Original Article

The relationship between habitual dietary phosphorus and calcium intake, and bone mineral density in young Japanese women: a cross-sectional study

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Phosphorus and calcium are essential for bone health. There is a concern that a low calcium/phosphorus intake ratio resulting from low calcium intake coupled with high phosphorus intake may have a negative effect on bone mineral status, especially in Western countries. The objective of this study was to examine cross-sectionally the influence of habitual phosphorus and calcium intake and the calcium/phosphorus intake ratio on the bone mineral density (BMD) in 441 young Japanese women (aged 18-22) whose calcium/phosphorus intake ratio was assumed to be lower than young Western women. We also ascertained the relationship between dietary intake and serum or urinary measurements of phosphorus and calcium. Parathyroid hormone (PTH) and 25-hydroxy vitamin D (25(OH)D) were also examined for 214 of the 441 subjects. Phosphorus and calcium intake and the calcium/phosphorus intake ratio had significant positive correlations with urinary phosphorus. Calcium intake and the calcium/phosphorus intake ratio independently had positive and significant associations with BMD in the distal radius adjusted for postmenarcheal age, body mass index, and physical activity. There were no significant associations with BMD in the lumbar spine and femoral neck. These results indicate that in young Japanese women, phosphorus intake did not have a significantly negative effect on bone mineral density, and calcium intake and calcium/phosphorus intake ratio had a small but significant association only in a site-specific manner with BMD.

Key Words: phosphorus, calcium, bone mineral density, parathyroid hormone, young Japanese women

INTRODUCTION

Current incidence of osteoporotic fracture has been increasing in both Japan and Western countries.[1] As peak bone mass (PBM) largely determines the risk of osteoporotic fracture,[2] maintaining PBM is important if osteoporosis is to be prevented in adults as they grow older. From a nutritional standpoint, phosphorus (P) and calcium (Ca) are essential for the formation of hydroxyapatite, the main crystalline component of bone.[3] However, Ca intake is low in Japan especially among young women.[4,5] On the other hand, excess intake of P has been a concern in Western countries.[6-8] There is evidence that a low Ca/P intake ratio caused by low Ca and simultaneously high P intake may have a negative effect on bone health.[3,9,10] This could be because low Ca/P intake promotes secretion of parathyroid hormone (PTH) and bone resorption.[11] Acute intervention studies among young women have shown that a low Ca/P diet led to higher serum PTH,[12-14] and also increased bone resorption and decreased bone formation.[12,14] In addition, habitual low Ca/P intake, estimated from dietary records or food frequency questionnaires, is associated with higher serum PTH and lower bone mineral density.[15,16] Metz *et al*. showed that calcium intake was positively associated whereas P intake was negatively associated with bone mass and density although the Ca/P intake ratio was not mentioned.[17] However, Teegarden *et al*. found that Ca and P intake were positively correlated with bone measurements.[18] Thus, the relationship between habitual P intake and bone status has yet to be elucidated, whereas current evidence appears to show that calcium intake or the Ca/P intake ratio may have a positive influence on bone health. However, these studies were conducted in Western countries, and not in Asia.

The average daily Ca/P mg intake ratio (Ca/P intake ratio) in young adult Japanese women aged 18 to 29 years was 0.50 (475/955).[19] Although there are some methodological differences in the survey, the average daily Ca/P mg intake ratio in Western countries was 0.70-0.76 (802-

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Manuscript received 8 November 2010. Initial review completed 1 March 2011. Revision accepted 11 April 2011.

1056/1056-1411).[15-18,20] This suggests that young Japanese women have a lower Ca/P intake ratio than young Western women because of low Ca intake in Japan.

The number of studies conducted among young Japanese women is limited. P and Ca intake and the Ca/P intake ratio measured using the duplicate sampling method had positive correlations with femoral neck bone mineral density (BMD) in Japanese women aged 19 to 25 years.[21] However, it would be difficult to assert that this nutritional survey accurately represents habitual intake because the study was conducted over 3 consecutive weekdays.

The objective of this study was to examine the influence of P and Ca intake and the Ca/P intake ratio on the BMD in young Japanese women whose Ca/P intake ratio was assumed to be lower than that of young Western women.

