

Dietary zinc intake is inversely related to subclinical atherosclerosis measured by carotid intima-media thickness

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The relationship between dietary Zn intake and the risk of atherosclerosis remains unclear, and no epidemiological studies have been reported on the effects of dietary Zn intake on morphological changes in the vascular wall. We examined the relationship between dietary Zn intake and common carotid intima-media thickness (IMT) as a marker of subclinical atherosclerosis among the middle-aged and elderly populations. A cross-sectional analysis of a prospective cohort baseline study was performed with 4564 adults aged 40–89 years and free of clinical CVD. Dietary data were collected by trained interviewers using an FFQ. Common carotid IMT was measured using a B-mode ultrasound imaging technique. Subclinical atherosclerosis was determined using carotid IMT, and defined as >80th percentile of carotid IMT or ≥ 1 mm of carotid IMT. After adjustment for potential confounders, the mean carotid IMT in the low Zn intake group was higher than that in the high Zn intake group. When subclinical atherosclerosis was defined as >80th percentile value of IMT or ≥ 1 mm of carotid IMT, after adjustment for potential confounders, Zn intake was inversely related to subclinical atherosclerosis (5th v. 1st quintile, OR 0.64, 95% CI 0.45, 0.90, P for trend=0.069; 5th v. 1st quintile, OR 0.34, 95% CI 0.16, 0.70, P for trend=0.005, respectively). In persons free of clinical CVD, dietary Zn intake was inversely correlated with subclinical atherosclerosis. The present findings suggest a putative protective role of dietary Zn intake against the development of atherosclerosis.

Zinc: Carotid intima-media thickness: Subclinical atherosclerosis

Atherosclerosis is an arterial disease that induces lumen narrowing and obstruction, and is the most common cause of heart attacks and strokes worldwide. Several lifestyle and nutritional factors have been identified to influence the formation of atherosclerotic plaque^(1–4), but the relationship between Zn intake and atherosclerosis has been inconclusive, although Zn has been regarded as an antioxidant⁽⁵⁾. The protective effects of Zn on the process of atherosclerosis have been suggested through *in vitro* and *in vivo* studies^(6–9). Zn has been shown to inhibit LDL oxidation by macrophages and endothelial cells *in vitro*⁽⁶⁾ and to prevent lesion development in hypercholesterolaemic animals⁽⁷⁾. Zn has also been shown to inhibit apoptosis of endothelial cells by inhibiting the activity of caspases^(8,9). Nevertheless, limited epidemiological studies have been done on the relationship between Zn intake and CVD, and the associations of Zn intake with CVD were not consistent^(10–15). A cross-sectional survey

showed an inverse relationship between dietary Zn intake and the prevalence of coronary artery disease⁽¹⁰⁾, and a cohort study reported that dietary Zn intake showed an inverse association with CVD mortality⁽¹¹⁾. Two case–control studies reported that CVD patients had higher Zn intakes and lower serum Zn than the controls^(12,13). However, other case–control studies demonstrated that serum Zn in individuals with atherosclerosis obliterans was higher than that in the healthy control groups^(14,15). Therefore, the effects of dietary Zn on atherosclerosis remain uncertain.

Intima-media thickness (IMT) of large artery walls is a non-invasive marker of early arterial wall alteration. Carotid IMT measurements assessed by B-mode ultrasound have been used as surrogate markers for atherosclerosis in observational studies and clinical research⁽¹⁶⁾. Increase in carotid IMT was directly associated with an increased risk of CHD including myocardial infarction and stroke in cohort studies^(17,18).

Abbreviation: IMT, intima-media thickness.

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To date, no epidemiological studies have been reported on the effect of dietary Zn intake on morphological changes in the vascular wall. Thus, we examined the relationship between dietary Zn intake and subclinical atherosclerosis as measured using carotid IMT among the middle-aged and elderly populations.

