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Zinc Nutritional Status in Korean Adults from Rural, Urban and Metropolitan Areas

Soo-Lim Lee, Eun-Hee Kwak, Jin-Sook Yoon[1], Chong-Suk Kwon, John H. Beattie[2] and In-Sook Kwun[†]

Department of Food Science and Nutrition, Andong National University, Gyeongbuk 760-749, Korea
[1]Department of Food Science and Nutrition, Keimyung University, Daegu 704-701, Korea
[2]Cellular Integrity Division, Rowett Research Institute, Aberdeen, Scotland, United Kingdom

Abstract

The zinc intake and status of South Koreans from rural, urban and metropolitan areas were compared to evaluate the zinc nutritional status in different regional areas in South Korea. The dietary habits of 721 healthy adult subjects (271 from rural, 240 from urban, 210 from metropolitan city) with an age range 30~64 (mean age 54±18) were assessed using a food frequency questionnaire. Mean daily Zn intake for rural, urban, and metropolitan areas was 6.5 mg, 7.3 mg, and 11.4 mg ($p<0.05$), respectively, which was 54%, 61% and 95% of the Korean RDA for man (12 mg/d). Mean phytate : zinc molar ratios for rural, urban, and metropolitan city were 41, 34, and 30, respectively ($p<0.05$), which were higher than the cutoff level of 20 for poor zinc status. The zinc intake and phytate : Zn molar ratio in the rural area were 0.5 and 1.3-fold compared to those of the metropolitan city, which can cause poor zinc nutriture in the rural area. Most of the zinc biomarkers were lower in the rural area than in the metropolitan city ($p<0.05$) (mean rural and metropolitan values for plasma Zn: 80.8 μg/dL and 119.8 μg/dL, respectively; RBC Zn: 7.8 μg/dL and 8.8 μg/dL, respectively; plasma alkaline phosphatase (ALP) activity: 87 mU/mL and 100.4 mU/mL, respectively). It seems that a lower zinc intake in the rural area decreased zinc biomarker levels, such as plasma and RBC zinc, and plasma alkaline phosphatase activity, and caused the poor zinc nutritional status in this area. Most of the zinc biomarkers, such as RBC zinc and urinary and plasma zinc levels, in the subjects from the three localities, were within the normal range even when zinc intake of rural and urban subjects was low. The exception was plasma ALP activity in the rural area, which was lower than the reference level. Thus, marked zinc deficiency in these subjects were not observed, however, the potential for marginal zinc deficiency should be considered, especially for the rural area, because of the low zinc intake and the biomarker levels for marginal zinc deficiency.

Key words: zinc intake, rural, metropolitan, phytate : zinc, plasma zinc, red blood cell zinc, plasma alkaline phosphatase, South Koreans

INTRODUCTION

Zinc is crucial to vital processes, playing a unique role in growth and development. This trace element is a catalytic component of more than 200 enzymes and a structural component of a large number of proteins, hormones, neuropeptides, hormone receptors, and nucleotides (1). The Korean Recommended Daily Allowance (RDA) for dietary zinc is 12 and 10 mg/d for males and females, respectively (2). However, there is evidence that zinc intakes in the rural population in South Korea are significantly lower than the RDA levels (3,4). Various grains and legumes, which contain a high level of phytate, are the main portions of the typical Korean diet, especially in the rural areas. Phytate as inositol hexaphosphate inhibits zinc absorption and is likely to exacerbate deficiency caused by low zinc intake (5-7). There is therefore a very significant risk that a proportion of South Koreans in the rural area are zinc deficient and that their zinc nutritional status is compromised.

We previously reported two studies for evaluating the potential for zinc deficiency in Koreans. The first study estimated dietary zinc intakes from the 214 most consumed foods over a 2-day period in 2000 households (3), using data from the 1995 National Nutrition Survery. The results of this study suggest that dietary zinc intake in large cities is around 80% of the Korean RDA for zinc, but that in rural areas, the mean zinc intake is only

[†]Corresponding author. E-mail: iskwun@andong.ac.kr
Phone: +82-54-820-5917, Fax: +82-54-823-1625

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around 60% of the RDA. In that study, zinc intake was lower and phytate intake was higher in rural areas compared to metropolitan city areas, which implied that zinc deficiency was prevalent in rural areas. However, in that study it was only possible to obtain data from foods included in the survey, and the estimation of zinc intake may have been incomplete. In addition, there was no assessment of zinc status using biochemical indicators. Therefore in the second study, we evaluated zinc nutritional status using biomarkers including plasma zinc, blood cell zinc, hair, nail and urinary zinc, in addition to dietary zinc intake estimation by food frequency questionnaire. In that study, we reported the zinc nutritional status of Korean adults, which did not account for regional differences between rural and large city localities. In addition to dietary assessment, conventional biochemical indices for evaluation of human zinc status are used, including zinc levels in plasma or serum, red blood cells (RBCs), white blood cells, urine, hair and nail, or zinc-dependent enzyme activities, such as alkaline phosphatase (8).

