### Short Communication

# Average daily intake of phosphorus in 3- to 5-year-old Japanese children as assessed by the duplicate-diet technique

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The present study aimed to determine whether there is excessive mean daily intake of phosphorus in 3- to 5year-old Japanese children and to ascertain relationships between phosphorus intake and various food and beverage groups. Subjects comprised of 90 children, with 15 boys and 15 girls in each age group from 3 to 5 years. The duplicate-diet technique was used to ascertain total dietary intake, including snacks and beverages over a 24-h period on 3 separate days from summer 1999 to winter 2000. After wet ashing food samples, phosphorus was quantified by colourimetry using a spectrophotometer. Median and 25th-75th percentile daily phosphorus intake for 3- to 5-year-old Japanese children was 674 mg (534-890 mg), and phosphorus intake correlated with the intake of many food groups and was closely correlated with total daily intake of food and beverage (rs=0.64). In addition, phosphorus intake correlated with the intake of magnesium and calcium (rs≥0.6, p<0.001). When assessed based on dietary reference intakes for the US, maximum intake did not exceed the tolerable upper intake level in any of the young children, but phosphorus intake was not more than the estimated average requirement (EAR) in 4.4% of subjects, which exceeded the target of 2.5% for the US EAR. We concluded that there is a risk of insufficient phosphorous intake, rather than excessive intake, for 4.4% of 3- to 5-year-old Japanese children.

## Key Words: phosphorus intake, duplicate-diet technique, preschool children, seasonal variation, nutrition survey

#### INTRODUCTION

Phosphorus (P) is an essential element for energy metabolism requiring phosphorylation. Shifts in dietary P intake are directly related to serum P levels and urinary P excretion and affect the absorption and metabolism of other minerals, such as calcium (Ca) and magnesium (Mg).<sup>1-4</sup> Shibniews<sup>5</sup> reported that the wide use of P as a food additive in processed foods carries a risk of excessive P intake, which has a negative impact on Ca metabolism. In addition, several researchers<sup>6-8</sup> have reported insufficient Ca/P ratio in the diet leads to osteoporosis and bone fractures. Abrams et al<sup>9</sup> reported that the balance between Ca and Mg is important in bone formation. The accurate assessment of mineral intake during infancy is also important. Shibata et al.<sup>10</sup> and Goshima et al.<sup>11</sup> have reported mineral intakes in preschool children. Nutritional studies conducted in many countries often assess food intake using food composition tables. Previous studies on P intake have thus employed the diet record technique<sup>12-15</sup> or the 24-h recall technique.<sup>16,17</sup> Kimura and Itokawa<sup>18</sup> have reported a large loss of minerals through cooking. Also, since food composition tables are generally based on the

data for raw foods, mineral intake cannot be accurately ascertained from cooked foods. In Japan, many foods are boiled, and mineral intake may likewise be affected by cooking methods. While the duplicate-diet technique<sup>5,19,20</sup> can be used to directly analyze foods that are actually consumed, few studies have applied this technique, and no study has examined young children.

In this study, we measured P intake in 3- to 5-year-old Japanese children using the duplicate-diet technique, calculated the Ca/P ratio using published data, and Ca/P ratio that may affect bone metabolism. We also investigated the relationship of P intake to the intakes of various food

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Manuscript received 18 March 2009. Initial review completed 29 July 2009. Revision accepted 14 August 2009.

groups and the intakes of soft drinks, which are believed to be a key cause of excess P intake.

#### MATERIAL AND METHODS

The study protocol was reviewed and approved by the ethics committee of Aichi-Gakuin University.

