J Food Sci Nutr
Vol 9, p 374~382 (2004)

J Food Science and Nutrition

Potential Antioxidant Trace Mineral (Zn, Mn, Cu and Fe) Concentrations Measured by Biochemical Indices in South Koreans

Young-Eun Cho, Young-Mee Byun, Eun-Hee Kwak, Jin-Sook Yoon[1], Hyun-Mee Oh[1], Jae-Wang Kim[2], Hyun-Soo Shin[3], Chong-Suk Kwon 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]Kim Jae-Wang Clinics, Gyeongbuk 760-749, Korea
[3]Andong Medical Center, Gyeongbuk 760-060, Korea

Abstract

The concern of the antioxidant micronutrient status in normal healthy people, including antioxidant trace minerals such as Cu, Zn, Mn, Fe and Se is focused since systemic oxidation is involved in various chronic diseases. In the present study, we evaluated the concentration of trace minerals (Cu, Zn, Mn, and Fe) which are considered as potential antioxidant minerals in plasma, red blood cells (RBCs) and urine in normal healthy Korean subjects. The 760 subjects (male 341, female 419; mean age 54.2±18.9) were recruited from the rural, urban and metropolitan city in South Korea. Dietary intake was evaluated using 24-hours recall for general major nutrient intake assessment. The trace elements (Cu, Zn, Mn, and Fe) concentrations in plasma, RBCs, and urine were measured by inductively coupled plasma spectrophotometer (ICP) and atomic absorption spectrophotometer (AAS). Cu and Zn levels in plasma, RBCs and urine in normal healthy South Koreans were within the normal range of those mineral levels, but Mn and Fe levels were higher compared to the normal range of those mineral levels. None of the selected trace mineral levels in plasma and RBC's was lower than the normal range value. The results showed that Zn and Cu levels in plasma and RBC's in Korean were within the normal range, and plasma and urinary Mn and Fe levels were higher than the normal reference values. Potential antioxidant trace mineral (Cu, Mn, Zn and Fe) levels in Koreans are within or a bit higher than the normal range.

Key words: trace minerals (Mn, Zn, Cu and Fe), plasma, red blood cells, urine, 24-hours recall, South Koreans

INTRODUCTION

The relationship between dietary or biochemical status or nutrients and chronic disease has been focused intensely on antioxidant nutrients, since systemic oxidation may be involved in a number of diseases, such as blood vascular disease (1), diabetes (2), cancer (3,4), stroke (5) and aging (6), etc. Thus, antioxidant status may influence the development of chronic diseases and deficiencies in antioxidant-related micronutrient nutrition are frequently implicated in the etiology of such chronic diseases (1). The etiology of such chronic diseases depends on the fact that a low intake of antioxidant nutrients might result in low blood concentration of those antioxidant nutrients, which in turn might be related to a high incidence of those chronic diseases (7). Not only some vitamins, such as β-carotene as provitamin A, vitamin C and E, but also some trace minerals, such as Cu, Zn, Mn, Fe, and Se, are considered to be the antioxidant nutrients, even though those metals can act as pro-oxidant property under high intake status.

Trace minerals such as Cu, Zn, Mn, Fe and Se, can have antioxidant properties or are incorporated into proteins that have an antioxidant role. Although Zn may have antioxidant properties in its own right (3), it has structural roles in antioxidant proteins such as superoxide dismutase (8) and metallothionein (9). Since Fe, Mn and Cu have two valency states in nature, they can participate in redox reactions which may have pro-oxidant or antioxidant consequences. As integral components of proteins, their role is usually to perform an antioxidant function.

The objective of this study was therefore to assess the antioxidant trace minerals (Mn, Zn, Cu and Fe) status of normal Korean subjects from the three areas of rural, urban and metropolitan areas, in order to evaluate the need for better dietary management and even supplementation. Biochemical indices of plasma, red blood

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

cells (RBCs) and urinary samples for those potential antioxidant minerals (Cu, Zn, Mn, and Fe) were measured. Actually, major changes in status can be assessed in this way in all cases, since marginal changes in status of some of these micronutrients might not be detected by analyzing plasma or urinary levels.

SUBJECTS AND METHODS

Participants and anthropometric assessment

The size and the sex ratio of the population for the study are shown in Table 1. Total 760 subjects (male 341, female 419; mean age 54.2±18.9) were recruited from the three different areas representing rural, urban and metropolitan city for the study. 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. Selected anthropometric measurements of height and body weight were made. Body fat was measured using a body composition analyzer (inBody 3.0 Body Composition Analyzer, Biospace, South Korea) using electronic impedance analysis. The protocol for the participants selections and anthropometric measurement was described in the previous studies (10,11).

