equiv [a]	4835(3049)	5704(3501)	4324(2914)	4103(2533)	6107(3645)		
Sodium (mg)	2398(954)	2319(908)	2891(1054)	2271(916)	2488(860)		/1600
Potassium (mg)	3483(978)	3444(860)	3741(1077)	3390(896)	3427(1493)	/2800	/4700
Magnesium (mg)	350(118)	345(116)	389(104)	340(102)	354(102)	265	320
Calcium (mg)	829(342)	814(281)	763(228)	839(377)	891(504)	1100	1300
Iron (ug)	14(6)	13.5(5)	16(5)	13(6)	14(7)	5	8
Zinc (mg)	11(4)	10(3)	12(4)	10(4)	10(4)	6.5	8
Servings/day*							
Fruit	1.99	2.0	2.1	2.2	1.7	≥2	
Vegetable	3.46	3.5	3.3	3.4	3.8	≥3	
Bread/cereals	4.4	4.1	4.9	4.5	4.6	≥6	
Dairy [b]	1.58	1.9	1.1	1.5	1.4	≥2	
Protein	1.9	1.8	1.7	2.0	2.0	1	
PRAL mEq/day†	-1.5	-2	-2	0	-6	0.74mEq/day	
NEAP mEq/day†	39.4	39.5	38.5	39.1	41.2	41mEq/day	

[a,b]. Anova: Values in the same row are significantly different at p<0 .02 for a and p<0 .005 for b. * All serving sizes: according to NZ Ministry of Health guidelines: fruit/vegetables = 50-80 grams, or 0.5 cup cooked or 1 cup raw (salad greens) or 1 medium fruit, starchy vegetables (135grams), protein includes meat, fish, eggs, nuts/seeds and beans. † Estimated PRAL (mEq/d) = 0.49 protein (gms/day) + 0.037 phosphorus (mg/day) - 0.021potassium (mg/day) – 0.026 magnesium mg/day -0.013calcium mg/day, Estimated NEAP (mEq/day) = PRAL (mEq/day) + Organic acid excretion (mEq/day).PRAL and NEAP values determined from a sample diet Ministry of Health, NZ (38). NZ reference values are estimated average requirement (EAR) (50% population requirements) except when EAR is not available and adequate intake (AI) is used instead. Recommended daily intake (RDI) (98% of population requirements) are given and "suggested dietary target" (SDT) if available.

dietary PRAL and NEAP in the 4 BMD groups. Partial correlations (correcting for age and years since menopause) showed inverse relationships between bone resorption marker CTX and energy adjusted potassium intake and dairy consumption (Table 3). Positive associations (r=0.18 - 0.23) were also found between areal BMD and intakes of dairy products, protein, potassium, zinc and B vitamins thiamine, niacin and cobalamin however, when these nutrients were combined in multiple regression models the significance only persisted for potassium and dairy intake. Analysis of a random subset of diaries for potassium demonstrated more than two thirds of potassium intake derived from fruit/vegetables.

Pearson's correlation showed the women's areal bone mineral density was related to soft tissue compartments of fat and lean mass, weight and BMI. Body weight had the most consistently strong positive relationship with BMD at all 3 sites measured (13) (Figure 1).

Bone turnover markers (CTX and P1NP) (Table 1) showed significant inverse correlations with BMD at all three measured sites spine, hip and neck of hip.

Increased levels of the pro-inflammatory marker CRP (produced in the liver) were found in group 2 with the highest percentage android (AFM) and total body fat, however, inflammatory cytokines (expressed per kg fat mass) associated with bone: OPG, IL-6, IL-10 and TNF (median) were all higher in group 4 (osteoporosis) with OPG significantly higher than other groups (Table 4, Figure 2).

