This article was downloaded by: [University of Toronto Libraries] On: 23 November 2014, At: 15:44 Publisher: Routledge Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



## Journal of the American College of Nutrition Publication details, including instructions for authors and subscription information: http://www.tandfonline.com/loi/uacn20

# Dietary Protein, Phosphorus and Potassium Are Beneficial to Bone Mineral Density in Adult Men Consuming Adequate Dietary Calcium

Susan J. Whiting PhD<sup>a</sup>, Jennifer L. Boyle MSc<sup>a</sup>, Angela Thompson PhD<sup>a</sup>, Robert L. Mirwald PhD<sup>a</sup> & Robert A. Faulkner PhD<sup>a</sup>

<sup>a</sup> College of Pharmacy and Nutrition and College of Kinesiology, University of Saskatchewan, Saskatoon, Saskatchewan, CANADA Published online: 19 Jun 2013.

To cite this article: Susan J. Whiting PhD, Jennifer L. Boyle MSc, Angela Thompson PhD, Robert L. Mirwald PhD & Robert A. Faulkner PhD (2002) Dietary Protein, Phosphorus and Potassium Are Beneficial to Bone Mineral Density in Adult Men Consuming Adequate Dietary Calcium, Journal of the American College of Nutrition, 21:5, 402-409, DOI: 10.1080/07315724.2002.10719242

To link to this article: <u>http://dx.doi.org/10.1080/07315724.2002.10719242</u>

## PLEASE SCROLL DOWN FOR ARTICLE

Taylor & Francis makes every effort to ensure the accuracy of all the information (the "Content") contained in the publications on our platform. However, Taylor & Francis, our agents, and our licensors make no representations or warranties whatsoever as to the accuracy, completeness, or suitability for any purpose of the Content. Any opinions and views expressed in this publication are the opinions and views of the authors, and are not the views of or endorsed by Taylor & Francis. The accuracy of the Content should not be relied upon and should be independently verified with primary sources of information. Taylor and Francis shall not be liable for any losses, actions, claims, proceedings, demands, costs, expenses, damages, and other liabilities whatsoever or howsoever caused arising directly or indirectly in connection with, in relation to or arising out of the use of the Content.

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden. Terms & Conditions of access and use can be found at <a href="http://www.tandfonline.com/page/terms-and-conditions">http://www.tandfonline.com/page/terms-and-conditions</a>

## Dietary Protein, Phosphorus and Potassium Are Beneficial to Bone Mineral Density in Adult Men Consuming Adequate Dietary Calcium

Susan J. Whiting, PhD, Jennifer L. Boyle, MSc, Angela Thompson, PhD, Robert L. Mirwald, PhD, and Robert A. Faulkner, PhD

College of Pharmacy and Nutrition and College of Kinesiology, University of Saskatchewan, Saskatoon, Saskatchewan, CANADA

Key words: bone mineral density, potassium, phosphorus, protein, calcium

**Objective:** The purpose of this study was to determine relationships of calcium (Ca), protein (Pr), phosphorus (P) and potassium (K) to measures of bone mineral density in adult men.

**Methods:** Cross-sectional analysis of 57 men ages 39 to 42 years who were participants in an ongoing study. Dietary assessment was conducted using the Block food frequency questionnaire (FFQ). BMD of total body (TB), hip and lumbar spine (LS) were measured with dual X-ray absorptiometry (DXA).

**Results:** Ca, Pr, P and K, as well as lean body mass (LBM), showed significant correlation with BMD at the total body, hip and lumbar spine. Stepwise forward regression selection method identified LBM, height and fat mass as significant predictors of TB-BMD, LBM and height as significant predictors of hip BMD, and LBM as a significant predictor of LS-BMD. As the nutrients tested correlated significantly with each other, only one nutrient was entered into the regression model at a time to accommodate the potential for multicollinearity. In regression analysis, adjusted for site-specific anthropometric variables and energy intake, K, Pr and P intake accounted for significantly to the prediction of TB-BMD and LS-BMD values by 7% to 13%. No bone-related nutrient added significantly to the prediction of hip BMD. Ca intake was not significantly associated with BMD at any site in the adjusted models.

**Conclusions:** Our analysis provides support that a moderate protein (1.2 g/kg) diet, plentiful in potassium (>100 mmol/day) and phosphorus (1741  $\pm$  535 mg) is beneficial for maintaining bone mineral density in adult men when Ca intake was adequate (1200  $\pm$  515 mg).

## **INTRODUCTION**

Failure to gain optimal bone density during adolescence and young adulthood contributes to low bone density, leading to osteoporosis later in life. Similarly, failure to reduce bone loss throughout adulthood also contributes to low bone density. Although efforts to prevent osteoporosis have focussed on young people in their growing years, efforts could be made to promote maintenance of bone mass by reducing bone loss throughout adulthood. Although calcium intake has been a main area of research, it is noteworthy that diets adequate in calcium are often adequate in many other essential nutrients for good bone health, including vitamin D, potassium, magnesium, phosphorus, and protein [1]. Determining dietary patterns is important for making food-based recommendations.