Dietary assessment for nutrient intakes

For the dietary assessment of the major nutrient intakes, 24-hours recall method was used. Nutrient intakes were calculated using Computer Aided Nutritional Analysis Program, version 2.0 (CAN Pro 2.0) (12).

Trace mineral (Mn, Zn, Cu and Fe) biochemical index assessment

All the tubes used for the trace mineral analysis were made of polyethylene which is free of trace mineral content, and lab consumables were soaked overnight in 10% nitric acid solution to avoid trace mineral contamination. Precautions were taken to avoid trace mineral contamination during the collection and analyses of the samples.

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). The blood was centrifuged with 2,000 rpm at 4°C for 10 min. The supernatant of plasma and red blood cell pellets was collected. Twenty four-hour urine collections were removed in the early morning. Hair samples (300 mg) for the zinc analysis 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 for zinc analysis were also collected. The collected hair and nail clippings were washed thoroughly in water and wet-digested for element analyses.

Sample analyses: Trace elements (Cu, Zn, Mn and Fe) concentration was 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. The plasma was diluted to 1:8 with 0.125 mol HCl. The accuracy and precision of all analytical methods were checked by analyzing a standard reference material obtained from National Institute of Standards and Technology (NIST SRM 1577b, bovine liver, Gaithersburg, USA). Protein levels in RBCs were measured using the Lowry et al. method (13).

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 one-way ANOVA and the difference between sex was analyzed by Student's *t*-test. If significant differences were found by ANOVA, a post hoc Turkey test was used for group comparisons using a significance level of $p<0.05$.

RESULTS

Anthropometric assessment

The mean age and anthropometric assessment of the participants are shown in Table 2. The mean age of the participants in the rural area was the highest among three localities, which represents early aging people. 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

Table 1. Population size and sex ratio of the subjects

Area	Rural area (Andong suburb)	Urban area (Andong city)	Metropolitan city (Daegu city)	Total
Population number	2,605 (100%)	184,108 (100%)	2,538,212 (100%)	2,724,925 (100%)
Male	1,327 (51%)	92,107 (50%)	1,276,725 (50%)	1,370,159 (50%)
Female	1,278 (49%)	92,001 (50%)	1,261,487 (50%)	1,354,766 (50%)

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 trace mineral status in a specific locality.

The mean height and weight values were higher in larger city areas, and increased in the order of rural, urban, and metropolitan. However, body fat was lower in the rural area, which is also a characteristic feature of elderly people. Mean body fat values ranged between 23~29%, which was nearly within the normal range of body fat values for men (10~20%) and for women (20~30%) (Table 2).

Nutrient intake assessment

Energy nutrients and major vitamin and mineral intakes are shown in Table 3~5. The nutrient intakes were analyzed using 24-hr dietary recall. Actually, the dietary assessment was also measured using food frequent questionnaire (FFQ) (data not shown) and most of the analyzed nutrient intakes using FFQ and 24-hr dietary recall were similar. Most of the energy nutrients and dietary fiber intakes were not different between men and women. However, energy and most of energy nutrient intakes were higher in the metropolitan city and dietary fiber intake showed no difference (Table 3).

CAN Pro 2.0 software can only be eligible for analyzing of intakes of Ca, P, Fe, Na, and K. Most of mineral (Ca, P, Fe and K) intakes were not different between men and women except that Na intake is higher in men (Table 4). Most of mineral intakes were higher in the order of rural, urban and the metropolitan city. Specially, Fe intake was higher in big city compared to rural.

Vitamin B_1, B_2 and niacin intakes were not different between men and women, but vitamin A and C intakes were higher in women ($p<0.05$) (Table 5). Vitamin A intake in rural was less than half of urban and metropolitan city. Vitamin B_1 intake in rural area was three times higher, compared to urban and metropolitan city. Unexpectedly, vitamin C intake was lower in rural area and it was higher in metropolitan city (Table 5).

Biochemical indices of potential antioxidant trace minerals (Zn, Cu, Mn and Fe)

Zn biochemical indices are shown in Table 6. Zn concentrations in plasma, RBCs, urine and plasma alkaline phosphatase (ALP) were not different between men and women. Plasma and RBCs zinc concentrations and plasma ALP activities were higher in urban and metropolitan city compared to rural. In particular, plasma Zn concentration was lower in rural area ($p<0.05$). Plasma Zn (86.0 μg/dL, 13 μmol/L) and urinary Zn (33.3 μg/dL, 5.1 μmol/L) concentrations in the total subjects were within the normal range of plasma Zn (51~165 μg/dL, 8~20 μmol/L) and urinary Zn (13~76 μg/dL, 2~12 μmol/L) (Zn atomic weight, 65.39) (14,15).