MATERIALS AND METHODS

Design

Subjects were first-year female students of Kagawa Nutrition University in 2006 and 2007. The purpose and protocol of this study were explained to the subjects in advance. Written informed consent was obtained from each subject. The procedures used in this study were approved by the Ethics Committee of Kagawa Nutrition University and the Japanese National Institute of Health and Nutrition. A total of 475 subjects (238 in 2006 and 237 in 2007) took part in the study. The following subjects were excluded from analysis; 9 subjects who were aged more than 23 years and 6 subjects who were smokers. Finally, 441 subjects (92.8%) who had all the required data except for PTH and 25-hydroxy vitamin D (25(OH)D) were analyzed. The number of subjects of PTH and 25(OH)D were 214 of the 441 subjects because PTH and 25(OH)D were measured only in 2006.

Measurements

Body height and weight were measured to the nearest 0.1 cm and 0.1 kg, respectively, while wearing light clothes and no shoes. Body mass index (BMI) was calculated as body weight (kg) divided by the square of body height (m). Serum samples were obtained from blood drawn under fasting conditions. Subjects were asked not to eat or drink with the exception of water after 10 pm on the day prior to blood sampling. A single 24-h urine sample was collected. Subjects were instructed both in writing and orally on the method of urine collection and the necessity of obtaining a complete 24-h urine collection. We requested the subjects to eat and drink normally during the collection and to follow their usual pattern of activity. Subjects were then provided with a bag, three or four 1-L plastic bottles (containing no additives) and 10 400-ml cups. A recording sheet was also provided. In the morning, subjects were asked to discard the first specimen and to record the time on the sheet (the start of the collection period). Subjects were asked to collect all specimens by the time of the start of the collection period in the next morning. When some specimens were missed, subjects were asked to record the estimated volume of missing urine and the time. In the next morning, subjects were asked to collect the last specimen at the time when the specimen was discarded last morning and to record the time on the sheet (the end of the collection period). The sheet was reviewed by staff when the collection bottles were handed in, and any missing information was obtained from subjects. The height of urine in each bottle was measured and later converted into volume with an empiric formula based on repeated measurements of volume in identical bottles.[22] All urine from the 24-h collection period was then combined and mixed thoroughly by vigorous stirring, and several urinary aliquots were taken for analysis. Total 24-h excretion of P and Ca calculated by multiplying the measured concentration with the total volume of urine collected. Serum and urinary inorganic P was determined with the direct method using molybdic acid.[23] Serum and urinary Ca was determined with the ortho-cresolphtalein complexone (OCPC) method.[24] Serum intact PTH was measured with the electrochemiluminescence immunoassay (ECLIA) method.[25] Serum 25(OH)D was measured with the double antibody radioimmunoassay (RIA-DA) method.[26] All serum and urinary samples were maintained at a temperature of −20°C while in transport for measurement at a laboratory in Tokyo (SRL Inc., Tokyo, Japan). Bone mineral density in the distal one-third of radius (R3) of non-dominant forearm, lumbar spine (L2−L4) and femoral neck of non-dominant leg was measured by dual-energy X-ray absorptiometry (DCS-600 for radius and DCS-900 for lumbar spine and femoral neck, Aloka Co., Ltd., Tokyo, Japan). Dietary habits during the preceding month were assessed using a self-administered diet history questionnaire (DHQ).[27] Estimates of dietary intake for energy, and nutrients including P and Ca were calculated using an ad hoc computer algorithm for the DHQ based on the Standard Tables of Food Composition in Japan.[28] Pearson's correlation coefficients between the DHQ and 3-d estimated dietary records were 0.59 for P and 0.49 for Ca.[27] Postmenarcheal age (age at the survey minus age at menarche) and total duration of physical activity (h/month) (total time of vigorous and moderate exercise and walking in a month) were reported in a lifestyle questionnaire.

Statistical analysis

Pearson's product-moment correlation coefficient was calculated to test for linear correlations of P and Ca intake and the Ca/P intake ratio with serum or urinary inorganic P and Ca and serum intact PTH. Multiple regression analysis was used to assess the influence of P and Ca intake and the Ca/P intake ratio on BMD, adjusting for postmenarcheal age, BMI and duration of physical activity. A p-value <0.05 was considered statistically significant. All statistical analyses were performed using SPSS version 15.0 (SPSS, Inc., Chicago, IL).