Subjects were 30 children (15 boys and 15 girls) in each of the 3-, 4- and 5-year old groups attending preschools in Yokkaichi and neighbouring cities in Mie Prefecture, Japan. Duplicate portions of all foods and drinks consumed by a child were collected by the duplicationportion technique for 3 days (1 day each in summer, autumn, and winter) during the school year from April 1999 to March  $2000^{21}$  and the total number of days of sample collection from all 90 children was 19 (7 days in summer, 6 days in autumn, and 6 days in winter). We excluded the spring collection because April is the beginning of the new school year in Japan. The body weight of each child was checked monthly by staff at the participating preschools. The total intake of all recovered foods was weighed using a scale (PE3600; Mettler, Greifensee, Switzerland) to determine daily food intake. Next, for each sample, distilled water was added and blended using an industrial blender (Warring HGB-SS; Waring, Atlanta, GA, USA). The resulting smooth and homogenized specimen was vacuum-packed (Tospack V-222; Tosei, Shizuoka, Japan) and stored at -30 °C. After thawing each stored sample, wet ashing was performed using nitric acid (UGR grade; Kanto Chemical, Tokyo, Japan) and 30% hydrogen peroxide (Wako Pure Chemical Industries, Osaka, Japan).<sup>10,11</sup> Each ashed sample was placed on a hotplate (Advantec HTP552AA; Toyo Seisakusho Kaisha, Tokyo, Japan) at 90 °C and left to stand for 24 h. A spectrophotometer (UV/Vis spectrometer Lambda 11; Perkin-Elmer, Ueberlingen, Germany) was used to determine phosphorus intake by colourimetry<sup>22</sup> Recovery rates were verified by the P addition method in which a set amount (80 µg) of P is added to food samples (n=20). For Ca, we used data obtained by atomic absorption spectrophotometry by Shibata et al.<sup>10</sup> on the same sample.

Statistical analysis was conducted using SPSS 11.0J. Normality was assessed using the Shapiro-Wilk test, but because a normal distribution was not confirmed, we expressed the numerical data as median and 25th-75th percentile ranges. To allow comparisons between the present results and the findings of other studies, mean, standard deviation (SD) and range were also determined. The Kruskal-Wallis test was used to analyze age differences, while the Mann-Whitney test was used for gender differences and the Friedman test was used to assess seasonal variations. Comparisons for Ca/P ratio between age groups were performed by the Kruskal-Wallis test followed by multiple comparison testing using the Mann Whitney U test with Bonferroni correction. Spearman rank correlation coefficients were used to assess correlations. Furthermore, measured phosphorus intake values were assessed based on Japanese<sup>23</sup> and US<sup>24</sup> dietary reference intakes (DRIs).

#### RESULTS

Mean recovery rate of P as assessed by the addition method was 101.0% (coefficient of variation, 5.9%).

Table 1 shows daily dietary P intake, daily intake per kg body weight, and Ca/P ratio for the 3 to 5 year olds in each age group. No significant differences existed in P intake among the 3 age groups, but a gender difference was identified (p < 0.05): median (25th-75th percentile) was 754 (574-948) mg for boys and 635 (475-804) mg for girls in all age groups. Mean (±SD) of Ca/P (w/w) ratio for 3, 4 and 5 year olds was 0.639±0.168, 0.635±0.188 and 0.512±0.165, respectively. Although no significant age difference between 3- and 4 year olds, significant age differences were seen in Ca/P ratio (p < 0.05). No significant gender difference was identified. There were differences in daily P intake for 3 to 5 years olds among three seasons, which were similar to those in total daily food intake (p < 0.001). Median (25th-75th percentiles) daily P intake was 718 (502-886) mg in summer, 735 (551-1200) mg in autumn, and 497 (374-654) mg in winter. Mean (±SD) total food intakes in summer, autumn and winter were 1340±288 g, 1430±337 g and 1150±261 g, respectively. Table 2 shows the correlation between mean daily P intake and intake of various food groups. Mean daily P intake correlated strongly with total food and beverage intake (rs=0.64, p<0.001). Also shown in Table 2, daily P intake exhibited a significant positive correlation to intake of the following foods and beverages: milk and dairy products (rs=0.47, p<0.001); meats (rs=0.32, p<0.001); beans and bean products (rs=0.41, p<0.001); green and yellow vegetables (rs=0.39, p<0.001); hypochromic vegetables (rs=0.25, p < 0.01); fruits (rs=0.27, p < 0.01); sugars (rs=0.23, p<0.05); and milk (rs=0.40, p<0.001).