Plasma, RBCs and urinary Cu concentrations are shown in Table 7. Plasma and urinary Cu concentrations were not different between men and women. Urinary Cu was higher in men than in women. Also, plasma and urinary Cu concentrations were higher in urban and metropolitan city compared to rural. Plasma Cu (90.4 μg/dL, 14 μmol/L) and urinary Cu (4.2 μg/dL, 0.7 μmol/L) concentrations in the total subjects were also within the normal range of plasma Cu (6.5~177.9 μg/dL, 1~28 μmol/L) and urinary Cu (0.6~5.1 μg/dL, 0.1~0.8 μmol/L) (Cu atomic weight, 63.55) (14).

The Cu:Zn ratio can be used for the estimation of systemic oxidant load. The Cu:Zn ratio of total participants was within the range of 0.9~1.3 for whole range of subject groups and the total subjects showed 1.08 of Cu:Zn, which is considered being normal and acceptable for protection of oxidative stress.

Mn concentrations in plasma, RBCs and urine are shown in Table 8. Plasma Mn concentration was higher in men. However, RBCs and urinary Mn concentrations were not different between men and women. Biochemical Mn level didn't show any consistency among the areas. Plasma Mn (0.65 μg/dL, 118 nmol/L) level in the total subjects was higher than the normal range of plasma Mn (49~110 ng/dL, 9~20 nmol/L) level. However, urinary Mn (0.1 μg/dL, 18 nmol/L) level was within the normal range (0~110 ng/dL, 0~20 nmol/L) (Mn atomic weight, 54.94) (14).

Fe concentrations in plasma, RBCs and urine are shown in Table 9. Plasma, RBCs and urinary Fe concentrations were not different between men and women. Unexpectedly, Fe concentrations in plasma, RBCs and urine were lower in metropolitan city than in rural or in urban. Plasma Fe (353.2 μg/dL, 63 μmol/L) and urinary Fe (13.5 μg/dL, 2.4 μmol/L) concentrations in the total subjects were higher compared to normal range of plasma Fe (50~175 μg/dL, 9~31 μmol/L) and urinary Fe (negligible) (Fe atomic weight, 55.85).

DISCUSSION

Zn and Cu are part of the antioxidant Cu-Zn SOD in cytosol, which protects against iron- and copper-catalyzed free-radical injury (16,17). Mn is also part of the antioxidant Cu-Zn SOD in mitochondria. Fe is a part of catalase which is one of the antioxidant metalloenzyme in systemic antioxidant function in the cells. All

Table 2. Age and anthropometric measurement of the subjects (mean±SD)[1,2]

Index \ Area	Rural area			Urban area			Metropolitan city			Total		
	Men	Women	Total	Men	Women	Total	Men	Women	Total	Men	Women	Total
Age (Year)	65.0±11.8 (n=135)	63.2±11.0 (n=175)	63.8±11.3^{a} (n=310)	44.6±13.7* (n=84)	36.3±20.5* (n=156)	38.7±19.1^{b} (n=240)	28.8±5.0 (n=122)	31.7±8.2 (n=88)	30.7±7.4^{c} (n=210)	56.8±16.9* (n=341)	53.0±19.6* (n=419)	54.2±18.9 (n=760)
Height (cm)	162.5±5.8* (n=134)	150.5±6.5* (n=116)	154.3±8.4^{a} (n=250)	168.9±5.7* (n=104)	157.5±6.7* (n=126)	161.0±8.3^{b} (n=230)	172.7±5.5* (n=80)	160.7±4.3* (n=129)	164.8±7.5^{c} (n=209)	165.3±6.8* (n=318)	153.4±7.4* (n=371)	157.2±9.1 (n=689)
Weight (kg)	60.0±9.1* (n=134)	54.3±9.3* (n=116)	56.1±9.6^{a} (n=250)	69.8±9.1* (n=104)	54.4±6.7* (n=126)	59.1±10.3^{b} (n=230)	71.4±9.5* (n=80)	54.3±7.3* (n=129)	60.2±11.5^{b} (n=209)	63.9±10.4* (n=318)	54.3±8.4* (n=371)	57.4±10.1 (n=689)
Body fat (%)	23.9±5.7* (n=129)	31.1±5.5* (n=121)	28.8±6.5^{a} (n=250)	22.6±5.7* (n=72)	28.5±5.7* (n=149)	28.5±5.9^{a} (n=221)	18.9±3.9* (n=58)	25.8±4.1* (n=92)	23.4±5.2^{b} (n=150)	23.2±5.7* (n=259)	29.9±5.7* (n=362)	28.2±6.4 (n=621)

[1]Values with different superscript letters in column 'Total' within the three different areas (rural area, urban area, metropolitan city) are significantly different at $p<0.05$ by Turkey, ANOVA.
[2]Values with asterisk are significantly different between men and women within each area at $p<0.05$ by Student's *t*-test.