RESULTS

The subject's demographic and physical characteristics, serum and urinary measurements, BMD, duration of physical activity and nutrient intakes are shown in Table 1. The body height and weight of the subjects were not significantly different from subjects aged 18 to 20 years that participated in the National Nutrition Survey, Japan.[19] Serum P and Ca levels were not significantly different from those found in young Japanese women aged

19.5±5.6 years in another cross-sectional study.[29] The daily Ca/P mg intake ratio was 0.57 (554/963).

Table 2 shows the correlation coefficients for P and Ca intake and the Ca/P intake ratio with serum or urinary inorganic P and Ca, and serum intact PTH. Phosphorus and Ca intake and the Ca/P intake ratio correlated positively only with urinary P.

Table 3 shows the results of linear regression models used to examine the relationship between P and Ca intake and the Ca/P intake ratio and BMD adjusted for postmenarcheal age, BMI, and duration of physical activity. A higher Ca intake and a higher Ca/P intake ratio were individually significantly associated with greater BMD in the distal radius, but not with BMD in the lumbar spine and femoral neck. The results did not change when the subject's age was added into each model. In this study, serum 25(OH)D had no association with any BMD. The results did not change when serum 25(OH)D was added into each model.

Table 1. Characteristics of study subjects (n = 441)[†, ‡, §]

Variable			
Age (y)	19.0	±	0.5
Postmenarcheal age (y)	7.0	±	1.5
Body height (cm)	158.3	±	5.5
Body weight (kg)	53.8	±	7.2
BMI[¶] (kg/m^2)	21.4	±	2.5
Serum Pi[††] (mg/dL)	4.0	±	0.4
Serum calcium (mg/dL)	9.8	±	0.3
Serum intact PTH[‡‡] (pg/mL)	22.4	±	8.8
Serum 25(OH)D[§§] (ng/mL)	12.6	±	4.1
Urinary Pi[††] (mg/day)	649	±	166
Urinary calcium (mg/day)	103	±	48
BMD[¶¶]			
Radius (mg/cm^2)	662	±	49
Spine (mg/cm^2)	1058	±	115
Femoral neck (mg/cm^2)	979	±	113
Physical activity (h/month)	26.3	±	38.9
Nutrient intakes			
Energy (kcal/day)	1785	±	434
Protein (% Energy)	13.7	±	1.9
Fat (% Energy)	29.7	±	4.9
Carbohydrate (% Energy)	56.6	±	5.7
Vitamin D (μg/1000 kcal)	3.5	±	1.8
Phosphorus (mg/1000 kcal)	537	±	104
Calcium (mg/1000 kcal)	309	±	109
Calcium/phosphorus ratio (mg/mg)	0.57	±	0.12

[†] Values are mean ± standard deviation.
[‡] n = 214 for serum intact PTH and 25(OH)D.
[§] Standard values; serum Pi 2.4-4.3 mg/dL, serum calcium 8.7-10.1 mg/dL, serum intact PTH 10-65 pg/mL and serum 25(OH)D 7-41 ng/mL.
[¶]Body mass index; [††] Inorganic phosphorus; [‡‡] Parathyroid hormone; [§§] 25-hydroxy vitamin D; [¶¶] Bone mineral density.

DISCUSSION

In this study, the relations of habitual P and Ca intake and the Ca/P intake ratio with bone mineral density were examined in young Japanese women. Calcium intake and the Ca/P intake ratio individually were positively associated only with BMD in the distal one-third of radius. Sasaki *et al*. showed that P intake had negative and Ca intake had positive associations with BMD in the calcaneus in premenopausal Japanese women.[29] This is inconsistent with our results. One of the reasons for this inconsistency may be due to the difference in terms of P intake levels, age and the site of BMD.

Orito *et a.l* reported that serum Ca was constant between the ages 12 and 30, while serum P gradually decreased from ages 12 to 18 and remained constant thereafter.[30] As the average age of the subjects in this study was 19.0 years, serum inorganic P and Ca levels in these subjects were expected to be stable. Also, serum inorganic P and Ca levels had no relationship to P and Ca intake. This is consistent with previous observations that major alterations in dietary P and Ca intake produce only small alterations in circulating levels due to the combined action of several hormones on bone, intestinal and renal transport in healthy individules.[31,32] Furthermore, there was no relationship between serum PTH and P and Ca intake and the Ca/P intake ratio. This differs from the findings of Kemi *et al.* who found that the 1st quartile of the Ca/P intake molar ratio ≤0.50 (mg ratio ≤0.65) had higher serum PTH than other groups.[20] The 1st quartile of the subjects in their study is equivalent to about 75% of this study. The difference in the results could be attrib uted to this difference in the Ca/P intake ratio due to P and/or Ca intake level.