Recent studies report a significant positive relationship between high protein intakes and bone density [2,3]; however, others espouse the view that a high protein diet, by increasing urinary calcium excretion, has a negative affect on bone [4,5]. On the other hand, there is growing evidence that low dietary

Journal of the American College of Nutrition, Vol. 21, No. 5, 402–409 (2002) Published by the American College of Nutrition

Address correspondence to: Susan J. Whiting, PhD, College of Pharmacy and Nutrition and College of Kinesiology, 110 Science Place, University of Saskatchewan, Saskatoon SK, S7N 5C9, CANADA. E-mail: susan.whiting@usask.ca

Presented in abstract form at the American Society form Bone and Mineral Research annual meeting, Toronto, September 23, 2000.

Abbreviations: BMD = bone mineral density, LS = lumbar spine, TB = total body, DXA = dual X-ray absorptiometry, LBM = lean body mass, FM = fat mass, SD = standard deviation, HHHQ = Health, Habits and History Questionnaire, FFQ = food frequency questionnaire, SGDFS = Saskatchewan Growth and Development Followup Study, CV = coefficient of variation.

protein negatively affects bone metabolism [6,7]. Studies of potassium intake indicate that fruit and vegetable intake may decrease the adverse effect of dietary acid load on bone [8,9,10]. While high phosphorus intakes may seriously alter calcium metabolism [11], one study reported that protein, phosphorus and calcium intakes were positively correlated with bone mineral density, suggesting a greater intake of each of these, including phosphorus, was beneficial [3]. None of these studies has looked at all three—protein, phosphorus and potassium—at the same time.

While many studies have examined dietary intakes of adult women in relation to bone mineral mass, few have examined men. The etiology of bone loss in adult men is multifactorial and includes heredity, lifestyle such as smoking, alcohol consumption and physical activity, hormonal function and different nutritional factors [12]. Since a well-balanced diet may be an important modifiable factor related to bone health [1], it is important to determine the role of nutrition in maintenance of bone mineral density in adult men. Therefore, we examined the effect of dietary factors on bone mineral density in adult men in a cross-sectional study in which dietary information was collected using a food frequency questionnaire. Three outcome variables were evaluated: total body bone mineral density (TB-BMD), total hip bone mineral density (hip BMD), and lumbar spine bone mineral density (LS-BMD).

## METHODS

## Subjects

A cross-sectional analysis of all male subjects from the Saskatchewan Growth and Development Follow-up Study (SGDFS) who completed both the dietary assessment and bone measurements was performed. Subjects recruited for the SGDFS had previously participated in the Saskatchewan Growth and Developmental Study between 1964 and 1973, a longitudinal study of 131 boys [13]. The initial sample was randomly stratified on a socioeconomic basis from elementary schools in Saskatoon. Dietary intakes were not obtained. In 1998, the SGDFS began, with the purpose to examine the relationship between physical activity and fitness in childhood/ adolescence and health habits, physical activity level and physical fitness in middle adulthood. Subjects were contacted through media. Upon agreement to participate in the follow-up study (SGDFS), the subjects were sent four questionnaires to self-administer: 1) demographics, 2) milk history [14], 3) food frequency and 4) health, habits and behaviors. Data on current physical fitness level, physical activity level, bone mineral density, health habits, anthropometric measures, dietary intake and demographics were collected. Seventy men (53% of original cohort) completed at least part of the follow-up study.

Usual dietary intake was assessed using the Health, Habits, and History Questionnaire (HHHQ), a semi-quantitative questionnaire also known as the "Block" FFQ [15–17]. Although mailed to subjects, there was opportunity to have questions answered by a trained undergraduate nutrition student at the time of bone scans. FFQs were analyzed using Dietsys Version 3.0, obtained from the National Cancer Institute. All FFQs were coded and double-entered by the same researcher. One subject had a low reported energy intake (below 1000 kcal) that remained low even after repeated measurement; this subject was excluded. Food group servings and amount of alcohol based on medium-sized drinks consumed per day were provided by Dietsys.

#### Anthropometric Assessment

Body measurements were taken by a trained anthropometrist according to published protocol [18] during the time the bone scan was performed, in a hospital setting. Measurements included height, weight and skinfolds of the triceps, subscapular, biceps, pectoral, supraspinale, iliac crest, abdominal, front thigh and medial calf skinfolds from the right side of the body. Height was measured twice and recorded to the nearest 0.1 mm. Weight was measured twice and recorded to the nearest 0.1 kg. Skinfold measurements were also taken twice with Harpenden calipers and recorded to the nearest 0.1 mm.

#### **Bone Mineral Measurement**

Bone measures were obtained using dual-energy X-ray absorptiometry using the Hologic QDR 2000 in array mode at the Department of Nuclear Medicine, Royal University Hospital, Saskatoon. Subjects (n = 57) wore t-shirts and loose fitting shorts, with shoes and metal objects removed. BMD was assessed at the lumbar spine, proximal femur and total body sites. Bone mineral content and bone area were also determined. All scans were analyzed by the same qualified individual. Short term precision values (expressed as coefficient of variation, CV%) were less than 1.1% at both the proximal femur and lumbar spine, and 0.51% for the total body BMD [19].