Therefore, in the present study, we evaluated zinc nutritional status in three different South Korean localities, namely rural, urban, and metropolitan city areas, using zinc intake from complete food intake data and conventional zinc biochemical indices. Along with the dietary zinc assessment, biochemical zinc indices would be beneficial to evaluate zinc nutritional status in adult Koreans. Also, a comparison between three different localities, rural, urban, and metropolitan city, would give a more comprehensive understanding of zinc nutritional status in South Koreans.

SUBJECTS AND METHODS

Subjects

The subjects in this study were recruited from three different areas (Andong County, Andong City, and Daegu City) representing rural, urban and metropolitan city populations, which were reported to be ca 2.6, 190, and 2,540 thousand people, respectively, at the time of the study. The subjects (total 721; 271, 240 and 210 from rural, urban and metropolitan city areas, respectively) aged between 21 ~ 69 yrs were randomly selected from the register of the 2000 ~ 2001 Health Promotion Project in the Andong and Daegu Public Center, supported by Ministry of Health and Welfare, and private clinics in Andong area and Daegu city. The study protocol was approved by the Ethical Committee of Andong National University and written consent was obtained from each subject after the aim of the study had been explained to them.

Anthropometric assessment

Selected anthropometric measurements were made; height and body weight were measured with the subjects wearing light clothing and no shoes. Body fat was measured using a body composition analyzer (inBody 3.0 Body Composition Analyzer, Biospace, South Korea) using electronic impedence analysis. Each measurement was taken by the same anthropometrist to avoid inter-examiner error.

Dietary assessment for Zn, Ca and phytate intakes

The food frequency questionnaire (FFQ) for zinc intake evaluation was developed. This FFQ, which contained 38 food items, included the major food sources and major zinc-containing food items which are commonly consumed by Koreans during the four seasons of each year. A FFQ was used for estimation of the intakes of zinc, calcium, phytate and other nutrients. In order to verify the reliability and reproducibility of the FFQ, a 24-h recall record was also used.

Nutrient intakes were calculated using Computer Aided Nutritional Analysis Program, version 2.0 (CAN Pro 2.0) (2). Intake for zinc and phytate, which were not present in CAN Pro 2.0 program, were analyzed using food composition tables, databases, a cross-referenced index, and various values from the literature (9,10). The procedure for calculation of zinc, calcium, phytate and phytate : zinc molar ratio has been described previously (3).

Zinc biochemical index assessment

All the tubes used for the analysis were made of polyethylene, and lab consumables were soaked overnight in 10% nitric acid solution to avoid zinc contamination. Precautions were taken to avoid zinc contamination during the collection and analyses of the samples. Samples from 10 ~ 15 subjects were pooled for analysis. The accuracy and precision of all analytical methods were checked by analyzing a standard reference material (1577b, bovine liver, National Institute of Standards and Technology, Gaithersburg, USA).

Sample collection : Blood samples were obtained by venepuncture in the morning after overnight fasting and were collected into trace element-free heparinized tubes (Becton Dickinson, Rutherford, NJ, USA). Twenty four-hour urine collections were removed in the early morning. Hair samples (300 mg) were collected from close to the occipital portion of the scalp with stainless steel scissors. Only the proximal 2 ~ 3 cm of the hair strands were retained to avoid collection of contaminated hair. Nail clippings were also collected.

Sample analyses : Zinc and calcium levels in plasma, red blood cells (RBCs) and urine were measured using inductively coupled plasma (ICP) emission spectroscopy (Boschstrasse 10 Spectro Analytical Instruments, Germany) after wet-digestion and appropriate dilution. In brief, RBCs were wet-digested with concentrated nitric acid and hydrogen peroxide. The wet-digested RBCs, plasma and urine were diluted using trace element analysis grade 0.125 M HCl (Fluka, Buchs, Switzerland). Protein levels in RBCs were measured using the Lowry method (11). The collected hair and nail clippings were washed thoroughly in water and wet-digested for element analyses. Plasma alkaline phosphatase was measured using a commercial kit (Sigma).

Statistical analyses

Statistical analyses were performed with the statistical package SPSS. The differences between the three localities (rural, urban and metropolitan city) were analyzed by ANOVA. If significant differences were found, a post hoc Turkey test was used for group comparisons using a significance level of $p<0.05$.