Table 3. Energy and macronutrient intake in subjects by 24-hours recall (intake/day, mean±SD)[1,2]

Nutrient \ Area	Rural area			Urban area			Metropolitan city			Total		
	Men (n=129)	Women (n=121)	Total (n=250)	Men (n=72)	Women (n=149)	Total (n=221)	Men (n=58)	Women (n=92)	Total (n=150)	Men (n=259)	Women (n=362)	Total (n=621)
Energy (kcal)	1383.4±402.5	1354.8±415.5	1363.8±411.2^{a}	1422.6±404.2	1731.8±532.6	1710.1±529.6^{b}	2238.1±951.4	1833.7±999.2	1973.7±992.7^{c}	1482.9±559.8	1508.8±551.2	1502.2±553.0
Protein (g)	50.9±22.9*	48.8±26.4*	49.4±25.3^{a}	47.3±16.1*	66.2±28.3*	64.9±28.0^{b}	48.4±37.8*	64.5±33.8*	72.8±36.7^{b}	54.9±27.2	55.4±28.8	55.3±28.4
Lipid (g)	23.3±15.4*	19.1±14.2*	20.1±14.7^{a}	30.2±23.1	40.7±21.8	39.9±22.0^{b}	60.2±29.3	45.5±29.7	50.8±30.2^{c}	27.2±21.6	27.9±21.4	27.7±21.4
Carbohydrate (g)	230.0±61.8	243.3±63.4	241.7±62.9^{a}	237.9±49.7	277.5±76.9	274.7±75.9^{b}	333.4±146.3	293.4±156.7	307.2±153.0^{c}	248.8±80.6	257.8±80.1	255.5±80.3
Dietary fiber (g)	6.1±3.1	6.4±3.8	6.3±3.6	5.0±3.1	6.8±3.4	6.7±3.4	7.5±3.3	7.0±3.8	7.1±3.6	6.2±3.1	9.6±3.7	6.5±3.5
Cholesterol (mg)	130.2±84.8*	89.7±47.9*	102.5±67.9^{a}	143.6±102.3	232.2±105.4	226.0±102.6^{b}	285.3±173.8	240.4±159.7	255.9±156.4^{b}	148.7±119.1	145.7±107.7	146.5±110.1

[1]Values with different superscript letters in column 'Total' within the three different areas (rural area, urban area, metropolitan city) are significantly different at $p<0.05$ by Turkey, ANOVA.
[2]Values with asterisk are significantly different between men and women within each area at $p<0.05$ by Student's *t*-test.

Table 4. Mineral intake in subjects by 24-hours recall (intake/day, mean±SD)[1,2]

Mineral \ Area	Rural area			Urban area			Metropolitan city			Total		
	Men (n=129)	Women (n=121)	Total (n=250)	Men (n=72)	Women (n=149)	Total (n=221)	Men (n=58)	Women (n=92)	Total (n=150)	Men (n=259)	Women (n=362)	Total (n=621)
Ca (mg)	429.0±262.8	426.4±268.8	427.2±266.6[a]	334.6±232.8*	539.2±335.7*	524.8±333.1[b]	643.5±303.2	497.2±276.2	547.9±291.4[b]	446.8±274.3	467.1±296.0	461.9±290.5
P (mg)	848.1±369.2	832.8±406.4	837.6±394.7[a]	724.4±284.0*	1036.6±432.5*	1014.7±430.7[b]	1309.7±578.9	994.6±508.6	1103.7±549.5[b]	891.8±419.4	908.94±432.7	904.6±4429.1
Fe (mg)	8.6±4.0	8.8±5.8	8.7±5.3[a]	8.9±3.2*	11.6±5.5*	11.4±5.4[b]	16.1±7.3*	12.9±10.7*	14.0±9.7[c]	9.5±5.0	10.0±6.4	9.8±6.1
Na (g)	4.9±2.1*	4.3±2.2*	4.5±2.15[a]	4.2±1.8	4.6±2.2	4.6±2.2[a]	6.5±3.3	4.8±3.3	5.4±3.4[b]	5.0±2.3*	4.4±2.3*	4.6±2.3
K (g)	2.1±0.9	2.1±1.2	2.1±1.1[a]	1.6±0.3*	2.6±1.1*	2.5±1.1[b]	3.3±1.4	2.7±1.6	2.9±1.6[c]	2.2±1.1	2.3±1.2	2.3±1.2

[1]Values with different superscript letters in column 'Total' within the three different areas (rural area, urban area, metropolitan city) are significantly different at $p<0.05$ by Turkey, ANOVA.
[2]Values with asterisk are significantly different between men and women within each area at $p<0.05$ by Student's *t*-test.