#### **Other Questionnaires**

Current activity levels were assessed using the Seven-Day Physical Activity Recall [21], administered at the time subjects had their bone scans. Each component of the activity questionnaire was multiplied by an appropriate weighting factor based on an estimate of energy expenditure and summed to arrive at an estimate of total kilocalories expended per day from activity for each subject. Information on current and past smoking habits was collected. The number of pack-years smoked (assuming 20 cigarettes per pack) was calculated for all past and current smokers.

#### **Statistical Analysis**

All statistical analyses were performed with SPSS (Statistical Package for the Social Science) Version 9.0 (SPSS Inc., Chicago). A *p*-value of  $\leq 0.05$  was considered significant. The data were initially explored for outliers and missing values. Descriptive statistics were determined for all variables. The relation between nondietary factors (age, weight, height, fat mass, lean body mass, physical activity levels, alcohol consumption, coffee consumption and pack-years smoked) and bone mass was examined using Pearson correlation coefficients: then, forward stepwise multiple regression was used to determine which factors significantly predicted BMD. Lean body mass, fat mass and height were identified as significant predictors of total body BMD; lean body mass and height were significant predictors of total hip BMD, and lean body mass was identified as a significant predictor of lumbar spine BMD. Thus, these factors were considered confounders and were controlled in all subsequent nutritional analyses of BMD. Because most nutrients correlate with energy intake, adjusting for energy was necessary to assess the independent effect contributed by the nutrient of interest [9,10]. Furthermore, controlling for energy intake may account for differences that may have been due to body size or activity levels, as well as correct for some of the measurement error inherent in the FFQ [21]. Energy was controlled by including it as a confounding variable within each regression model. All distributions were checked for normalcy.

To better understand the potential of collinearity among energy, calcium, potassium, protein and phosphorus intakes, simple and partial (adjusted for identified non-dietary confounders) correlations among these variables were performed. Because these analyses showed evidence of high collinearity (r > 0.8) among most variables, it was not possible to assess the independent effects of calcium, potassium, protein and phosphorus on BMD in the same model. Intake for each nutrient (calcium, potassium, protein or phosphorus) and energy was entered into each BMD regression model of non-dietary predictors/confounders by the forward stepwise regression procedure using a staged approach with only one nutrient entered at a time [9,10]. Partial F tests [22] were calculated to determine if the addition of the nutrient to the model significantly contributed to the prediction of BMD over and above that achieved by the non-dietary predictors already in the model. This method allowed the strongest independently predictive factors of BMD to be identified.

#### RESULTS

Mean values ( $\pm$  SD) for subject characteristics including lifestyle factors, BMD, and anthropometric measurements are described in Table 1. Forty-nine per cent of subjects were within a BMI range of 20–26, 18% were marginally above, 28% were overweight and the remaining 5% were underweight. The average hip BMD of the subjects, ages 39 to 42 years, was similar to that

Table 1. Characteristics of Subjects (n = 57)

Measurement	Mean $\pm$ SD	Range
Age (years)	$39.6 \pm 0.6$	39–42
Weight (kg)	$84.3 \pm 15.3$	57.5-127.3
Height (cm)	$178 \pm 7$	157-195
Physical Activity (kcal/day)	$2260 \pm 1479$	0-7092
Smoking (pack-years <sup>a</sup> )	$16.7 \pm 12.2$	0.1-57.5
Alcohol consumption		
(drinks/day)	$0.8 \pm 0.2$	0-8.4
Coffee intake (cups/day)	$2.7 \pm 2.4$	0-10
BMI (kg/m <sup>2</sup> )	$26.5 \pm 4.2$	19.1-39.3
Body Fat (%)	$23.7 \pm 7.8$	9.3-42.3
Fat Mass (kg)	$20.9 \pm 10.3$	5.3-53.8
Lean Body Mass (kg)	$60.4\pm6.8$	49.3-76.7
Total Body BMD (g/cm <sup>2</sup> )	$1.15 \pm 0.09$	0.94-1.43
Hip BMD (g/cm <sup>2</sup> )	$1.01\pm0.14$	0.61-1.38
Lumbar BMD (g/cm <sup>2</sup> )	$1.03\pm0.13$	0.77-1.36

BMD = bone mineral density

 $^a$  average pack-years (#cigarettes per day/20  $\times$  #years smoked) for former and current smokers, n = 32; value does not include 25 subjects who never smoked.

of young adult non-Hispanic men, ages 20 to 29 years, measured in NHANES III [23]. One subject had a hip BMD of 2.5 standard deviations below the mean hip BMD of the young adult standard reference group. The majority of the subjects, 82.5%, had normal hip BMD values. Physical activity, alcohol consumption, coffee consumption and smoking were considered possible confounders in diet-bone relationships; however, no significant correlations were found between these lifestyle factors and bone density measurements (data not shown).

Mean ( $\pm$  SD) and 5th and 95th percentiles of nutrient intake for energy and nutrients used in analyses and for servings/day of the four food groups are presented in Table 2. Seventy-two per cent of the subjects were below the average energy requirement (2700 kcal/day) for adult men ages 25 to 49 [24]. Average protein intake per kilogram body weight was 1.17 g/kg body weight, with 19% of subjects having an intake below the current Canadian recommendation of 0.86 g of protein/kg body weight [24]. Average reported calcium intake was above the AI of 1000 mg/day [25]. All subjects (100%) reported phosphorus intakes above the 1997 RDA of 700 mg/day [25]. The average intake for potassium was above the U.S. 1989 RDA desirable intake of 90 mmol/day [26]. Average servings of fruit and vegetables, milk products, breads and cereals, and meat, fish and poultry were close to recommended servings for Canadians (i.e., 5-10, 2-4, 5-12, 2-3, respectively) considering Dietsys serving sizes were generally larger than Health Canada sizes [27].