Table 1. Age and anthropometric measurement of adult Korean subjects in the rural, urban and metropolitan city areas

Index	Subjects (n=721)		
	Rural (n=271)	Urban (n=240)	Metropolitan city (n=210)
Age (year)	53.8±11.3[1)a2)]	43.7±19.1[b]	47.7±7.4[c]
Height (cm)	154.3±8.4[a]	161.0±8.3[b]	164.8±7.5[c]
Weight (kg)	56.1±9.6[a]	59.1±10.3[b]	60.2±11.5[b]
Body fat (%)	28.8±6.5[a]	28.5±5.9[a]	23.4±5.2[b]

1)Values are mean±SD.
2)Means within a row with different superscript letters are significantly different, $p<0.05$ (one-way post hoc ANOVA, Turkey).

RESULTS

Anthropometric assessment

The mean age of the subjects showed differences among the areas (Table 1). When recruiting the subjects in each area, most of residents in the rural area were the early aging people, while the main residents of the urban and metropolitan cities were younger. This is the natural situation for each locality and so subjects were chosen at random without ensuring intra-area uniformity of their characteristics. This sampling rationale was considered more beneficial for reflecting the actual zinc intake and status in a specific locality. The mean age of the subjects in the rural area was the highest among three localities (53.8±11.3 yrs), which represents early aging people. The mean age in the urban and metropolitan city areas was 43.7 yrs and 35.7 yrs, respectively, which indicates the younger age of populations in larger cities.

The mean height and body fat values were higher in larger city areas, and increased in the order rural, urban, and metropolitan, which parallels the differences in age. However, body fat was lower in the rural area, which is also a characteristic feature of elderly people (Table 1). Mean body fat values ranged between 23~29%, which was within the normal range of body fat values for men (10~20%) and for women (20~30%) (Table 1).

Dietary assessment and diet pattern

Energy nutrients and dietary fiber intakes are shown in Table 2. The nutrient intakes analyzed by FFQ were verified for reliability and reproducibility using 24-hr

Table 2. Energy and macronutrient intakes in Korean adults in three different areas of the rural, urban, and metropolitan city areas (intake/day)

Nutrient	Subjects[1)] (n=721)			
	Rural (n=271)	Urban (n=240)	Metropolitan city (n=210)	Korean RDA[2)]
Energy (kcal)	1389.5±393.9[3a4)]	1581.9±775.3[a]	1869.1±1254.2[b]	1700~2500
Protein (g)	45.9±19.6[a]	59.9±36.9[b]	75.4±67.2[c]	55~70
Animal protein (g)	14.3±13.3[a]	27.2±24.3[b]	36.9±34.1[c]	-
Plant protein (g)	31.7±10.4[a]	32.7±18.5[a]	38.4±26.9[b]	-
Lipid (g)	20.7±12.7[a]	33.5±24.3[b]	46.3±24.6[c]	-
Animal fat (g)	7.7±7.4[a]	18.1±15.8[b]	25.4±16.7[c]	-
Plant oil (g)	13.0±8.1[a]	15.3±12.2[a]	20.9±11.9[b]	-
Carbohydrate (g)	251.4±64.2[a]	257.8±122.9[ab]	284.6±156,1[b]	-
Dietary fiber (g)	5.1±2.6	4.9±3.6	5.0±3.6	20~25[5)]
Cholesterol (mg)	92.7±87.0[a]	185.4±168.6[b]	275.8±196.3[c]	<300[6)]

Nutrient intaked were measured using food frequency questionnaire.
1)The range for the subjects' age in this study was 21~69 yrs.
2)RDA for each nutrient were presented with the range for covering subjects' age range (21~69 yrs). 7th Korean RDAs for each nutrient are as follow: Energy (2500 kcal for 20~49 yrs, 2300 kcal for 50~64 yrs, 2000 kcal for 65~74 yrs for men; 2000 kcal for 20~49 yrs, 1900 kcal for 50~64 yrs, 1700 kcal for 65~74 yrs for women), protein (70 g for 20~64 yrs, 65 g for 65~74 yrs for men; 55 g for 20~74 yrs for women).
3)Values are mean±SD.
4)Means within a row with different superscript letters are significantly different, $p<0.05$ (one-way post hoc ANOVA, Turkey).
5)Recommended total dietary fiber intake for Koreans (from 7th RDA for Korean, 2000).
6)Recommended cholesterol intake for Koreans (from 7th RDA for Korean, 2000).

dietary recall. Most of the analyzed nutrient intakes using FFQ and 24-hr dietary recall were similar, except for Na, vitamin A, and vitamin C, and this confirms the reliability of using FFQ for dietary assessment in this study.

Most of the nutrient intakes, including zinc intake, were not different between men and women. We therefore presented data on a regional basis only. Energy intake was lower in the rural area and higher in the metropolitan city, as would be expected from the prevalence of older subjects in the rural area. Protein and lipid from animal sources and cholesterol were lower in the rural area. This can be interpreted that the subjects in the rural area might eat less meat or other animal products, which can result in low zinc intake (Table 2). However, nutrient intake from plant food sources showed less discrepancy among localities, as with carbohydrate, or no difference, as with dietary fiber.