Table 5. Vitamin intake in subjects by 24-hours recall (intake/day, mean±SD)[1,2]

Vitamin \ Area	Rural area			Urban area			Metropolitan city			Total		
	Men (n=129)	Women (n=121)	Total (n=250)	Men (n=72)	Women (n=149)	Total (n=221)	Men (n=58)	Women (n=92)	Total (n=152)	Men (n=259)	Women (n=362)	Total (n=621)
Vitamin A (μgRE[3])	344.1±219.7	351.3±264.9	349.0±224.2[a]	397.7±314.5	837.0±748.8	803.5±734.8[b]	838.6±447.9	840.9±765.0	840.1±667.5[b]	403.8±368.5*	539.5±336.8*	504.8±382.9
Vitamin B_1 (mg)	11.0±6.6	0.8±0.4	4.0±2.4	0.9±0.5	1.1±0.4	1.1±0.4	1.6±0.7	1.2±0.7	1.3±0.7	9.3±5.4	0.9±0.4	3.1±2.3
Vitamin B_2 (mg)	0.6±0.4	0.6±0.4	0.6±0.4[a]	0.7±0.5*	1.0±0.5*	1.0±0.5[b]	1.6±1.0	1.1±0.6	1.3±0.8[c]	0.7±0.6	0.8±10.5	0.8±0.5
Niacin (mgNE[4])	11.5±5.7	10.7±5.4	11.0±5.5[a]	10.1±4.1*	14.4±7.0*	14.1±6.9[b]	20.5±9.9*	15.1±8.7*	17.0±9.4[c]	12.4±6.8	12.2±6.5	12.2±6.6
Vitamin C (mg)	82.1±47.1	90.2±58.8	87.6±55.5[a]	55.6±38.4*	106.2±62.1*	102.7±62.0[a]	123.2±76.2	129.0±87.8	127.0±83.2[b]	84.9±52.7*	98.1±63.3*	94.7±60.9

[1]Values with different superscript letters in column 'Total' within the three different areas (rural area, urban area, metropolitan city) are significantly different at $p<0.05$ by Turkey, ANOVA.
[2]Values with asterisk are significantly different between men and women within each area at $p<0.05$ by Student's *t*-test.
[3]RE (retinol equivalent unit).
[4]NE (niacin equivalent unit).

Table 6. Zn concentration in blood, urine, hair and nail, and plasma alkaline phosphatase activity (mean±SD)[1,2]

Index \ Area	Rural area			Urban area			Metropolitan city			Total			Normal range[6]
	Men (n=129)	Women (n=121)	Total (n=250)	Men (n=72)	Women (n=149)	Total (n=221)	Men (n=58)	Women (n=92)	Total (n=152)	Men (n=259)	Women (n=362)	Total (n=621)	
Plasma Zn (μg/dL)	77.4±51.2	82.7±74.7	80.2±70.6[a]	95.1±39.4	86.4±34.6	89.4±36.4[a]	111.7±20.9	127.1±42.0	121.9±36.7[b]	83.6±48.4	87.8±69.3	86.0±61.3	50.8~164.5 (8~20 μmol/L)
RBC[3] Zn (μg/g protein)	8.3±1.7[a]	7.8±1.3*	8.0±1.5[a]	8.0±1.0	7.9±1.3	7.9±1.2[a]	7.7±1.4*	8.9±2.1*	8.5±1.9[b]	8.2±1.5	7.9±1.4	8.0±1.4	-
Urinary Zn (μg/dL)	34.9±25.8	30.3±28.1	31.6±27.5	45.9±30.7	37.5±28.6	38.2±28.7	32.3±12.6	27.2±13.4	28.9±13.2	35.7±25.1	32.6±27.7	33.3±27.1	12.7~76.3 (2~12 μmol/L)
Hair Zn (μmol/g)	-	-	2.2±1.5[a]	-	-	1.8±1.1[a]	-	-	0.5±0.3[b]	-	-	1.7±0.8	-
Nail Zn (μmol/g)	-	-	1.2±0.4	-	-	0.9±0.2	-	-	0.8±0.1	-	-	1.0±0.1	-
Plasma ALP[4] activity (mU[5]/mL)	79.2±53.9	90.6±64.5	87.1±61.5[a]	113.1±35.1	116.4±48.5	115.3±44.5[b]	111.3±19.3	94.9±27.8	100.1±26.4[ab]	99.2±45.3	102.7±55.3	101.6±52.3	-

[1]Values with different superscript letters in column 'Total' within the three different areas (rural area, urban area, metropolitan city) are significantly different at $p<0.05$ by Turkey, ANOVA.
[2]Values with asterisk are significantly different between men and women within each area at $p<0.05$ by Student's *t*-test.
[3]Red blood cell.
[4]Alkaline phosphatase.
[5]mU: milliunit.
[6]Reference 14 & 15.