Energy intake was significantly and highly correlated with calcium, phosphorus, potassium and protein (r = 0.56, 0.76, 0.80 and 0.84, respectively); therefore, we adjusted for energy in all subsequent analyses. Correlations among dietary calcium, phosphorus, potassium, protein, adjusted for energy were strong, indicating collinearity (Table 3). Lean body mass was significantly correlated (p < 0.01) with all three BMD sites (r = 0.37-0.45)

Nutrient/Food Group <sup>1</sup>	Mean $\pm$ SD	5th	50th	95th
Energy (kcal)	2343 ± 715	1370	2202	3458
Protein (g)	$97 \pm 27$	61.1	91.7	147.4
Ca (mg)	$1200 \pm 515$	508	1050	2112
P (mg)	$1741 \pm 535$	1098	1669	2723
Ca:Pr (mg/g)	$12.1 \pm 3.9$	6.2	11.6	19.5
Ca:P (mg/mg)	$0.66 \pm 0.16$	0.45	0.67	0.89
K (mg)	$3787 \pm 1093$	2283	3572	5578
Fruit & Vegetables (serving)	$4.2 \pm 2.1$	1.7	3.5	8.2
Milk Products (serving)	$3.4 \pm 2.5$	0.8	2.7	10.2
Breads and Cereals (serving)	$3.1 \pm 1.4$	1.0	2.9	5.8
Meat <sup>2</sup> (serving)	$2.1 \pm 0.7$	1.0	2.1	3.8

Table 2. Daily Intake of Bone-Related Nutrients of Male Subjects (n = 57)

<sup>1</sup> Food Groups according to HHHQ [16-18].

 $^{2}\ \mathrm{Alternates}$  such as eggs, peanut butter, beans and lentils not included.

Ca = calcium, P = phosphorus, Ca:Pr = calcium:protein ratio, Ca:P = calcium:phosphorus ratio, K = potassium.

 Table 3. Pearson Correlation Coefficients between Dietary

 Calcium, Phosphorus, Potassium and Protein Adjusted for

 Energy Intake<sup>1</sup>

	Calcium	Phosphorus	Potassium	Protein
Calcium	1.00	0.881	0.587	0.519
Phosphorus		1.00	0.733	0.761
Potassium			1.00	0.566
Protein				1.00

 $^{1}$  n = 57; all Pearson correlation coefficients are significant, p < 0.001.

while weight approached significance (r = 0.258, p = 0.053) at the total body BMD site. No significant correlations between age, height, percent body fat, fat mass and bone mineral density measures were found. Lean body mass, height and fat mass were selected to be included in the predictive model of total body BMD. Lean body mass and height were significant predictors of total hip BMD, and only LBM was significantly related to spine BMD. These factors were considered confounders in the relationship between calcium/bone-related nutrients and BMD and were controlled for in all subsequent analyses.

Dietary protein, phosphorus and potassium were significantly

correlated with all three BMD sites, while calcium intake was significantly correlated with total body and spine BMD. These are shown as *unadjusted* data in Table 4. When adjusted for energy intake and appropriate anthropometric measures, all correlations among protein, potassium and phosphorus and BMD measures remained significant, while associations between calcium and BMD measures became insignificant (*adjusted* values, Table 4). Fruit and vegetable intake was significantly correlated with total body BMD but became insignificant once adjusted for anthropometric measures and energy intake. No other food group was significantly associated with BMD at any site.

Calcium, potassium, phosphorus and protein intakes were added individually to three BMD models—total body, hip and spine. The complete display of regression models for total body BMD only is shown in Table 5. Models containing a combination of two or more of calcium, potassium, phosphorus and protein tended not to show independent effects because of the high collinearity between dietary nutrients of interest (data not shown). Generally, potassium, protein and phosphorus were determined to significantly contribute to the prediction of TB BMD and spine BMD, but not hip BMD, over and above the models consisting of non-dietary predictors (Table 6). The

 Table 4. Pearson Correlations Coefficients for Nutrient Intakes and Bone Mineral Density Measures Unadjusted and Adjusted for

 Non-Dietary Factors and Energy Intake

	Total body BMD		Hip BMD		Spine BMD	
	Unadjusted	Adjusted <sup>1</sup>	Unadjusted	Adjusted <sup>2</sup>	Unadjusted	Adjusted <sup>3</sup>
Calcium	0.285*	0.218	0.213	0.115	0.253	0.189
Phosphorus	0.373**	0.385**	0.301*	0.272*	0.296*	0.331*
Protein	0.358**	0.383**	0.289*	0.322*	0.284*	0.419**
Potassium	0.404**	0.401**	0.346**	0.309*	0.325**	0.400**
Fruit & Vegetable Intake	0.297*	0.187	0.201	0.122	0.141	0.129

n = 57.