Mean mineral and vitamin intakes are shown in Table 3. Calcium intake (410 mg/d), which was about 60% of the RDA, was the lowest and was half of the calcium intake in metropolitan city. Other animal food sources for minerals (animal calcium, iron, vitamin A, retinol) intakes were lower in the rural area as expected. However, vitamin C intake was not different among three areas. Even accounting for the lower energy intake in rural area, the lower intake of nutrients from animal food sources described above, would be the potential risk for poor zinc intake.

Zinc intake and molar ratio of phytate : Zn and phytate × Ca : Zn

The calculated daily Zn, Ca, phytate intake and their molar / millimolar ratios are shown in Table 4 and Fig. 1 and 2. Mean Zn intake in rural, urban, and metropolitan city areas was 6.5 mg, 7.3 mg and 11.4 mg per day, respectively, which is 54%, 62%, and 95% of Korean RDA. Zn intake in the rural area was about 57% of the Zn intake in metropolitan city. Since energy intake was lower in the rural area (Table 4 and Fig. 1), Zn intake was corrected using the amount of energy intake and Zn intake was calculated on the basis of 4.2 MJ (1000

Table 3. Mineral and vitamin intakes in Korea adults in three different areas of the rural, urban and metropolitan city areas (intake/day)

Nutrient	Subjects[1] (n=721)			
	Rural (n=271)	Urban (n=240)	Metropolitan city (n=210)	Korean RDA[2]
Ca (mg)	410.1±291.4[3)a4)]	569.4±432.4[b]	717.4±649.9[c]	700
Animal Ca (mg)	156,6±73.8[a]	3166.3±295.7[b]	401.7±324.4[c]	-
Plant Ca (mg)	253.6±183.4	252.8±211.6	315.7±296.9	-
P (mg)	813.8±362.6[a]	1101.4±667.2[b]	1314.9±1069.2[c]	700
Fe (mg)	7.6±4.2[a]	9.7±6.6[b]	12.0±11.0[c]	12~16
Animal Fe (mg)	1.2±1.2[a]	2.4±2.3[b]	3.7±4.5[c]	-
Plant Fe (mg)	6.5±3.6[a]	7.4±5.3[ab]	8.4±6.4[b]	-
Na (mg)	2894.5±1517.8	2558.8±1657.0	2758.0±2052.2	<2400[7]
K (mg)	1708.8±858.7[a]	2160.4±1451.9[b]	2374.0±1856.0[b]	-
Vitamin A (μg RE[5])	451.0±393.5[a]	929.1±797.8[b]	897.8±782.8[b]	700
Retinol (mg)	34.1±26.9[a]	77.3±63.4[b]	115.9±109.7[c]	-
Carotene (mg)	2437.2±2467.8[a]	4682.7±4699.1[b]	4008.9±4307.5[b]	-
Vitamin B_1 (mg)	0.79±0.32[a]	0.98±0.57[b]	1.13±0.87[c]	1.0~1.3
Vitamin B_2 (mg)	0.60±0.38[a]	0.98±0.71[b]	1.33±1.25[c]	1.2~1.4
Niacin (mg NE[6])	10.8±5.5[a]	14.8±9.5[b]	17.4±16.2[b]	13~17
Vitamin C (mg)	53.6±49.8	53.2±44.6	48.0±44.9	70

Nutrient intaked were measured using food frequency questionnaire.
[1)]The range for the subjects' age in this study was 21~69 yrs.
[2)]From 7th Korean dietary recommendation, 2000. RDA for each nutrient were presented with the range for covering subjects' age range (21~69 yrs). RDA for each nutrient is as follow: Ca (700 mg for 30~74 yrs for both men and women), P (700 mg for 20~74 yrs for both men and women). Fe (12 mg for 20~74 yrs for men; 16 mg for 20~49 yrs, 12 mg for 50~74 yrs for women), vitamin A (700 μg RE for 20~74 yrs for both men and women), vitamin B_1 (1.3 mg for 20~49 yrs, 1.2 mg for 50~64 yrs, 1.0 mg for 65~74 yrs for men; 1.0 mg for 20~74 yrs for women), vitamin B_2 (1.5 mg for 20~49 yrs, 1.4 mg for 50~64 yrs, 1.2 mg for 65~74 yrs for men; 1.2 mg for 20~74 yrs for women), niacin (1.7 mg for 20~49 yrs, 1.5 mg for 50~64 yrs, 1.3 mg for 65~74 yrs for men; 1.3 mg for 20~74 yrs for women), vitamin C (70 mg for 20~74 yrs for both men and women).
[3)]Values are mean±SD.
[4)]Means within a row with different superscript letters are significantly different, $p<0.05$ (one-way post hoc ANOVA, Turkey).
[5)]RE (retinol equivalent, 1 RE = 1 μg retinol = 3.33 β-carotene).
[6)]NE (niacin equivalent, niacin 1 mg = tryptophane 60 mg).
[7)]Recommended sodium intake for Koreans (from 7th RDA for Korean, 2000).