Table 2. Pearson's correlation coefficients for phosphorus and calcium intake, and calcium/phosphorus intake ratio with serum and urinary measures (n = 441)[†]

	Phosphorus intake (mg/1000 kcal)		Calcium intake (mg/1000 kcal)		Calcium/phosphorus intake ratio(mg/mg)	
	r	*p*	*r*	*p*	*r*	*p*
Serum Pi[‡] (mg/dL)	0.055	0.248	0.048	0.318	0.026	0.580
Serum calcium (mg/dL)	0.001	0.983	0.025	0.603	0.039	0.416
Serum intact PTH[§] (pg/mL)	-0.020	0.772	0.006	0.928	0.005	0.939
Urinary Pi[‡] (mg/day)	0.206	< 0.001	0.202	< 0.001	0.140	< 0.01
Urinary calcium (mg/day)	0.070	0.140	0.033	0.486	-0.004	0.938

[†] n = 214 for serum intact PTH.
[‡] Inorganic phosphorus.
[§] Parathyroid hormone.

Table 3. Multiple regression analysis of BMD† against postmenarcheal age, BMI‡, physical activity, and phosphorus and calcium intake or calcium/phosphorus intake ratio (n = 441)

Dependent variable/Predictors	Model 1§		Model 2¶	
	b (SE) ††	*p*	b (SE) ††	*p*
Radius BMD† (mg/cm^2)				
Postmenarcheal age (y)	3.85 (1.54)	< 0.05	4.00 (1.54)	< 0.05
BMI‡ (kg/m^2)	4.28 (0.92)	< 0.001	4.17 (0.92)	< 0.001
Physical activity (h/month)	-0.08 (0.06)	0.179	-0.08 (0.06)	0.188
Phosphorus intake (mg/1000 kcal)	-0.02 (0.04)	0.553	-	-
Calcium intake (mg/1000 kcal)	0.08 (0.04)	< 0.05	-	-
Calcium/phosphorus intake ratio (mg/mg)	-	-	48.11 (19.21)	< 0.05
R^2‡‡	0.091		0.085	
Spine BMD† (mg/cm^2)				
Postmenarcheal age (y)	12.52 (3.41)	< 0.001	12.49 (3.40)	< 0.001
BMI‡ (kg/m^2)	16.09 (2.04)	< 0.001	16.25 (2.03)	< 0.001
Physical activity (h/month)	-0.13 (0.13)	0.335	-0.13 (0.13)	0.328
Phosphorus intake (mg/1000 kcal)	-0.13 (0.09)	0.158	-	-
Calcium intake (mg/1000 kcal)	0.14 (0.09)	0.093	-	-
Calcium/phosphorus intake ratio (mg/mg)	-	-	51.90 (42.47)	0.222
R^2‡‡	0.180		0.177	
Femoral neck BMD† (mg/cm^2)				
Postmenarcheal age (y)	0.23 (3.66)	0.950	0.04 (3.66)	0.991
BMI‡ (kg/m^2)	6.34 (2.20)	< 0.01	6.62 (2.18)	< 0.01
Physical activity (h/month)	-0.08 (0.14)	0.587	-0.08 (0.14)	0.569
Phosphorus intake (mg/1000 kcal)	-0.11 (0.10)	0.257	-	-
Calcium intake (mg/1000 kcal)	0.07 (0.09)	0.448	-	-
Calcium/phosphorus intake ratio (mg/mg)	-	-	6.34 (45.70)	0.890
R^2‡‡	0.025		0.022	

† Bone mineral density. ‡ Body mass index.
§ Predictors are postmenarchael age, BMI, physical activity, phosphorus intake and calcium intake.
¶ Predictors are postmenarchael age, BMI, physical activity and calcium/phosphorus intake ratio.
†† Multiple regression coefficient (Standard Error)
‡‡Coefficient of determination.