Multiple regression analysis was used to assess the combined effects of OPG, weight, CTX, dairy and potassium intake on BMD. The equation that predicts hip BMD from the independent variables was found to be:

Hip BMD= -.221 +.026(dairy) +0.006(weight) -0.001(OPG) +.18(K) -0.003(CTX)

The value of R^2 (for Hip BMD) was 0.39 (adjusted R^2 was 0.36) a value that was highly significant (p<0.00). F (5,119) = 15.14, MS residual was 1.2, p< 0.00. The standard error of the estimate was 0.10. Although each independent variable alone correlated significantly with hip BMD, only weight, dairy

Table 3
Correlations between selected nutrients, bone mineral density and bone turnover markers in postmenopausal study women (n=142)

	Protein	K	Zn	Mg	Thiamine	Niacin	Cobalamin	Dairy
FN Hip BMD (g/cm^2)	.19*	.23*	.24*	.14*	.16	.2*	.24*	.19*
FN Hip T-score	.17*	.18*	.20 *	.11	.14	.15	.20*	.19*
CTX	-.18 *	-.18*	-.21*	-.09	-.12	-.13	-.17*	-.2*
P1NP	-.23*	-.24*	-.24*	-.20*	-.19*	-.2*	-.12	-.19*

K = potassium, Zn = zinc, Mg = magnesium, Dairy = servings/day, FN = Femoral Neck, BMD = bone mineral density, CTX = C-terminal telopeptide of type 1 collagen, P1NP = Procollagen type I N propeptide, *Correlations r = ± .17 and above are significant at the p<0.05. Nutrients are energy adjusted values.

Table 4
Comparison of cytokine production and body composition in postmenopausal study women based on bone density classification

	1 Normal BMD (n=51)	2 Mild Osteopenia (n=21)	3 Significant Osteopenia (n=53)	4 Osteoporosis (n=17)	P <
Weight (kg)	76(13)	72(9)	67(10)	61(9)	0.001
BMI (kg/m^2)	28(6)	28(3)	24(4)	22(3)	0.001
Lean mass (kg)	45(5.4).	43(5.3)	42(4.2)	39(5.1)	0.001
Fat mass (kg)	31(9.8)	29(9.1)	25(7.2)	22(8.2)	0.001
Body fat %	40(7)	41(7)	37(6)	37(7)	0.01
Android fat %	37(9)	41(8)	34(9)	32(7)	0.002
Gynoid fat %	42(6)	44(6)	41(6)	41(6)	0.25
CRP mg/l(kg FM) †	0.9 (0.9) (0.3-1.5,0.5)	1.3(1.3) 1.0(.3-20)	0.77(0.88) 0.49(0.20-0.93).	0.91(1.2) (0.17-1.30,0.42,)	0.73
OPG ng/l(kg FM) †	0.07(0.06) (0.03-0.08,0.05)	0.07(0.04) (0.04-0.08,0.06,)	0.07(0.04) (0.05-0.93,0.06)	0.13(0.12) (0.17-1.3,0.08)	0.007
IL-6 pg/ml(kg FM)†	0.06(0.05) (0.03-.08,0.05)	0.05(0.03) (0.04-0.08,0.05)	0.07(0.07) (0.02-0.08,0.05).	0.08(0.07) (0.04-0.09,0.07)	0.56
IL-10 pg/ml(kgFM)†	0.11(0.11) (0.05-.15,0.08)	0.11(0.07) (0.05-0.16,0.09)	0.10(0.09) (0.05-0.12,0.08)	0.20(0.3) (0.07-0.19,0.1).	0.53
TNF pg/ml(kg FM) †	0.31(0.28) (0.12-0.43,0.24)	0.29(0.19) (0.14-0.37,0.23)	0.29(0.25) (0.12-0.37,0.25)	0.30(0.16) (0.16-0.42,0.30)	0.74

Values are means (standard deviation) except inflammatory markers which given as both means (SD) and (range, median). † C - reactive protein (CRP), interleukin 6 and 10 (IL-6, IL-10), tumour necrosis factor (TNF) and osteoprotegerin (OPG) expressed per kilogram of fat mass (kg FM). P values derived from ANOVA. P values <0.05 are considered statistically significant.