Table 4. Ca, Zn, and phytate intakes and their molar/millimolar ratios in Korean adults in three different areas of the rural, urban and metropolitan city areas

	Subjects[1] (n=721)			
	Rural area (n=271)	Urban area (n=240)	Metropolitan city (n=210)	Korean RDA[2]
Zn intake (mg/d)	6.5±3.7[3)a4)]	7.3±4.4[a]	11.4±3.1[b]	10~12
Zn intake (mg/d/1000 kcal)	4.6±2.6[a]	4.6±2.8[a]	6.1±1.7[b]	
Ca intake (mg/d)	441.3±313.5[a]	535.6±381.8[a]	693.3±605.6[b]	700
Phytate intake (mg/d)	2334.5±821.7[a]	2212.0±1054.6[a]	2712.7±1621.9[b]	-
Phytate:Zn (molar)	41.3±14.5[a]	33.5±12.6[b]	30.4±11.6[b]	-
Phytate×Ca:Zn (millimolar)	397.3±253.9	389.8±250.5	431.8±266.6	-
(Phytate×Ca:Zn)/4.2 MJ[5] (millimolar)	275.0±135.3	254.4±115.5	297.2±278.6	-

Nutrient intaked were measured using food frequency questionnaire.
[1]The range for the subjects' age in this study was 21~69 yrs.
[2]From 7th Korean dietary recommendation, 2000. RDA for each nutrient were presented with the range for covering subjects' age range (21~69 yrs). RDA for each nutrient is as follow: Zn (12 mg for 20~74 yrs for men and 10 mg for 20~74 yrs for women).
[3]Values are mean±SD.
[4]Means within a row with different superscript letters are significantly different, $p<0.05$ (one-way post hoc ANOVA, Turkey).
[5]4.2 megajoule (1000 kcal).

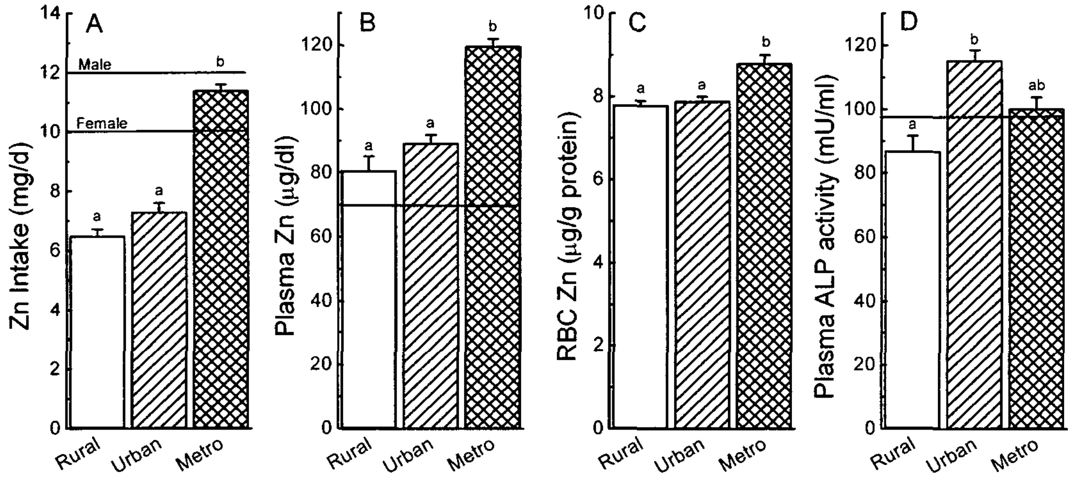

Fig. 1. Dietary zinc intake and biochemical zinc indices in Korean subjects from rural, urban and metropolitan city areas. A. Zn intake: the Korean RDA values for men (12 mg/d) and for women (10 mg/d) are shown as horizontal lines. B. Plasma Zn: Minimum reference value for normal plasma zinc level in adult humans (70 g/dL) is shown. C. RBC Zn: It has been reported that erythrocytes contain at least ten times more zinc than plasma (Milne, 2000). D. Plasma alkaline phosphatase activity: Minimum reference value for normal plasma alkaline phosphatase activity (97.3 mU/mL) in human is shown.

kcal), taking into account differences in the amount of food consumed, in order to compare it with the data from the different survey units. Zn intake in the rural area can be supplemented if the subjects in the rural area consume adequate energy intake. Zn intake per 4.2 MJ also showed lower rural intake (4.6 mg Zn/d), compared to the metropolitan city intake (6.1 mg Zn/d).