Table 3 shows annual mean daily P intake in percentiles. Adequate intake (AI) of P (800 mg) in Japanese children was found at the 55.4 percentile for boys and 74.6 percentile for girls in our study. In addition, daily mean P intake was compared to the US Estimated Average Requirement (EAR) of P (380 mg for 1-3 years, 405 mg for 4-8 years) and the percentage of subjects whose P intake was not more than the US EAR target (2.5%) was 4.4%.

#### DISCUSSION

The target subjects in this study are the same as in the study by Goshima et al.<sup>11</sup> As stated by Goshima et al.,<sup>11</sup> body type and energy intake for subjects were comparable to the results of the National Health and Nutrition Survey (2003),<sup>25</sup> suggesting that the present study could be compared to those survey findings.

The National Health and Nutrition Survey (Ministry of Health, Labour and Welfare, Japan) is conducted in autumn (November 1) using the weighed food record method (a diet recording method). The AI for P according to the Japanese DRI is set at the median intake as ascertained by the 2001 National Health and Nutrition Survey.<sup>25</sup> Median autumn intake in the present study was slightly lower than the AI (800 mg). Japan experiences clear seasonal variations in weather. Different foods are consumed in different seasons, and foods are also cooked differently in different seasons. Since the daily P intake of the total daily beverage intake was not measured, the relationship between daily P intake and total daily beverage intake on the seasonal variations was not confirmed but there were significant differences among summer, autumn

**Table 1.** Daily intake of phosphorus and Ca/P ratio in 3- to 5-year-old Japanese children (n=90)<sup>†</sup>

Group <sup>‡</sup>	n	Body Weight <sup>10</sup>	mg/day							mg/kg/day						
			Percentile					Ca/P ratio	p	Percentile			Mean±SD	p	Ca/P ratio	p
1		kg	50 (Median)	25-75	Range	Mean±SD	value	Mean±SD	value	50 (Median)	25-75	Range	Wiedn±5D	value <sup>™</sup>	Mean±SD	value
3 year olds	30	15.1±2.2	643	477-752	320-1650	677±289		0.639±0.168		39.9	33.9-57.7	23.8-115	44.9±18.1		0.578±0.147	
4 year olds	30	17.0±1.3	702	536-973	325-1640	778±317	n.s.	0.635±0.188	*	41.2	31.5-56.8	21.6-94.3	45.8±18.8	n.s.	0.579±0.209	*
5 year olds	30	19.1±2.7	771	549-923	456-1730	783±284		0.512±0.165		38.4	28.6-48.1	26.9-93.4	41.6±16.7		0.623±0.185	
Boys	45	17.0±2.8	754	574-948	363-1650	806±290	*	$0.600 \pm 0.208$		43.5	37.5-58.7	23.5-115	48.2±18.3	*	0.570±0.169	
Girls	45	17.2±2.7	635	475-804	320-1730	686±297		0.592±0.160	n.s.	33.6	28.6-46.8	21.6-93.4	40.0±16.5		$0.620{\pm}0.194$	n.s.
Total	90	17.1±2.7	674	534-890	320-1730	746±298		0.595±0.183		39.4	31.1-50.9	21.6-115	44.1±17.8		0.595±0.183	

<sup>†</sup> All data have three significant digits.
<sup>‡</sup> As of the beginning of the preschool year (April 2, 1999).
<sup>¶</sup> The kruskal-wallis test was used to compare age differences, and the Mann-Whitney test was used to compare gender differences.
\*: p <0.05, \*\*: p <0.01, \*\*\*: p <0.001, n.s.: not significant</li>