Table 5
Regression coefficients β for independent predictors of BMD in study women

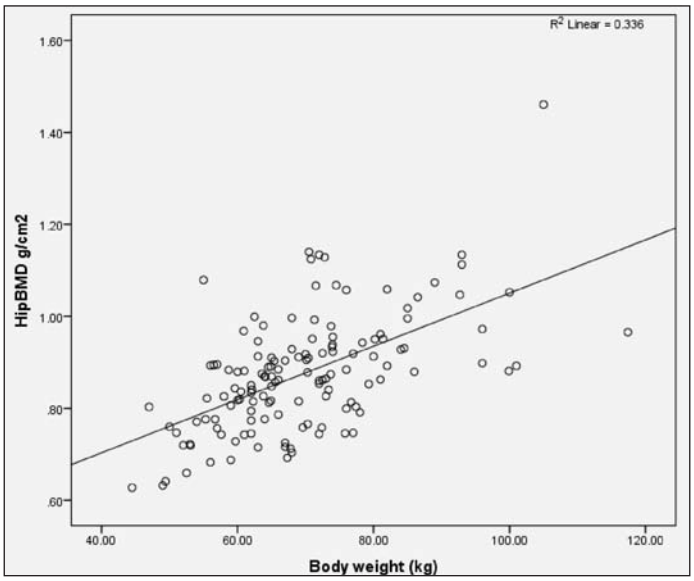
	Hip BMD R^2 =.39				Femoral Neck of Hip BMD R^2 =.27				Lumbar spine BMD R^2 =.28			
	Semi partial r	β	T (124)	P value	Semi partial r	β	T (124)	P value	Semi partial r	β	T (124)	P value
OPG	.001	.001	.015	.99	-.02	-.03	-.3	.77	.01	-.02	.18	.86
Weight	.450	.60	6.3	.00	.31	.40	4.0	.00	.35	.45	4.6	.00
Potassium †	.166	.17	2.30	.02	.21	.22	2.7	.01	.14	.15	1.8	.07
Dairy	.171	.18	2.40	.02	.16	.17	-.76	.05	.15	.16	1.9	.06
CTX	-.003	-0.004	-.049	.96	-.06	-.07	-1.03	.30	-.09	-.02	.18	.86

β coefficient = standardised regression coefficient. † Energy adjusted value. Coefficient of variation for Hip BMD 10.7%, Neck of hip BMD 12.2% and Spine BMD 13.7%.

intake and potassium accounted for a significant amount of unique variance of hip BMD. This multiple regression shows when body weight, dairy and potassium intake are used to predict a woman's hip BMD 39% of the variance in BMD is removed. The coefficient of variation for the hip BMD model was 10.7% indicating a fair to good fit (a good fit being 10% or less). Semi partial r values and values of Beta (standardised coefficients) for all the independent variables are shown together with results of the significance tests.

Figure 1

Relationship between body weight and hip bone mineral density in post-menopausal study women (n=142)



Regression relationship between weight in kilograms and hip bone mineral density (g/cm^2) in 142 postmenopausal women from three New Zealand regions. "r" is the Pearson correlation coefficient.

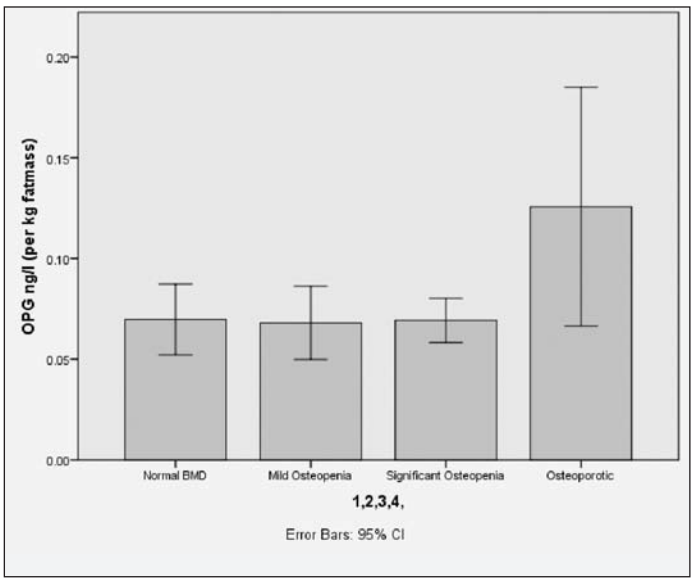
Discussion

The women in this study were predominantly European (98%) and 12% were classified with osteoporosis (T score ≤-2.5 SD below normal). As prevalence of osteoporosis varies with ethnicity (40) this is higher than reported population estimates for white PM women in Canada (8%) but less than the U.S (17%), U.K and Sweden (21%) (40, 44).