Since phytate and Ca interfere with Zn absorption, molar phytate×Ca : Zn and millimoar phytate Ca : Zn ratios were calculated. A higher ratio indicates a lower efficiency of Zn absorption, which can cause poor zinc nutriture. The phytate : Zn molar ratio was higher in the rural area, compared to the urban or metropolitan city areas (Table 4). Unexpectedly, phytate intake in the metropolitan city area (2713 mg/d) was higher than in the rural area (2334 mg/d). However the phytate : Zn molar ratio was still higher in the rural area, since Zn intake in the rural area was much lower than that in the metropolitan city area. Since the cutoff ratio for detrimental effect of phytate : Zn molar ratio on zinc absorption was reported to be 20, all of the subjects from the three localities showed a potential risk for lower zinc absorption due to the high ratio of phytate : Zn. Considering the Ca effect along with that of phytate, the millimolar ratio of phytate×Ca : Zn in the three areas was around 400, which was double the cutoff level of 200 which might be expected to result in zinc deficiency (Fig. 2).

Biochemical indices

The zinc concentrations in plasma, RBCs, urine, hair and nail, and also plasma alkaline phosphatase activity, are shown in Table 5 and Fig. 1. Mean plasma zinc concentrations in rural, urban, and metropolitan city areas were 81, 89 and 120 μg/dL, respectively (Table 5 and Fig. 1). The pattern of RBC Zn concentration was similar to that of plasma zinc, being the lowest (7.8 μg/g protein) in the rural area, and the highest in the metropolitan area (8.8 μg/g protein). Thus, plasma and RBC zinc concentrations reflected the lower Zn intake of the rural area. Again, plasma alkaline phosphatase activity was also lower in the rural area, compared to the urban or metropolitan city areas (Table 5 and Fig. 1).

Even with low zinc intake in rural and urban areas (Table 4), biochemical zinc indices (plasma and RBC zinc, plasma ALP activity) were within the normal range, except for plasma alkaline phosphatase activity in the rural area, which was lower than the reference value (Table 5). Urinary and hair zinc concentration was also within the normal range. Normally, urinary zinc is not completely reliable to explain dietary zinc status, and hair zinc is considered as being easily contaminated. Urinary zinc was the highest in the urban area and hair zinc was highest in the rural area, which was not consistent with the pattern of other zinc indices. Nail zinc concentration ranged between 0.8 ~ 1.2 μmol/g.

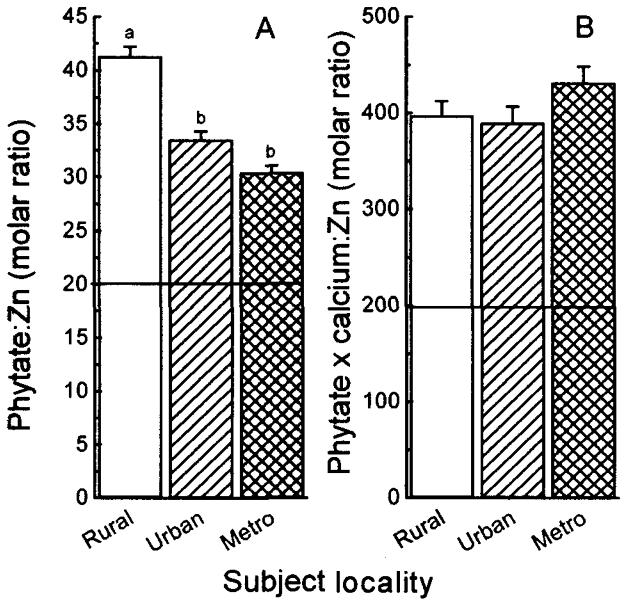

Fig. 2. The ratio of phytate : Zn (molar) and phytate Ca : Zn (millimolar). The cutoff levels causing poor zinc nutriture is a phytate : Zn ratio of 20, (A) and a phytate Ca : Zn of 200, (B), which are shown as horizontal lines.

DISCUSSION

Overt zinc clinical deficiency is quite rare, although subclinical signs of marginal deficiency is prevalent even

Table 5. Zinc biochemical indexes in adult Korean subjects in three different areas of the rural, urban, and metropolitan city areas

Zn concentration	Subjects			
	Rural	Urban	Metropolitan city	Reference value
Plasma Zn (μg/dL) (n=657)	80.8 ± 69.2[1)a2)] (n=234)	89.4 ± 36.4[a] (n=230)	119.8 ± 35.7[b] (n=193)	70 ~ 150[5)]
RBC[3)] Zn (μg/g protein) (n=564)	7.8 ± 1.5[a] (n=217)	7.9 ± 1.2[a] (n=189)	8.8 ± 2.7[b] (n=158)	-
Urinary Zn (μg/dL) (n=497)	31.5 ± 27.5[a] (n=206)	38.2 ± 28.7[a] (n=144)	28.8 ± 13.6[b] (n=147)	15.8 ~ 94.8[6)]
Hair Zn (μmol/g wet tissue) (n=463)	2.2 ± 1.5[a] (n=163)	1.7 ± 1.1[a] (n=161)	1.5 ± 0.3[b] (n=139)	1.8 ± 0.7[7)]
Nail Zn (μmol/g wet tissue) (n=461)	1.2 ± 0.4 (n=158)	1.0 ± 0.2 (n=162)	0.8 ± 0.1 (n=141)	-
Plasma ALP[4)] activity (mU/mL) (n=427)	87.1 ± 61.4[a] (n=176)	115.4 ± 44.2[b] (n=145)	100.4 ± 26.5[ab] (n=106)	97.3 ~ 102.3[8)]