	Daily intake of each food	Phosphorus intake (mg/day)				
Food groups (g/day) and beverages	group and beverage (g/day) Mean±SD	Rs	<i>p</i> -value			
Milk and dairy products	178±119	0.47	***			
Eggs	31±19	-0.04	n.s.			
Fish and shellfish	27±20	0.20	n.s.			
Meats	44±22	0.32	**			
Beans and bean products	29±23	0.41	***			
Green and yellow vegetables	42±27	0.39	***			
Hypochromic vegetables	70±37	0.25	**			
Mushrooms and seaweeds	13±8	0.14	n.s.			
Potatoes	19±19	-0.01	n.s.			
Fruits	$72 \pm 48$	0.27	**			
Cereals	246±60	0.09	n.s.			
Sugars	5±4	0.23	*			
Fats and Oils	12±6	-0.04	n.s.			
Confectioneries and others	168±89	0.09	n.s.			
Beverages without milk	412±137	0.17	n.s.			
Total intake	1370±245	0.64	***			
All beverages (g/day)						
Milk	135±102	0.40	***			
Milky and lactic acid beverages	33±44	0.17	n.s.			
Green tea	72±96	0.21	n.s.			
Oolong tea	23±94	-0.11	n.s.			
Black tea	4±13	-0.05	n.s.			
Blended teas	30±77	-0.03	n.s.			
Barley tea	183±138	0.07	n.s.			
Other beverages and tap water	67±65	0.05	n.s.			
All beverages	547±160	0.42	***			

**Table 2.** Correlation coefficients between daily phosphorus intake from diets (mg/day) and daily intake of each food group and beverage (g/day) (Spearman)

\*: p < 0.05, \*\*: p < 0.01, \*\*\*: p < 0.001, n.s.: not significant

Age group	sex	mean±SD	Percentiles													
			1		5		10		25		50	75	90	95	99	
3 years <sup>†</sup>	boys	741±299	388	$\Delta$	492		548		567		654	764	982	1220	1570	
	girls	613±274	330		370		396		432	$\Delta$	518	715	934	1090	1290	
4-5 years <sup>‡</sup>	boys	839±284	444		465	$\Delta$	512		585		856	1020	1150	1250	1550	
	girls	722±306	362		422		472	Δ	506		653	809	1050	1340	1630	

Table 3. Distribution (percentiles) of daily intakes of phosphorous by 3- to 5-year-old Japanese children and evaluation based on DRIs

 $\Delta$  is the U.S recommended dietary allowance (RDA) of P, which is 460 mg for 1- to 3-year-old children and 500 mg for 4- to 8-year-old children.

▲ is the U.S. estimated average requirement (EAR) of P, which is 380 mg for 1- to 3-year-old children and 405 mg for 4- to 8-year-old children.

<sup>†</sup>The number of children with P intake less than the U.S. RDA (460 mg/day) was 1(6.7%) of 15 boys and 6 (40.0%) of 15 girls. The children with P intake less than the U.S. EAR (380 mg/day) was 1 (6.7%) of 15 boys and 1(6.7%) of 15 girls.

<sup>1</sup>The number of children with P intake less than the U.S. RDA (500 mg/day) was 3 (10.0%) of 30 boys and 7 (23.3%) of 30 girls. The children with P intake less than the U.S. EAR (405 mg/day) was 0 (0%) of 30 boys and 2 (6.7%) of 30 girls, respectively.

and winter results in both the daily P intake and the total daily dietary intake. We presume that these differences may be responsible for the following findings in the dietary survey: 1) the intake of Milk and dairy products, a major source of P, was higher in summer than that in winter, and 2) P is contained in many food and beverages, P intake had a strong correlation with the total dietary intake, and the total dietary intake of P was the highest in autumn.

The present study showed seasonal differences in P intake, and seasonal variations in Japan must therefore be taken into account.

According to the 2003 National Health and Nutrition Survey,<sup>25</sup> the mean ( $\pm$ SD) P intake for 3 to 5 year old Japanese children is 758±222 mg, similar to our result. In the US, mean (±SEM) P intake was 982±301 mg/day for 5-year-old children<sup>12</sup> and 968±21.5 mg/day for children <6 years of age.<sup>15</sup> In Greece, mean (±SD) P intakes for 4to 5-year-old boys and girls were 1120±379 mg/day and 1150±346 mg/day, <sup>13</sup> respectively. Mean daily P intake in Western countries is thus higher compared those in the present Japanese study. Nakamura et al.<sup>26,27</sup> conducted studies in adult women using the duplicate-diet technique and reported that the mean daily P intake (±SD) was  $1020\pm267$  mg and  $996\pm208$  mg. Mean ( $\pm$ SD) P intake as measured by the duplicate-diet technique was 2010±142 mg/day for adults in Poland<sup>5</sup> and 1440±79 mg/day for adults in Italy.<sup>28</sup> These figures are clearly higher than those for Japan, and differences such as higher intake of processed meat products containing polyphosphates (ham, sausages, etc.) may be attributable to food intake volume and dietary habits.