Table 7. Cu concentration in plasma, RBC and urine (mean±SD)[1,2]

Index \ Area	Rural area			Urban area			Metropolitan city			Total			Normal range[4]
	Men (n=129)	Women (n=121)	Total (n=250)	Men (n=72)	Women (n=149)	Total (n=221)	Men (n=58)	Women (n=92)	Total (n=152)	Men (n=259)	Women (n=362)	Total (n=621)	
Plasma Cu (μg/dL)	94.8±66.6*	83.2±28.2*	88.8±50.6[a]	79.7±14.3*	93.4±36.4*	88.8±31.4[a]	101.8±13.7*	111.9±11.1*	106.8±12.4[b]	91.7±56.9	89.2±31.4	90.4±44.1	6.5~177.9 (1~28 μmol/L)
RBC[3] Cu (μg/g protein)	0.7±0.3	0.8±0.8	0.7±0.7[a]	0.9±0.4	0.8±0.4	0.9±0.4[a]	1.3±0.5	1.8±1.4	1.6±1.0[b]	0.8±0.4	0.9±0.5.	0.9±0.5	-
Urinary Cu (μg/dL)	6.0±4.4	5.2±4.5	5.4±4.5[a]	2.5±0.9	2.5±1.7	2.5±1.7[b]	0.9±0.4	1.4±1.0	1.2±0.7[c]	5.0±4.3*	4.0±3.9*	4.2±4.0	0.6~5.1 (0.1~0.8 μmol/L)
Plasma Cu:Zn (μM)	1.25	1.00	1.17	0.87	1.15	1.00	0.94	0.95	0.89	1.08	1.08	1.08	-

[1]Values with different superscript letters in column 'Total' within the three different areas (rural area, urban area, metropolitan city) are significantly different at $p<0.05$ by Turkey, ANOVA.
[2]Values with asterisk are significantly different between men and women within each area at $p<0.05$ by Student's *t*-test.
[3]Red blood cell.
[4]Reference 14.

Table 8. Mn concentration in plasma, RBC and urine (mean±SD)[1,2]

Area / Index	Rural area			Urban area			Metropolitan city			Total			Normal range[4]
	Men (n=129)	Women (n=121)	Total (n=250)	Men (n=72)	Women (n=149)	Total (n=221)	Men (n=58)	Women (n=92)	Total (n=152)	Men (n=259)	Women (n=362)	Total (n=621)	
Plasma Mn (μg/dL)	1.12±0.76*	0.27±0.16*	0.68±0.20[a]	0.30±0.02	0.46±0.12	0.41±0.07[a]	1.39±0.13	1.35±0.19	1.36±0.20[b]	0.95±0.35*	0.43±0.26*	0.65±0.36	49~110 ng/dL (9~20 nmol/L)
RBC[3] Mn (μg/g protein)	4.08±2.59	4.22±2.26	4.18±2.47[a]	1.97±1.05*	4.24±2.54*	3.49±2.41[a]	3.41±1.10	3.33±1.09	3.37±1.08	3.15±1.10	3.93±1.03	3.68±1.05	-
Urinary Mn (μg/dL)	0.13±0.10	0.13±0.11	0.13±0.10[a]	0.08±0.05	0.09±0.06	0.09±0.06[b]	0.01±0.00	0.01±0.00	0.01±0.00[c]	0.11±0.10	0.10±0.09	0.10±0.09	0~110 ng/dL (0~20 nmol/L)

[1]Values with different superscript letters in column 'Total' within the three different areas (rural area, urban area, metropolitan city) are significantly different at $p<0.05$ by Turkey, ANOVA.
[2]Values with asterisk are significantly different between men and women within each area at $p<0.05$ by Student's *t*-test.
[3]Red blood cell.
[4]Reference 14.