Phosphorus and Ca intake and the Ca/P intake ratio had no relationship with urinary Ca, but has with urinary phosphorus. Dietary P and Ca are absorbed from the intestine and the excess is excreted into urine. If the homeostasis of P or Ca in the body is maintained, the absorbed amount is nearly equal to or correlates closely with the amount excreted in the urine.[33] Unlike Ca, P is readily and efficiently absorbed at all levels of dietary intake.[34] This would suggest that levels of urinary P are more strongly influenced by intake than urinary Ca.

In this study, Ca intake had small but positive effect on BMD in the radius not in the spine or the femoral neck. This is inconsistent with previous findings in the U.S. that Ca intake was correlated with both radius (measurement site was not mentioned) and spine BMD in women aged 18 to 31 years.[18] However, we could not find the reason for this inconsistency. BMD in the distal one-third of radius measured using the same device (DCS-600) showed peak BMD at 34 years among Japanese women aged 20 to 39 years (data not published). In another study among Japanese women, BMD of the lumbar spine and total hip peaked at age 18 years although the device used to measure BMD was different from ours.[30] In Swedish men, peak BMD of the radius occurred later than peak BMD of the lumbar spine and femoral neck.[35] Thus, in this study, subjects may have attained peak BMD of the lumbar spine and femoral neck but not of the radius. Also, bone at different site has different properties; radius is cortical and non-weight bearing bone, while lumbar spine and femoral neck are trabecular and weight bearing bone. Those could be attributed to the fact that, in this study, the effects of dietary Ca and the Ca/P intake ratio were seen only in the radius. Sexual maturity, physical size and physical activity are assumed to contribute to building peak bone mass.[36] In this study, the effects of Ca intake and the Ca/P intake ratio on the BMD in the radius were seen independently of postmenarcheal age, BMI and physical activity. Since radius is a non-weight bearing bone, we couldn't find why BMI had a positive association with BMD in the radius.

The limitation of this study is that intake of P may be underestimated because much of the P contained in food additives is not included in nutrient composition tables.[7,34,37] There are several studies undertaking oral P loading tests.[13,14,38,39] In these studies, the exact amount of added P is known but the duration of these studies was short. A long-term loading test is not feasible in consideration of the burden this would impose on the subjects. The short-term changes could be mediated by processes that are distinct from those required for longer term adaptations to changes in dietary P.[40] It is meaningful to examine the long-term effects of habitual dietary intake as if there is a limitation.

In conclusion, we found that in young Japanese women, P intake did not have a significantly negative effect on bone mineral density, and Ca intake, and the Ca/P intake ratio had a small but significant association only in a site-specific manner with BMD. These results are different from the results of the studies conducted in Western countries. Further studies to clarify the relationship be-

tween more accurately determined amounts of dietary P and Ca, and bone mineral status in the Asian population are required.

ACKNOWLEDGMENTS

I would like to thank the participating students. KU was responsible for BMD measurement and data collection. SS was responsible for data collection. KM was responsible for analysis of DHQ. SI and SS were responsible for statistical analysis. SI and HI were responsible for designing the study and writing the paper.

AUTHOR DISCLOSURES

No personal financial interest in the work or with a commercial sponsor involved.

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Original Article

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日本年輕女性日常膳食磷與鈣攝取與骨密度之相關性

磷與鈣在骨骼健康上扮演著重要的角色。過去西方國家研究發現，低鈣與高磷攝取，會導致較低的鈣磷比，而對於骨骼密度狀態有負面的影響。本篇橫斷型研究目的，在探討 441 位日本年輕女性(18-22 歲)，其日常磷與鈣攝取情形及鈣磷比與骨密度之相關性。本研究並假設日本年輕女性相較於西方年輕女性有較低的鈣磷比。此外，本研究也嘗試釐清，飲食鈣與磷攝取量與血清或尿液鈣與磷之相關性，同時也檢測其中 214 位女性的血清副甲狀腺素與 25(OH)D。結果發現，磷與鈣攝取及鈣磷比與尿液磷呈現顯著正相關。在調整了初經後年齡、身體質量指數及體能活動後，鈣攝取及鈣磷攝取比與橈骨遠端骨密度呈現顯著正相關，但與腰椎骨及股骨頸的骨質密度沒有顯著相關性存在。本篇研究結果指出，日本年輕女性之磷攝取對於骨質密度沒有顯著的負面影響，而鈣攝取與鈣磷比僅與某些特定部位之骨質密度有顯著的相關。

關鍵字：磷、鈣、骨質密度、副甲狀腺素、日本年輕女性