Subjects and methods

Study population

The Korean Multi-Rural Communities Cohort Study in Rural Communities is part of the Korean Genome Epidemiology Study. The Korean Multi-Rural Communities Cohort Study in Rural Communities was initiated in 2004 to construct a genomic cohort and identify risk factors for CVD. As baseline, 7818 subjects aged ≥ 40 years were recruited from the centres located in Yangpyeong, Namwon and Goryeong between January 2005 and November 2007. Yangpyeong is located 45 km east of Seoul, the capital of South Korea, and Namwon and Goryeong are located in southwestern and southeastern areas of South Korea, respectively. The majority of the subjects were farmers and housewives. Among 7818 subjects, we excluded subjects who had hypertension (n 2131), hyperlipidaemia (n 348), diabetes (n 734), heart disease (n 502), cerebrovascular disease (n 273) or cancer (n 173) at the baseline survey, and those who reported implausible dietary intake (<2091 or $>16\,726$ kJ/d or 1000 g of alcohol/d or more than ten missing food items) (n 67), and those who reported that they had changed their usual diet during the previous year (n 277). A total of 4564 subjects aged 40–89 years were included in the final data analysis. The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Institutional Review Boards of Hanyang University, Chonnam National University and Keimyung University. Written informed consent was obtained from all subjects.

General characteristics, anthropometrics and biochemical variables

To overcome the limitations of the multicentre studies, we developed standardised protocols for a questionnaire and each examination procedure including measurements of height and weight and blood pressure and blood sampling. All interviewers and examiners were trained by the same personnel at the coordinating centre.

A structured questionnaire, including information on demographics, education, smoking, alcohol intake, exercise, medical history and female reproductive history, was administered by trained interviewers. Height was measured using a standard height scale to the nearest 0.1 cm, and weight was measured using a metric weight scale to the nearest 0.01 kg in light clothing without shoes. BMI was calculated as weight (kg)/height (m²). Waist circumference was measured half way between the lowest rib margin and the iliac crest. Blood pressure was measured from the right arm by auscultation using a standard sphygmomanometer and a standard cuff. Two consecutive measurements of blood pressure were done after each subject had been sitting for at least 5 min.

Systolic blood pressure and diastolic blood pressure were recorded to the nearest 2 mmHg. If two systolic or diastolic blood pressures were more than 5 mmHg apart, an additional measurement was done, and the mean value of the closest two measurements was used for the subsequent analyses. Blood samples were collected in the morning after at least 8 h of fasting, and all biochemical markers were analysed on the same day. Plasma total cholesterol, TAG, glucose and HDL-cholesterol were measured using the ADVIA1650 Automatic Analyser (Siemens, New York, NY, USA). If plasma TAG were <4000 mg/l, LDL-cholesterol was calculated as described by Friedewald *et al.*⁽¹⁹⁾.

Dietary assessment

Dietary data were collected by trained interviewers using a food-based FFQ in which each participant was asked to identify the usual frequency of consumption of 106 food items during the previous year and the average serving size consumed. The FFQ consisted of nine frequency categories ranging from 'never or rare' to '3 times/d', and three serving sizes were specified. For food items with different seasonal availability, the participants were asked to mark how long they had eaten among four categories: 3, 6, 9 and 12 months. The validity and reproducibility of the FFQ have been reported in detail elsewhere⁽²⁰⁾. Briefly, the estimated nutrient intakes from the FFQ were compared with those from 12 d food records during the four seasons. Pearson's correlation coefficient for Zn was 0.33. Based on the cross-classification analysis, with respect to Zn intake, 71.8% of the subjects were classified into the same or adjacent quartiles, and 6.5% of the subjects were classified into opposite quartiles. Nutrient intake was calculated using a weighted frequency per day and a portion size per unit in each food item. The seventh edition of the Food Composition Table of Korea was used as the nutrient database⁽²¹⁾. The major contributing food sources of Zn were cereal grains (56%) followed by oysters (3%) and milk (3%) (data not shown). Nevertheless, oyster ingestion explained 54% of the total variation in dietary Zn intake, which is attributed to the high Zn content of oysters and large variation in oyster consumption among the subjects.

Measurement of intima-media thickness

IMT of common carotid artery was evaluated with the subject in the supine position using high-resolution B-mode ultrasound (SonoAce-9900; Medison Company Limited, Seoul, South Korea) equipped with a 7.5 MHz linear-array transducer. From the longitudinal view of the carotid bifurcation at a point 10 mm proximal to the common carotid artery, the maximal value of the IMT in a region free of plaque was measured in both carotid arteries. End-diastolic images were captured and saved as files for offline analysis by five sonographers at the three centres. A single trained reader at the reading centre in the Department of Preventive Medicine at Chonnam National University analysed the still images using the Sigma Scan Pro 5.0 (SPSS Inc., Chicago, IL, USA). Between- and within-sonographer reliabilities were evaluated using 180 cases and thirty cases, respectively, and within-reader reliability was tested using thirty-six cases.