In the present study, Ca/P ratio (w/w) for the 3 to 5 year old children was 0.6, comparable to Suzuki's study<sup>29</sup> in which the ratio for 3 to 6 year old children was 0.7. Nakamura et al.<sup>26,27</sup> in studies using the duplicate-diet technique reported that Ca/P ratios in adult women were 0.51 and 0.52. In addition, Ueno et al.<sup>20</sup> found that the Ca/P ratio of 19- to 23-year-old female university students was 0.4, which is markedly lower when compared to the present study. As suggested by Ueno et al.,<sup>20</sup> the Ca/P (w/w) ratio for female university students reflects low intake of milk and dairy products and is caused by low Ca intake.

P is found in many food groups, and as shown in Table 2, P intake is closely correlated with total food and beverage intake. Although P intake was correlated with sugar intake, we speculated that the correlation between P intake and sugar intake may result from intakes of foods cooked using sugar because sugar is often used as a seasoning in Japan. The relationship between sugar intake and P intake will need to be studied further in the future.

In Japan, dietary habits have markedly changed in the last few decades, with decreased consumption of grains, beans and fish and increased consumption of animal products such as meats as well as foods rich in oils and fats. Also, since food additives are widely used in processed foods, excessive intake must be considered. Excessive P intake has a negative impact on the absorption and metabolism of Ca and Mg, and thus has been examined as a causative factor for osteoporosis.<sup>1,5</sup>

In recent years, increasing amounts of processed foods, soft drinks and fast foods have become available in Western countries, and their consumption has also increased markedly. Harnack et al.<sup>8</sup> and O'Connor et al.<sup>30</sup> reported that consumption of milk has markedly decreased in the US, while consumption of soft drinks has markedly increased. Lyytikainen et al.<sup>14</sup> documented that children and teenagers in Scandinavian countries consume more soft drinks due to changes in dietary habits. In the US, studies have reported higher incidences of bone fractures due to high consumption of carbonated beverages, particularly colas,<sup>31,32</sup> and higher incidences of obesity due to increased consumption of soft drinks.<sup>29</sup> However, these trends are not universal. Adair and Popkin<sup>33</sup> compared 4 countries, the US, Cuba, Russia and China, and reported that consumption of soft drinks has almost doubled over the last 20 years in the US and Cuba, while no marked changes have been seen in Russia and China in the last 10 years. In Japan, the consumption of tea-based soft drinks, rather than carbonated beverages, has increased in recent years.<sup>34</sup> Age differences were also seen in soft drink consumption. In the US and Russia, the degree of increase among 2 to 5.9 year olds was lower when compared to 11 to 18.9 year olds. The reason for this was that mothers and family members have greater control over what young children drink. As the subjects of the present study were young children, we assumed that their mothers and family members managed what the children were drinking. Furthermore, nutritionally balanced meals are provided in most Japanese preschools. Children in the present study also attended preschools that provide school lunches.