Nutrient intaked were measured using food frequency questionnaire.
1) Values are mean ± SD.
2) Means within a row with different superscript letters are significantly different, $p < 0.05$ (one-way post hoc ANOVA, Turkey).
3) RBC: Red blood cell.
4) ALP: Alkaline phosphatase.
5) Milne DB. 2000. In *Clinical nutrition of the essential trace elements and minerals.* Bogden JD and Klevay LM, eds. Human Press, Totowa, New Jersey (ref. 8).
6) Burgess et al. 1999. Proceeding of 10th Trace Element in Man and Animal (TEMA 10) (ref. 26).
7) Gibson and Huddle. 1998. *Am J Clin Nutr* 67: 702-709 (ref. 27).
8) Cavan et al. 1993. *Am J Clin Nutr* 57: 334-343 (ref. 28).

in the Western developed countries. Nevertheless, zinc is still important in cellular memebrane integrity, immunity, as an antioxidant and in the gene expression of growth factors (1).

In this study, zinc intake of the subjects in rural (6.5 mg), urban (7.3 mg) and metropolitan city (11.4 mg) areas was 54%, 62% and 95% of the Korean RDA (12 mg for men, 10 mg for women) (7th RDA, 2000), and a significant proportion of the rural and urban populations should be considered as being at risk of marginal zinc deficiency. There may be two possible explanations for a low zinc intake in subjects in rural or urban areas. Firstly, the major subjects of the rural area were early ageing subjects, and older people generally eat less food. Actually, mean energy intakes of the subjects in rural (1390 kcal) or urban (1582 kcal) areas were lower than in the metropolitan city area (1869 kcal) ($p<0.05$), indicating a lower food intake and therefore a low intake of all nutrients, including zinc. It is well recognized that intakes below 1 mg Zn/d in adult humans cause severe deficiency and that marginal deficiency effects may be found between 1 and 10 mg/d (12). When considering that the subjects in this study consumed insufficient daily energy as compared to the RDA, zinc intake may be improved if the subjects could consume a more appropriate energy intake. Thus, we calculated zinc intake per 4.2 MJ for compromising the insufficient dietary zinc supplying. However, zinc intakes per 4.2 MJ (1000 kcal) still showed lower levels in rural (4.6 mg/d) and urban areas (5.6 mg/d/1000 kcal) as compared to those in the metropolitan city area (6.1 mg/d/1000 kcal). Thus, the food sources for poor zinc nutriture, in addition to insufficient energy intake, needed to be explained.

The second possible reason for poor zinc intake in rural or urban areas, is that subjects in these areas consumed mainly plant food-dominated diets, which is known to be the case. The food frequency questionnaire used in this study was specially made for zinc intake assessment. Thus the questionnaire contained various animal food sources, since zinc is usually rich in protein-rich food sources, including meat, shell fish, etc. Most of the nutrients intake from the animal food origins, such as animal protein, animal Ca and Fe, retinal, were lower in the rural area in this study. Even corrected for the low amount of energy intake in the rural area, intake of these animal source nutrients were still lower in the rural area. Therefore, a low intake of animal foods would be the main reason for low zinc intake in the rural area. In addition, the insufficient animal food intakes in the rural area was confirmed that dietary fiber and vitamin C intake in rural area was about the same compared to urban and metropolitan city, even lower energy intake in rural area. The another potential for poor zinc intake in the rural area may be the reduced zinc absorption in elderly subjects Marginal zinc deficiency due to reduced zinc absorption in elderly subjects has been reported in several studies (1).

On the basis of these proposed reasons for poor zinc intake in rural area, a higher intake of animal foods would be recommended for the subjects to achieve optimal zinc intake. The zinc intake of Koreans in rural (6.5 mg/d) and urban (7.3 mg/d) areas in this study is two thirds of, and the intake in the metropolitan city area (11.4 mg/d) is about the same as, the intake levels of normal healthy Americans (11.9~12.3 mg/d) (13,14).