CV between and within sonographers (9.4–9.8 and 4.7–4.9 %, respectively), intraclass correlation coefficients between and within sonographers (r 0.85–0.88 and r 0.90–0.91, respectively), a CV within readers (3.8–4.1 %) and an intraclass correlation coefficients within readers (r 0.92–0.94) all indicated that the IMT measures taken from the three centres by five sonographers and one reader yielded highly reproducible values of common carotid IMT.

Statistical analysis

Nutrient intakes were adjusted for total energy intake by the residual method. Subjects were categorised into quintiles by dietary Zn intake. Stratification analysis was performed according to age of the subjects. The subjects aged <65 years were considered 'the middle-aged', and the subjects aged \geq 65 years were considered 'the elderly'. The general linear model and the Cochran–Mantel–Haenszel analysis with adjustment for age and sex were used to determine differences in means or distribution and the linear trends across the Zn intake groups. The trend tests were conducted by treating the median value of each group as continuous variable in a multivariate model. The common carotid IMT was calculated as the mean IMT measurement of both carotid arteries. The differences in the means of IMT between Zn intake groups were assessed using general linear model. When statistically significant effects were demonstrated, Tukey's *post hoc* comparison test was used to identify group differences at $P < 0.05$. The normal values of carotid IMT have been established based on the distribution of IMT values in a general healthy population^(22,23), so that normal values are different depending on the methodology used for the measurement, age and sex. Subclinical atherosclerosis was defined by the value >80th upper percentile of common carotid IMT (0.8232 mm) similar to the study done by He *et al.*⁽⁴⁾. However, since IMT tends to increase with age, this upper normal limit (0.8232 mm) can be too strict for elderly subjects. When this limit was applied to elderly subjects, 36 % of the elderly subjects aged \geq 65 years were categorised as having subclinical atherosclerosis. Therefore, another criterion (IMT = 1 mm) was also applied to determine the risk of subclinical atherosclerosis. A value of IMT \geq 1 mm at any age is related to the significant increase in myocardial infarction and/or cerebrovascular disease⁽²⁴⁾. Non-conditional logistic regression analysis was applied to obtain the OR and corresponding 95 % CI for subclinical atherosclerosis. Potential confounders were identified by examining the distributions of characteristics that can affect atherosclerosis by Zn intake groups. The variables that showed significantly different distributions among Zn intake groups or showed significant linear trends across Zn intake groups were considered as potential confounders and adjusted in the analyses. Three different models were applied to examine the associations of Zn intake with IMT. Age and sex were adjusted as confounders in the first model, and education, alcohol intake (g/d), smoking status (current, former or non-smoker), regular exercise (yes/no), waist circumference and serum HDL-cholesterol were further adjusted in the second model. The final model additionally adjusted dietary factors such as quintiles of total energy, protein, fat, carbohydrate, β -carotene, vitamins C and E, folate, dietary fibre and cholesterol intakes.

All statistical analyses were performed using the SAS software (version 9.1; SAS Institute, Inc., Cary, NC, USA), and P values <0.05 were considered significant.

Results

The present study subjects consisted of Koreans aged 40–89 years sampled in three rural areas. General characteristics of the subjects are given in Table 1. Men made up 40.2 % of the total number of subjects. The mean ages of men and women were 61.3 and 59.7 years, respectively. The proportion of the subjects aged \geq 65 years was 38.5 % of the total number

Table 1. General characteristics of the study subjects (Mean values and standard deviations or frequency)