There were no significant differences between the four groups for reported nutrient intake or dietary acid load (Table 2) except for dairy intake with the group with normal BMD having the highest intake. Dairy products provide calcium, protein and potassium (18) and positive associations with BMD were found for protein, potassium, vitamin B12 and zinc, all linked to improved bone status and reflective of higher meat/dairy and fruit/vegetable intake (7, 45, 46). Reported measures of dietary acid load (PRAL and NEAP) (Table 2) were similar to other studies (47, 48) and no significant association with BMD was found, however, an inverse relationship existed between levels of bone resorption marker CTX and both dairy servings and potassium intakes reflecting the positive correlation of fruit/vegetables and dairy on bone health which has previously been noted (18, 45, 49-53).

Figure 2

Osteoprotegerin (OPG) and bone density classification (DXA)



Osteoprotegerin is expressed per kilogram of fat mass. DXA groups are based on W.H.O classifications of T scores (33): Normal T-score <1.0 SD below normal, osteopenia T - score 1.0-2.49 SD below normal and osteoporosis T- score ≥2.5 SD below normal. This study divided the osteopenic group into mild and significant osteopenia with mild osteopenia those participants with a T score ≥1-1.49 SD below normal and significant osteopenia participants with a T score ≥1.5 - ≤2.49 SD below normal.

Increased levels of obesity in postmenopausal women are now seen as a risk factor for osteoporosis (31). While half (49%) of the women in this study had a BMI within the normal range, the other half were either were overweight (30%) or obese (21%). Body weight, BMI and soft tissue compartments of fat and lean mass were investigated for their relationships with BMD with body weight being the most positively associated with BMD. Body weight is made up of both lean and fat mass with lean mass said to have more significant influence on BMD in pre menopausal women while fat mass is more significant in postmenopausal women (12, 54). In our study, women's fat mass was more highly correlated with hip BMD than lean mass. Fat and lean mass are thought to impact bone independently through quite different means (12). We found lower levels of both fat and lean tissue were associated with increased bone loss as seen in group 3 and 4 (Table 5).

Fat is an endocrine organ therefore obesity and increased fat mass particularly visceral or android fat (34, 55) are now considered a potent source of cytokines (adipokines). Increased secretion of TNF and IL-6 and other inflammatory cytokines by macrophages which infiltrate fat tissue may induce a chronic low grade inflammatory state which contributes to inflammatory bone loss leading to osteoporosis (30, 56).

Increased production of pro-inflammatory markers such as TNF and IL-6 affect bone formation particularly osteoclastogenesis (55) however, a moderate inverse relationship was found between BMI and osteoclastogenesis in this study with resorption marker CTX (r= - 0.3, P<0.00) and formation marker P1NP (r= - 0.22, p<0.00) (57) indicating increased body mass in this study was associated with reduced levels of bone turnover.

There was a significant difference seen between OPG production with higher levels in the osteoporotic group OPG (Figure 2). Group 2 had the highest level of CRP which is produced in the liver and is a measure of systemic inflammation (29). CRP production has been strongly linked to fat mass and in particular, abdominal fat mass (android) (29, 58) and in this study Group 2 had a significantly higher percentage body and android fat. Higher CRP values may be a result of systemic inflammation associated with higher abdominal adiposity and therefore represent an additional cardiometatabolic risk factor (58). OPG production reflects a variety of metabolic processes (59) and the increased levels seen in the osteoporotic group may be due to elevated bone turnover with a higher pool of osteoblasts and precursors releasing OPG (59). This group also had significantly higher levels of resorption marker CTX reflecting increased osteoclastogenesis which may be due to less OPG attached to receptor activator of nuclear factor kappa B ligand (RANKL) therefore increasing osteoclastic activity (59, 60) which is central to bone loss. The significant positive relationship seen in this study between CTX and OPG and the inverse seen with both these markers and weight/BMI reinforces the protective relationship between fat and bone (12).

Conclusion

This study of PM women demonstrated significantly increased OPG (per kilogram of fat mass) and lower bodyweight were associated with osteoporosis indicating increased body weight was protective of bone loss. Higher CRP levels were seen with higher abdominal fat mass and increased dairy and potassium intake (fruit/vegetables) predicted lower levels of bone resorption marker CTX as well as higher BMD. Our findings highlight the importance of dietary intake of dairy and potassium sourced from fruit/vegetables to maintain bone health at midlife.

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