<sup>1</sup> Controlled for lean body mass, fat mass, height, and energy intake.

<sup>2</sup> Controlled for lean body mass, height, and energy intake.

<sup>3</sup> Controlled for lean body mass, and energy intake.

Significance: \* p < 0.05, \*\* p < 0.01.

Variable	Coefficient	SE	t	р
Model $1^1$ : TB-BMD = Cons	stant + LBM + Ht + FM			
Constant	1.373	0.265	5.18	< 0.01
LBM	0.011	0.002	5.12	< 0.01
Ht	-0.0046	0.002	-2.57	0.01
FM	-0.0024	0.001	-2.11	0.04
Adjusted $R^2 = 0.296$				
Model 2: $TB-BMD = Const$	tant + LBM + Ht + FM + Ener	$gy + Ca^2$		
Constant	1.42674	0.26727	5.34	< 0.01
LBM	0.01029	0.00215	4.78	< 0.01
Ht	-0.00478	0.00178	-2.69	0.01
FM	-0.00273	0.00115	-2.37	0.02
Energy	-0.00002	0.00002	-0.98	0.33
Ca	0.00004	0.00002	1.60	0.12
Adjusted $R^2 = 0.303$				
Model 3: $TB-BMD = Const$	tant + LBM + Ht + FM + Ener	$gy + K^3$		
Constant	1.2860	0.2509	5.13	< 0.01
LBM	0.0086	0.0021	4.11	< 0.01
Ht	-0.0037	0.0017	-2.57	0.01
FM	-0.0030	0.0011	-2.11	0.04
Energy	0.00006	0.00002	-2.57	0.01
K	0.00005	0.00002	3.13	0.01
Adjusted $R^2 = 0.386$				
Model 4: $TB-BMD = Const$	tant + LBM + Ht + FM + Ener	$gy + Protein^3$		
Constant	1.36000	0.25142	5.41	< 0.01
LBM	0.00941	0.00207	4.54	< 0.01
Ht	-0.00439	0.0017	-2.61	0.01
FM	-0.00298	0.00108	-2.14	0.04
Energy	-0.00006	0.00002	-2.52	0.02
Protein	0.00193	0.00065	2.96	< 0.01
Adjusted $R^2 = 0.376$				
Model 5: $TB-BMD = Const$	tant + LBM + Ht + FM + Ener	$gy + P^3$		
Constant	1.41683	0.25144	5.63	< 0.01
LBM	0.00970	0.00205	4.73	< 0.01
Ht	-0.00467	0.00168	-2.67	0.01
FM	-0.00291	0.00109	-2.77	0.01
Energy	0.00005	0.00002	-2.31	0.02
Р	0.00008	0.00003	2.98	< 0.01
Adjusted $R^2 = 0.377$				

Table 5. Final Multiple Linear Regression Models of Total Body BMD with and without Addition of Bone Related Nutrients

n = 57.

<sup>1</sup> Model of non-nutrient predictors of BMD determined by forward stepwise regression (variables entered: age, LBM, FM, height, education level, physical activity, alcohol consumption, coffee consumption, and pack years smoked).

<sup>2</sup> Addition of nutrient to model is not significant (Partial F Test)

<sup>3</sup> Addition of nutrient to model significant (Partial F Test), p < 0.05.

TB-BMD = total body bone mineral density, LBM = lean body mass, Ht = height, FM = fat mass, Energy = energy intake, Ca = calcium, K = potassium, P = phosphorus.

addition of calcium to the models did not significantly contribute to the prediction of BMD at any site.

Potassium was significantly associated with total body and spine BMD sites; there was a 4.3% increase in total body BMD and a 7.3% change in lumbar spine BMD with every 1000 mg increase in potassium intake. The differences in adjusted R<sup>2</sup> values for the non-dietary model and the model containing potassium indicate that about 9% of the variation in TB BMD and 11.6% of the variation in spine BMD can be explained by dietary potassium intake. Similarly for phosphorus intake, the change in BMD associated with a 1000 mg increase in phosphorus intake was 6.9% in total body BMD and 11.7% in lumbar spine BMD with every 1000 mg increase in phosphorus intake. Approximately 8.1% of the variation in total body BMD and 7.0% of the variation in spine BMD can be explained by dietary phosphorus intake. Protein intake also made a significant contribution to the prediction of TB BMD and spine BMD. There was a 1.7% change in total body BMD and a 3.4% change in lumbar spine BMD for every 10 g increase in dietary protein. About 8.0% of the variation in total body BMD and

Bone Site	Model 1:	Model 2:	Model 3:	Model 4:	Model 5:
	no nutrient	+ calcium	+ potassium	+ protein	+ phosphorus
Total Body BMD Hip BMD Spine BMD	$R^{2}$ 0.296 <sup>1</sup> 0.1882 0.127 <sup>3</sup>	R <sup>2</sup> 0.303 0.169 0.131	R <sup>2</sup> 0.3864 0.238 0.2434	R2     0.3764     0.245     0.2564	$R^{2}$ 0.377 <sup>4</sup> 0.22 0.197 <sup>4</sup>

**Table 6.** Summary Table of Multiple Regression Models Showing  $R^2$  Values for Models with and without Addition of Bone Related Nutrients

<sup>1</sup> R<sup>2</sup> with lean body mass (LBM), fat mass (FM), and height in the model.