Table 9. Fe concentration in plasma, RBC and urine (mean±SD)[1,2]

Area / Index	Rural area			Urban area			Metropolitan city			Total			Normal range[4]
	Men (n=129)	Women (n=121)	Total (n=250)	Men (n=72)	Women (n=149)	Total (n=221)	Men (n=58)	Women (n=92)	Total (n=152)	Men (n=259)	Women (n=362)	Total (n=621)	
Plasma Fe (μg/dL)	368.8±336.6	346.4±127.2	357.1±250.2[a]	414.6±131.1*	352.3±143.1*	373.3±141.9[a]	251.5±49.5	239.3±83.3	243.4±73.3[b]	372.3±292.2	339.0±133.1	353.2±216.2	50~175 μg/dL (9~31 Nmol/L)
RBC[3] Fe (μg/g protein)	296.4±68.0	301.9±75.1	300.2±72.9[a]	233.9±44.8*	212.3±64.9*	219.5±59.8[b]	207.2±44.8	204.6±48.0	205.5±46.6[b]	264.6±67.8	261.7±82.9	262.6±78.4	-
Urinary Fe (μg/dL)	13.2±6.5	14.2±12.8	14.0±11.4[a]	10.8±6.6	14.3±11.3	14.0±11.0[a]	7.1±1.3	7.6±2.4	7.4±2.1[b]	12.6±6.4	13.7±11.9	13.5±12.0	Negligible

[1]Values with different superscript letters in column 'Total' within the three different areas (rural area, urban area, metropolitan city) are significantly different at $p<0.05$ by Turkey, ANOVA.
[2]Values with asterisk are significantly different between men and women within each area at $p<0.05$ by Student's *t*-test.
[3]Red blood cell.
[4]Reference 14.

of these four trace elements have a potential antioxidant functions in the cells (7,18).

Cu and Fe, on the other hands, can be potent catalysts of the Fenton reaction and, at high concentrations, may act as pro-oxidant nutrients (19). However, reports of oxidative damage or increased susceptibility to oxidative stress during Cu deficiency (20) and, presumably, Fe deficiency suggest that Cu and Fe, at certain dietary intakes, be characterized as antioxidant nutrients. Zn can be pro-oxidant and has antioxidant properties similar to those of Cu, Fe and Mn, so that its imbalance may be expected to contribute to an oxidative stress condition.

In the present study, plasma and urinary Zn and Cu levels (Table 6 and 7, respectively) are within the normal range of plasma and urinary Zn and Cu level in the normal healthy Korean subjects. The subject selection for this study actually was arranged for the wide range of age (30s~70s years old) and for both men and women. Thus, the potential antioxidant trace mineral Zn and Cu levels in Koreans are considered as being normal status, in general. Plasma Zn level (86 mg/dL) in this study is somewhat less and similar with the other plasma Zn level (61~75 mg/dL) in Koreans compared to the previous study (21).

It has also been shown that Cu:Zn ratio is a metabolic imbalance in regard to Zn, and Cu is a major factor in the etiology of oxidative chronic disease (22). In the present study, plasma micromolar Cu:Zn ratio is within the range of 0.87~1.25 through the all subject groups by sex and areas, and the total mean Cu:Zn ratio is 1.08 (Table 7), which implies the optimal Cu:Zn ratio to prevent the systemic oxidative stress.

One major concern from the results of the present study is Mn status in these subjects. Plasma Mn (0.65 μg/dL, 118 nmol/L) level in the total subjects was higher than the normal range of plasma Mn (49~110 ng/dL, 9~20 nmol/L) level (Table 8). However, to be looked over in detail, plasma Mn level in the subjects in large city showed almost twice higher than the rural and urban areas, which implies the potential Mn contamination in the larger city from the environment factors.

Plasma and urinary Fe levels also showed higher than the normal reference level (Table 9). Unexpectedly, plasma and urinary Fe levels were higher in the rural area than in the large city. Plasma Fe level (353 μg/dL) of the total subjects in this study was almost four times higher than the healthy American female adults (85~88 μg/dL) (23). Further study is needed for clarifying of the possibility of the higher plasma Fe level in Koreans. Actually, major changes in status can be assessed in this way in all cases, since marginal changes in status of some of these micronutrients might not be detected by analyzing plasma or urinary levels.

In conclusion, the results showed that Zn and Cu biochemical levels in Korean were within the normal range. Plasma and urinary Mn and Fe levels were higher than the normal reference values. Thus, it seems that potential antioxidant trace mineral (Cu, Mn, Zn and Fe) levels in plasma and urine in Koreans are within the normal range or somewhat higher than the normal reference level for each trace element. Further study with specific and relevant assays for each trace element would be needed to evaluate the antioxidant trace mineral status in Koreans.

ACKNOWLEDGEMENT

This research was supported by a grant of the Korea Health 21 R&D project, Ministry of Health & Welfare, Republic of Korea (HMP-00-B-22000-00153). Authors also gratefully thank to the group of Health Promotion in Andong Medical Center, and the group of Health Promotion in North Daegu Public Medical Center.