Characteristics	Men (n 1837)		Women (n 2727)	
	Mean	SD	Mean	SD
Age (years)	61.3	9.9	59.7	10.1
Height (cm)	165.0	6.2	152.5	6.0
Weight (kg)	64.4	10.1	56.1	8.8
BMI (kg/m ²)	23.6	3.0	24.1	3.2
Waist circumference (cm)	84.5	8.3	82.4	9.0
Seated blood pressure (mmHg)				
Systolic	124.2	16.5	121.0	17.6
Diastolic	79.8	10.2	77.6	10.1
Total cholesterol (mg/l)	1900	352	2021	355
LDL-cholesterol (mg/l)	1165	315	1299	316
HDL-cholesterol (mg/l)	443	117	458	104
TAG (mg/l)	1544	1138	1346	781
Fasting blood glucose (mg/l)	980	168	940	140
Alcohol intake (g/d)	25.9	59.0	3.2	27.1
Dietary intake*				
Energy (kJ/d)	7261.8	2113.8	6370.7	1840.3
Protein (g/d)	46.4	8.6	45.2	8.5
Fat (g/d)	19.2	7.8	16.7	8.0
Carbohydrate (g/d)	287.6	30.7	295.5	30.1
β -Carotene (μ g/d)	1766.0	1282.7	1785.1	1394.5
Vitamin C (mg/d)	75.1	39.9	89.3	51.1
Folate (μ g/d)	159.6	67.2	170.4	75.4
Vitamin E (mg/d)	5.8	1.9	6.0	2.3
Fibre (g/d)	4.7	1.8	4.9	1.9
Cholesterol (mg/d)	107.3	89.9	101.4	87.5
Zn (mg/d)	6.1	1.7	6.0	1.2
Frequency (n and %)				
Menopausal women			2137	78.4
Education				
Less than elementary	285	15.6	1106	40.7
Elementary	686	37.5	901	33.1
Middle school	365	20.0	322	11.8
High school	338	18.5	311	11.4
College or higher	155	8.4	82	3.0
Smoking status				
Non-smoker	428	23.3	2579	94.6
Former smoker	696	37.9	49	1.8
Current smoker	712	38.8	97	3.6
Alcohol intake				
Non-drinker	430	23.5	1757	64.5
Former drinker	180	9.8	66	2.4
Current drinker	1224	66.7	901	33.1
Regular exercise				
No	1387	75.5	2028	74.4
Yes	450	24.5	698	25.6

* All nutrients were total energy adjusted by residual method after log transformation.

of subjects. The proportions of the subjects whose BMI was $>25 \text{ kg/m}^2$ were 31.9% of the men and 36.1% of the women, respectively. The proportion of postmenopausal women was 78.4%. Men were more likely to have a higher education, and to be current smokers and current drinkers than women. Means of total daily energy intakes were 7261.8 kJ (1736.6 kcal) for men and 6370.7 kJ (1523.5 kcal) for women. Means of daily Zn intake for men and women were 6.1 and 6.0 mg/d after adjustment for total energy intake by the residual method.

To determine the potential confounding factors, the distributions of selected characteristics were examined across five Zn intake groups, which are given in Table 2. The median Zn intakes of the first, second, third, fourth and fifth quintiles were 5.00, 5.48, 5.85, 6.29 and 7.19 mg/d, respectively. Age, total energy intake, carbohydrate and proportion of current smokers decreased across the Zn intake groups. The proportions of higher education, current drinkers, regular exercise, waist circumference, serum HDL-cholesterol, and intakes of alcohol, protein, fat, β -carotene, vitamin C, folate, vitamin E, fibre and cholesterol increased across Zn intake groups. Differences in the proportion of males were significant. All variables for which significant linear trends or significant differences in means were used as covariates are given in Tables 3 and 4.

Adjusted IMT means by Zn intake group are given in Table 3. The middle-aged (<65 years) and the elderly (≥ 65 years) populations showed similar median Zn intakes. Means of IMT in the elderly population were higher than those in the middle-aged population. After adjustment for age, sex, education, alcohol intake, smoking, regular exercise, waist circumference, HDL-cholesterol and dietary factors (quintiles of total energy, protein, fat, carbohydrate, β -carotene, vitamins C and E, folate, dietary fibre and cholesterol intakes), adjusted IMT mean in the first quintile of Zn intake group was significantly higher than that in the fifth quintile group among the total subjects. In the middle-aged population, the first quintile group showed higher adjusted IMT means than the fifth quintile group in the first and second models. With respect to the elderly population, after adjusting for potential confounders, adjusted IMT mean in the first quintile group was higher than that in the fifth quintile group, but the difference was NS. The interaction between quintile of Zn intake and age group was not statistically significant (data not shown). Adjusted IMT means by Zn intake from seafood and grain showed the same direction with the relationship between total dietary Zn and IMT (data not shown).