 $^{2}$  R<sup>2</sup> with LBM, and height in the model.

 $^{3}$  R<sup>2</sup> with LBM in the model.

<sup>4</sup> Addition of nutrient and energy intake to model is significant (Partial F Test), p < 0.05.

12.9% of the variation in spine BMD can be explained by protein intake.

#### DISCUSSION

The primary focus of this study was to examine the effect of calcium and bone-related nutrients (potassium, protein and phosphorus) on bone mineral density in male subjects ages 39 to 42 years. The average daily intake of calcium, as well as potassium, protein and phosphorus, exceeded recommended intakes in our subjects. A diet high in calcium intake appears to be a marker of a diet which is high in most or all other essential nutrients [28]. Indeed, this group of middle-aged men could be described as an above average group of individuals in terms of eating a well-balance diet, at an ideal body weight for height, and having good bone density.

Many factors, such as age, weight, height, BMI, physical activity, are reported to play a role in the degree of bone density obtained in men [29]. Age was not a confounder because our group had a narrow age range, 39 to 42 years. Our stepwise procedure identified lean body mass, fat mass and height as significant predictors of TB BMD. Lean body mass and height were significant predictors of hip BMD, and lean body mass was a significant predictor of spine BMD. Nutrient intakes were adjusted for total energy intake. No significant relationships between the nutrients of interest (calcium, phosphorus, protein, potassium) and BMD were found at the three sites when all four were added simultaneously; therefore; each nutrient was entered individually into the models, as others have done in situations of collinearity [9,10,31].

The evidence of a positive relationship between dietary calcium and bone density is well accepted from clinical randomized control trials [25] and increasingly from observational studies [32]. Our study did not find an association between calcium and bone mineral density at any of the three sites, once anthropometric factors were controlled for, a finding consistent with calcium's being a threshold nutrient [33]. Our subjects' mean intake of calcium exceeded the Adequate Intake level of 1000 mg [25]. As discussed below, the fact that calcium intake was adequate may have impacted on the findings for the other nutrients examined [34].

Dietary potassium was determined a significant predictor of TB BMD when LBM, FM, height and energy intake were controlled and a significant predictor of spine BMD when LBM and energy intake were controlled. Others have reported that greater potassium intakes, from fruit and vegetables, were associated with increased BMD at hip sites in elderly men and women [9] and with spine BMD in premenopausal women [8]. The beneficial effects of dietary potassium on bone health is well established in the literature [35-38]. Experimentally, supplemental KHCO<sub>3</sub> has been shown to be hypocalciuric in subjects consuming moderate protein diets [37], to reduce high-protein induced hypercalciuria [38] and reduce the rate of bone resorption [36] by improving calcium balance. Epidemiological research has also been successful in showing a positive relation between dietary potassium and BMD [8-10]. In the final linear regression models for TB BMD and spine BMD. potassium was able to explain 9% of the variation in TB BMD and 12% of the variation in spine in our male subjects. These subjects had a diet with good variety, which was relatively high in fruits and vegetables. With an increase in fruit and vegetables in the diet, there is also an increase in a variety of other nutrients, such as magnesium, vitamin C and vitamin K, that may also have an effect on bone health [39]. Magnesium was not directly investigated in this study; however, it was positively correlated with TB BMD and spine BMD in a crude analysis (data not shown). As both potassium and magnesium have been reported to be positively related to BMD in studies that indicated that increased fruit and vegetable intake was positively related to BMD [8-10], our findings on potassium may also apply to magnesium.

Protein was a significant predictor of both TB BMD (p < 0.01) and spine BMD (p < 0.01), but not hip BMD, when appropriate anthropometric variables and energy intake were controlled. The average protein intake in this group of men was 96 g/day or 1.17 g/kg body weight, a level considered "moderate" [40-42]. Until recently the focus has been on the negative effects of excess protein in the diet, yet low dietary intakes of protein (below the 1989 RDA of 0.8 g/kg body

weight) negatively affect bone [41,43]. Kerstetter and colleagues [42] suggested that calcium metabolism is normal when subjects consumed a well-balanced diet containing moderate amounts of protein ( $\sim$ 1.0 g/kg body weight/day). Animal protein and vegetable protein may have differential effects on calcium balance [5]; however, not all studies are in agreement [6,34]. Recently, a study examining the effect of dietary protein on BMD in elderly men and women was able to show low calcium absorption with high protein intakes when calcium intakes were low and higher BMD with high protein when calcium intakes were high [34]. Together, this study and our data suggest that protein exerts a positive effect on BMD when calcium intake is adequate.