REFERENCES

1. Brown AA, Hu FB. 2001. Dietary modulation of endothelial function: implications for cardiovascular disease. *Am J Clin Nutr* 73: 673-686.
2. Disilvestro RA. 2000. Zinc in relation to diabetes and oxidative disease. *J Nutr* 130 (Suppl): S1509-S1511.
3. McDermott JH. 2000. Antioxidant nutrients: current dietary recommendations and research update. *J Am Pharm Assoc* 40: 785-799.
4. Ching S, Ingram D, Hahnel R, Beilby J, Rossi E. 2002. Serum levels of micronutrients, antioxidants and total antioxidant status predict risk of breast cancer in a case control study. *J Nutr* 132: 303-306.
5. Suter PM. 2000. Effect of vitamin E, vitamin C, and beta-carotene on stroke risk. *Nutr Rev* 58: 184-187.
6. Gonzalez-Gross M, Marcos A, Pietrzik K. 2001. Nutrition and cognitive impairment in the elderly. *Br J Nutr* 86: 313-321.
7. Diplock AT. 1991. Antioxidant nutrients and disease prevention: an overview. *J Nutr* 153 (Suppl): S189-S193.
8. Anderson RA, Roussel AM, Zouari N, Mahjoub S, Matheau JM, Kerkeni A. 2001. Potential antioxidant effects of zinc chromium supplementation in people with type 2 diabetes mellitus. *Am J Coll Nutr* 20: 212-218.
9. Maret W. 2003. Cellular zinc and redox states converge in the matallothionein/thionein pair. *J Nutr* 133: 1460S-1462S.
10. Lee SL, Kwak EH, Yoon JS, Kwon CS, Beattie JH, Kwun IS. 2004. Zinc nutritional status in Korean adults from rural, urban and metropolitan areas. *J Food Sci Nutr* 9: 174-182.
11. Lee SL, Kwak EH, Yoon JS, Kwon CS, Beattie JH, Kwun IS. 2004. Zinc intake and status of the selected Korean adults. *J Food Sci Nutr* 9: 79-84.
12. Korean Nutrition Society, Computer Aided Nutritional Anlysis Porgram, version 2.0 (CAN Pro 2.0) software,

Seoul, South Korea.
13. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. 1951. Protein measurement with the Folin phenol reagent. *J Biol Chem* 193: 265-275.
14. Burgess E, Audette R, Knudtson M, Wyse G. 1999. Trace elements as determined by ICP-MS analysis in patients with coronary artery disease (abstract). Proceedings of trace elements in men and animals 10 (TEMA 10). Evian, France. 1999, May 2-7.
15. Milne DB. 2000. Laboratory assessment of trace element and mineral status. In *Clinical nutrition of the essential trace elements and minerals*. Bogden JD, Klevay LM, eds. Human Press, Totowa, New Jersey.
16. Powell SR. 2000. The antioxidant properties of zinc. *J Nutr* 10: S1447-S1454.
17. Wang XL, Adachi T, Sim AS, Wilcken DE. 1998. Plasma extracellular superoxide dismutase levels in an Australian population with coronary artery disease. *Arterioscler Thromb Vasc Biol* 18: 1915-1921.
18. Machlin LJ, Bendich A. 1987. Free radical tissue damage: protective role of antioxidant nutrients. *FASEB J* 1: 441-445.
19. Sokol RJ, McKim JM, Devereaux MW. 1996. Alpha-tocopherol ameliorates oxidant injury in isolated copper-overloaded rat hepatocytes. *Pediatr Res* 39: 259-263.
20. Johnson MA, Fischer JG, Kays SE. 1992. Is copper an antioxidant nutrient? *Crit Rev Food Sci Nutr* 32: 1-31.
21. Mezzetti A, Pierdomenico SD, Costantini F, Romano F, De Cesare D, Cuccurullo F, Imbastaro T, Riario-Sforza G, Di Giacomo F, Zulani G, Fellin R. 1998. Copper-zinc ratio and systemic oxidant load: Effect of aging and aging-related degenerative diseases. *Free Radic Biol Med* 6: 676-681.
22. Yoon JS, Yu KH. 1998. The effect of weekly iron supplementation in iron and zinc nutritional status in pregnant women. *J Korean Nutr* 31: 1270-1282.
23. Volpe SL, Huang HW, Larpadisorn K, Lesser I. 2001. Effect of chromium supplementation and exercise on body composition, resting metabolic rate and selected biochemical parameters in moderately obese women following an exercise program. *J Am Coll Nutr* 20: 293-306.

(Received October 19, 2004; Accepted December 7, 2004)