Other dietary factors promoting marginal zinc deficiency in Koreans are the high phytate and Ca intake, which decrease zinc absorption. Normally, the adverse effect of phytate and Ca on Zn absorption is evaluated by calculating the molar ratio of phytate : Zn or phytate $\times$ Ca : Zn. The molar ratio of phytate : Zn, above which zinc status may be compromised, is 20, and this is considered as a factor for zinc deficiency (15,16). In this study, the mean phytate : Zn ratio ranged from the highest in the rural area, 41, to the lowest in metropolitan city area, 30, which may cause poor zinc nutriture in Koreans, especially in the rural area. The phytate : Zn molar ratios are reported with levels ranging from 5 in normal Western people (17) through to 67 for vegetarian monks in the USA (18). Unexpectedly, phytate intake was highest in the metropolitan area and was the lowest in the rural area in this study. However, the discrepancy in phytate intake was not so great, and the zinc intake in the rural area was much lower than in the metropolitan city area, which still yielded a higher phytate : Zn molar ratio in rural area. Actually we previously reported the same pattern of phytate intake (3). The reason for higher phytate intake in larger cities would be that phytate is rich in whole grains and legumes, and people in large cities are concerned about eating healthy whole grains rather than the milled ones. The estimated daily phytate intake in the rural area (2334 mg/d) was 3~5 times higher than in Canadian children (422 mg/d) (15,19), or Americans (395~781 mg/d) (14,20,21). Millimolar ratios of phytate $\times$ Ca : Zn $\geq$ 200 are indicators of marginal zinc deficiency in men (16). The phytate $\times$ Ca : Zn millimolar ratio in the present study was almost twice the level thought to be a risk factor for zinc deficiency. The ratio was still $>$200, when corrected for energy intake [(phytate $\times$ Ca : Zn) / 4.2 MJ], which is likely to cause poor zinc nutriture.

The conventional methods for human zinc status assessment include determination of the zinc content of the plasma or serum, blood cells, urine, hair, nails and saliva, unfortunately, there is no single reliable indicator of zinc status (22). Plasma zinc levels generally reflect zinc status but are influenced by other variables such as stress and infection. This indicator is also less useful for assessment of marginal status because plasma zinc levels may not be greatly reduced, if they are reduced at all. Plasma or serum zinc does not reflect whole body zinc status in all cases (8). The situation with urinary zinc is much the same as for plasma zinc and efforts have been made to find other biomarkers. However, decreased urinary zinc excretion usually accompanies zinc deficiency. Correlations between hair zinc and blood or tissue zinc are usually poor (8). Thus, hair zinc is unreliable as a measure of zinc status. Alkaline phosphatase, a zinc-dependent enzyme, is readily measured and its activity is reduced in severe zinc deficiency. However, the sensitivity of this assay to detect marginal zinc deficiency is questionable (12). All these bioindices are used for zinc assessment, in spite of their limitations for measurement for zinc status, since other sensitive and reliable zinc indicators have not been developed.

In the present study, the mean results for plasma zinc, urinary zinc and hair, and plasma alkaline phosphatase were all within the normal range, except plasma alkaline phosphatase activity in the rural area which was below the minimum reference value, suggesting that the level of zinc deficiency was not sufficiently severe to affect these biomarkers, even when zinc intake was so low. However, plasma and RBC zinc, and plasma alkaline phosphates activity reflected the dietary zinc intake. The subjects in the rural area showed the lowest zinc intake, and also the lowest level in plasma, RBC zinc and plasma alkaline phosphatase activity (Table 4 and Fig. 1). The range of mean plasma zinc concentration (81 ~ 120 μg/dL) in Korean adults was similar to that found in Western populations (23-25), even with a low intake of zinc and high intake of phytate. The RBC zinc concentration in Koreans (7.8 ~ 8.8 μg/g protein) was one quarter of the level reported in subjects from the USA (31 μg/g protein) (23). It is a strong possibility that zinc deficiency is prevalent in the rural localities in Korea. However, bearing in mind the low dietary zinc and high phytate intakes, it is highly likely that a significant proportion of the subjects were marginally zinc deficient in all of three areas in Korea.

CONCLUSIONS

Zinc intake and status of 721 healthy adult South Koreans in the three different localities of rural, urban, and metropolitan city areas, were assessed using a food frequency questionnaire and zinc biochemical markers. Dietary Zn intake and biochemical zinc indices (plasma zinc, RBC zinc and plasma alkaline phosphatase activity) were the lowest in the rural area, which may indicate zinc deficiency in this area. The main reason for lower zinc intake in the rural area is probably the low intake of animal foods. The phytate : zinc and phytate × calcium : zinc molar ratios were much higher than the cutoff for negative effects on zinc absorption. However, generally, plasma zinc, RBC, urinary, hair zinc and plasma alkaline phosphatase levels were within the normal range even with low zinc intake in rural and urban areas, which indicates that these zinc status markers were not sufficiently sensitive to detect marginal zinc deficiency in these subjects. It is likely that the subjects in the rural and urban areas were at least marginally zinc deficient and the wider consumption of zinc rich animal foods and phytate deficient foods would be beneficial.

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