Phosphorus intake, similar to potassium and to protein, was also a significant predictor of both TB BMD and spine BMD, but not hip BMD. Another study found a similar relation between phosphorus and BMD, using the same FFQ to gather dietary intake data [3]. There has been concern about high phosphorus intakes as current intakes in the U.S. are very high while at the same time calcium intakes are very low [11]. Furthermore, an individual's actual phosphorus intake may be higher than estimated due to inaccurate nutrient composition tables for phosphorus values of processed foods [44]. To compound the issue, an FFQ does not differentiation between the use of processed foods (high in phosphates) or unprocessed foods. For example, there is only one descriptor for soft drinks, which can be phosphate-containing cola or not (and the phosphorus analysis of this food was 0 mg). Therefore, the usefulness of an FFQ for determining phosphorus in diet-disease relationships is limited. Protein and phosphorus intakes were highly correlated, even when adjusted for energy intake (r =0.761, p < 0.001). Generally, foods high in protein are also high in phosphorus, so that dietary phosphorus intakes measured by the FFQ are most likely reflecting protein intake and are not likely to be a true reflection of phosphorus in the diet.

Our study suggests that for adult men, a dietary pattern of adequate calcium intake, moderate protein, and generous potassium contributes to higher BMDs than a pattern where calcium is adequate but protein and potassium are low. Because of the collinearity in intake among these nutrients it is difficult to separate individual effects. Nevertheless, the former dietary pattern appears to be beneficial to BMD maintenance in men and can be achieved by incorporating fruit, vegetables and dairy products into the diet.

#### ACKNOWLEDGMENT

This work was supported by a grant from the Canadian Fitness and Lifestyle Research Institute (#961R001) to RLM and RAF. Preliminary dietary interviews and dietary assessments were conducted by Carolyn Medernach and Michelle Mackenzie.

## REFERENCES

- 1. Miller GD, Groziak SM, Dirienzo D: Age considerations in nutrient needs for bone. J Am Coll Nutr 15:553–555, 1996.
- Cooper C, Atkinson EJ, Hensrud DD, Wahner HW, O'Fallon WM, Riggs BL, Melton LJ: Dietary protein intake and bone mass in women. Calcif Tissue Int 58:320–325, 1996.
- Teegarden D, Lyle RM, McCabe GP, Proulx WR, Michon K, Knight AP, Johnston CC, Weaver C: Dietary calcium, protein, and phosphorus are related to bone mineral density and content in young women. Am J Clin Nutr 68:749–754, 1998.
- Freskanich D, Willett WC, Stampfer MJ, Colditz GA: Protein consumption and bone fractures in women. Am J Epidemiol 143: 472–479, 1996.
- Sellmeyer DE, Stone KL, Sebastian A, Cummings SR, for the Study of Osteoporotic Fractures Research Group: A high ratio of dietary animal to vegetable protein increases the rate of bone loss and the risk of fracture in postmenopausal women. Am J Clin Nutr 73:118–122, 2001.
- Hannan MT, Tucker KL, Dawson-Hughes B, Cupples LA, Felson DT, Kiel DP: Effect of dietary protein on bone loss in elderly men and women. The Framingham Study. J Bone Miner Res 15:2504– 2512, 2000.
- Kerstetter JE, Svastisalee CM, Caseria DM, Mitnick, ME, Insogna KL: A threshold for low-protein-diet-induced elevations in parathyroid hormone. Am J Clin Nutr 72:168–173, 2000.
- New SA, Bolton-Smith C, Grubb DA, Reid DM: Nutritional influences on bone mineral density: a cross-sectional study in premenopausal women. Am J Clin Nutr 65:1831–1839, 1997.
- Tucker KL, Hannan MT, Chen H, Cupples LA, Wilson PWF, Kiel DP: Potassium, magnesium, and fruit and vegetable intakes are associated with greater bone mineral density in elderly men and women. Am J Clin Nutr 69:727–736, 1999.
- New SA, Robins SP, Campbell MK, Martin JC, Garton MJ, Bolton-Smith C, Grubb DA, Lee SJ, Reid DM: Dietary influences on bone mass and bone metabolism further evidence of a positive link between fruit and vegetable consumption and bone health? Am J Clin Nutr 71:142–151, 2000.
- Calvo MS, Park YK: Changing phosphorus content of the U.S. diet: potential for adverse effects on bone. J Nutr 126:1168S– 1180S, 1996.
- Elmstahl S, Gullberg B, Janzon L, Johnell O, Elmstahl B: Increased incidence of fractures in middle-aged and elderly men with low intakes of phosphorus and zinc. Osteoporos Int 8:333–340, 1998.
- Mirwald RL: The Saskatchewan Growth and Development Study. In Ostyn M, Beunen G, Simons J (eds): "Kinanthropometry II," International Series on Sport Sciences, vol. 9. Baltimore: University Park Press, 1978.
- Sandler RB, Slemenda CW, Laporte RE, Cauley JA, Schramm MM, Barresi ML, Kriska AM: Postmenopausal bone mineral density and milk consumption in childhood and adolescence. Am J Clin Nutr 42:270–274, 1985.
- Block G, Woods M, Potosky A, Clifford C: Validation of a self-administered diet history questionnaire using multiple diet records. J Clin Epidemiol 43:1327–1335, 1990.
- 16. Block G, Thompson FE, Hartman AM, Larkin FA, Guire KE:

Comparison of two dietary questionnaires validated against multiple dietary records collected during a 1-year period. J Am Diet Assoc 92:686–693, 1992.