Median daily intakes for both boys and girls in the present study were lower than the AI for Japan. However, deficient subjects cannot be identified based solely on this parameter. In the present study, 4.4% of subjects had P intake not more than the US EAR, and although this exceeds the recommended value of 2.5%, no problems were observed, possibly due to the differences in age classification and body shapes between Japanese and US children. Intake of other minerals, including Ca and Mg, is also low for the children with low P intake. For 4.4% of subjects whose mean daily P intake was not more than the EAR, Ca intake ranged from 100 to 373 mg, which is below the AI for Japanese boys and girls (600 mg and 550 mg, respectively). Mg intake also ranged from 62.7 mg to 74.4 mg, below the EAR for Japanese boys and girls (85 mg and 80 mg, respectively). Equilibrium intake is the daily intake minus output. As far as Japanese equilibrium intakes (EIs) of P, as assessed by balance tests, are concerned, Nishimuta et al.<sup>35,36</sup> reported a mean P intake of 22.6 mg/kg/day for adults, while Suzuki<sup>29</sup> reported a mean of 27.2 mg/kg/day for 3- to 6-year-old children. Nishimuta et al.<sup>35,36</sup> have expressed "equilibrium intake alone" with an equation: Balance = (Intake) -((Urine output) + (Fecal output) + (Sweat loss)) mg/kg/day. However, these figures represent equilibrium intake alone, and growth-related accumulation in bones and tissues must be taken into account for children. Subsequently, Suzuki<sup>29</sup> conducted a balance test on 3- to 6year-old children and documented a positive balance of 34.7±0.97 (SE) mg/kg/day for boys and 32.7±1.28

mg/kg/day for girls. The previous Japanese DRIs (sixth revision: used from 2000 to 2005)<sup>37</sup> determined recommended daily P intake for 1- to 8-year-old children by multiplying body weight, and the EAR for 3- to 5-year-old children was set at 560 mg. Therefore, using either the Japanese or US EARs, a risk of insufficient P intake is seen for some Japanese 3- to 5-year-old children. In the present study of Japanese children, the highest daily P intake was 1730 mg, and the tolerable upper intake level (UL) for 1 to 8 year olds in the US (3 g/day) should thus be applicable. In the future, as these children get older and gain more control over what they drink, consumption of soft drinks (including carbonated beverages) is expected to increase due to westernization of diets. Follow-up studies are thus considered necessary.

#### CONCLUSION

Our study found that 4.4% of 3-to 5-year-old children in this study sample of Japanese children had a mean daily P intake not more than the U.S. EAR, which exceeded the target value of 2.5%. We conclude that some children potentially have a P deficiency. We considered that there is currently no risk of excessive intake due to processed foods and food additives in Japan, based on comparison of the maximum value of mean daily intake found in this study population with that of the U.S. UL.

#### ACKNOWLEDGEMENT

We would like to thank all participants, their parents and the staff of the preschools for their support. The present study was supported in part by the Strategic Research AGU-Platform Formation (2008-2012) from Ministry of Education, Culture, Sports, Science and Technology.

#### AUTHOR DISCLOSURES

There are no conflicts of interest.

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## Short Communication

## Average daily intake of phosphorus in 3- to 5-year-old Japanese children as assessed by the duplicate–diet technique

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# 使用雙重飲食取樣技術評估日本三到五歲小孩平均每日磷攝取情況

本篇研究目標為探討日本 3 至 5 歲小孩每日磷攝取是否過量,並確認磷攝取與 食物及飲料之相關性。共有 90 位小孩參與研究,在 3 到 5 歲各年齡層中,男 女各 15 位。於 1999 年夏天至 2000 年冬天選擇非連續 3 天,使用雙重飲食取 樣技術評估 24 小時飲食攝取,包含了點心及飲料。將食物樣本濕灰化後,接 著使用分光光譜儀,以比色法將磷定量。3 到 5 歲日本小孩磷攝取量中位數及 25-75 百分位分別為 674 mg 及 534-890 mg。磷的攝入與許多食物類別相關,並 且與食物及飲料總攝取量密切相關 (rs=0.64)。另外磷的攝取與鎂和鈣有相互 關聯 (rs>0.6, p<0.001)。所有兒童磷攝取最大量均未超過美國膳食建議最高 上限攝取量,且 4.4%受試者磷攝取低於美國建議的估計平均需要量 (EAR),這個比例大於 EAR 的設定目標即 2.5%。綜合上述,3 至 5 歲的日 本兒童,非但不是磷過量,反而 4.4%兒童有磷攝取不足之風險。

關鍵字: 磷攝取量、雙重飲食取樣技術、學齡前兒童、季節變異、營養調查