- Hartman AM, Block G, Chan W, Williams J, McAdams M, Banks WL, Robins A: Reproducibility of a self-administered diet history questionnaire administered three times over three different seasons. Nutr Cancer 25:305–315, 1996.
- Ross WD and Marfell-Jones MJ: Kinanthropometry. In MacDougall JD, Wenger HA, Green JJ (eds): "Physiological Testing of the High-Performance Athlete." Champaign: Human Kinetics, pp 223–308, 1990.
- McKay H, Bailey DA, Wilkinson A, Houston CS: Familial comparison of bone mineral density at the proximal femur and lumbar spine. Bone Miner 24:95–107, 1994.
- Sallis JF, Haskell WL, Wood PD, Fortmann SP, Rogers T, Blair SN, Paffenbarger Jr RS: Physical activity assessment methodology in the Five-City Project. Am J Epidemiol 121:91–106, 1985.
- Willett WC, Stampfer MJ: Total energy intake: implications for epidemiologic analyses. Am J Epidemiol 124:17–27, 1986.
- Kleinbaum DG, Kupper LL, Muller KE: "Applied Regression Analysis and Other Multivariate Methods," 2nd ed. Belmont, CA: Duxbury Press, pp 206–217, 1988.
- Looker AC, Orwoll ES, Johnston CC, Lindsay RL, Wahner HW, Dunn WL, Calvo MS, Harris TB, Heyse SP: Prevalence of low femoral bone density in older U.S. Adults from NHANES III. J Bone Miner Res 12:1761–1768, 1997.
- Health and Welfare Canada: "Nutrition Recommendations: The Report of the Scientific Review Committee." Ottawa: Supply and Services Canada, 1990.
- Institute of Medicine, Food and Nutrition Board: "Dietary Reference Intakes: Calcium, Magnesium, Phosphorus, Vitamin D, and Fluoride." Washington, DC: National Academy Press, 1997.
- National Research Council: "Recommended Dietary Allowances," 10th ed. Washington DC: National Academy Press, 1989.
- Health and Welfare Canada: "Canada's Food Guide to Healthy Eating." Ottawa: Minister of Supply and Services, 1992.
- Barger-Lux MJ, Heaney RP, Packard PT, Lappe JM, Recker RR: Nutritional correlates of low calcium intake. Clin Appl Nutr 2:39– 44, 1992.
- Nordstrom P, Nordstrom R, Lorentzon R: Correlation of bone density to strength and physical activity in young men with a low or moderate level of physical activity. Calcif Tissue Int 60:332– 337, 1997.
- 30. Kanis J, Johnell O, Gullberg B, Allander E, Elffors L, Ranstam J,

Dequeker J, Dilsen G, Gennari C, Vaz AL, Lyritis G, Mazzuoli G, Miravet L, Passeri M, Perez Cano R, Rapado A, Ribot C: Risk factors for hip fractures in men from Southern Europe: The ME-DOS study. Osteoporosis Int 9, 45–54, 1999.

- Elmstahl S, Gullberg B: Bias in diet assessment methods consequences of collinearity and measurement errors on power and observed relative risks. Int J Epidemiol 26:1071–1079, 1997.
- Heaney RP: Calcium, dairy products and osteoporosis. J Am Coll Nutr 19:83S–99S, 2000.
- Matkovic V, Heaney RP: Calcium balance during human growth: evidence for threshold behavior. Am J Clin Nutr 55:992–996, 1992.
- Dawson-Hughes B, Harris SS: Calcium intake influences the association of protein intake with rates of bone loss in elderly men and women. Am J Clin Nutr 75:773–779, 2002.
- Lemann J: Relationship between urinary calcium and net acid excretion as determined by dietary protein and potassium: a review. Nephron 81:18–25, 1999.
- Morris RC, Schmidlin O, Tanaka M, Forman A, Frassetto L, Sebastian A: Differing effects of supplemental KCl and KHCO<sub>3</sub>: Pathophysiological and clinical implications. Semin Nephrol 19: 487–493, 1999.
- Lemann J, Pleuss JA, Gray RW: Potassium causes calcium retention in healthy adults. J Nutr 123:1623–1626, 1993.
- Green TJ, Whiting SJ: Potassium bicarbonate reduces high proteininduced hypercalciuria in adult men. Nutr Res 7:991–1002, 1994.
- Ilich JZ, Kerstetter JE: Nutrition in bone health revisited: a story beyond calcium. J Am Coll Nutr 19:715–737, 2000.
- Remer T, Manz F: Estimation of the renal net acid excretion by adults consuming diets containing variable amounts of protein. Am J Clin Nutr 59:1356–1361, 1994.
- Kerstetter JE, Caseria DM, Mitnick ME, Ellison AF, Gay LF, Liskov TAP, Carpenter TO, Insogna KL: Increased circulating concentrations of parathyroid hormone in healthy, young women consuming a protein-restricted diet. Am J Clin Nutr 66:1188–1196, 1997.
- Kerstetter JE, O'Brien KO, Insogna KL: Dietary protein affects intestinal calcium absorption. Am J Clin Nutr 68:859–865, 1998.
- Kerstetter JE, Looker AC, Insogna KL: Low dietary protein and low bone density. Calcif Tissue Int 66:313, 2000.
- Calvo MS: Dietary phosphorus, calcium metabolism and bone. J Nutr 123:1627–1633, 1993.

Received September 24, 2001; revision accepted May 24, 2002.