The relationships between Zn intake and the risk of subclinical atherosclerosis are given in Table 4. When subclinical atherosclerosis was defined by >80 th percentile value of IMT (0.8232 mm), 10.4% of the middle-aged population and 35.7% of the elderly population were classified as having subclinical atherosclerosis. After adjustment for potential confounders in the third model, Zn intake was inversely related to subclinical atherosclerosis (5th v. 1st quintile, OR 0.64, 95% CI 0.45, 0.90, P for trend=0.069). In the middle-aged population, a significant inverse correlation between Zn intake and subclinical atherosclerosis was found in the third model (5th v. 1st quintile, OR 0.44, 95% CI 0.26, 0.76, P for trend=0.048). However, no significant relation was found in the elderly population.

When subclinical atherosclerosis was defined by ≥ 1 mm of IMT, 1.6% of the middle-aged population and 7.9% of the elderly population were classified as having subclinical atherosclerosis. Zn intake was inversely correlated with the risk of subclinical atherosclerosis after adjusting for potential confounders (5th v. 1st quintile, OR 0.34, 95% CI 0.16, 0.70, P for trend=0.005). The elderly subjects showed an inverse relationship between Zn intake and the risk of subclinical atherosclerosis (5th v. 1st quintile, OR 0.33, 95% CI 0.14, 0.77, P for trend=0.013). No significant correlations were found in middle-aged adult subjects.

When the subjects who were diagnosed as having hypertension, hyperlipidaemia, diabetes, heart disease, cerebrovascular disease or cancer were included and diagnosed diseases were adjusted in the model, similar trends were found between Zn intake and the risk of carotid atherosclerosis (results not shown).

Discussion

Carotid IMT is known as a surrogate marker for carotid atherosclerosis. In the present cross-sectional study, the low Zn intake group displayed a higher mean IMT than the high Zn intake group. An inverse relationship between dietary Zn intake and common carotid atherosclerosis was demonstrated in the middle-aged and elderly populations. The present findings suggest that adequate Zn intake may have a beneficial effect on the risk of atherosclerosis among the middle-aged and elderly populations.

In the present study, daily Zn intake was estimated using the FFQ. However, it is inappropriate to compare the absolute dietary Zn intake estimated using the FFQ to the estimated average requirements or dietary Zn intakes estimated by other dietary assessment methods. Therefore, we conducted 3 d 24 h recalls in a subgroup of the subjects (n 880) (data not shown). The mean energy intakes for men and women in the subgroup were 7439 (SD 2273) kJ and 5890 (SD 1750) kJ, which were similar to the energy intakes estimated by the FFQ. The means of daily Zn intakes for men (n 350) and women (n 530) were 8.7 and 7.4 mg/d, which were higher than the Korean estimated average requirements for Zn⁽²⁵⁾, but lower than Zn intakes estimated by multiple day 24 h recalls from a healthy Chinese sample (8.9–11.9 mg/d)⁽²⁶⁾, an older European sample (11.0–11.3 mg/d) and an adult sample from the United States (9.7–14.2 mg/d)^(27–29).

Carotid IMT has been used as an intermediate or proxy end point for CVD. Carotid IMT has been consistently associated with the incidence of CHD in cohort studies^(17,18,30). The association between carotid IMT and diet, such as consumption of fruit, olive oil, fat, alcohol, fish and isoflavone, has been determined^(1–4,31), but the relationship between carotid IMT and Zn intake has not been investigated.

In the present study, the risk of subclinical atherosclerosis in the high Zn intake group was significantly lower than that in the low Zn intake group. In particular, when 80th upper percentile values of common carotid IMT were used to determine the OR, after adjusting for potential confounders, the risks of subclinical atherosclerosis in the fifth quintile group were lower than those in the first quintile group among the middle-aged populations (5th v. 1st quintile, OR 0.44, 95% CI 0.26, 0.76, P for trend=0.048). The elderly population

Table 2. Age- and sex-adjusted characteristics of the study population by zinc intake group (Mean values with their standard errors)

Characteristics	Zn intake										P for difference	P for linear trend*
	Q1		Q2		Q3		Q4		Q5			
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE		
n	912		913		912		913		914			
Median Zn intake (mg/d)	5.00		5.48		5.85		6.29		7.19			
Range of Zn intake	2.81–5.27		5.27–5.65		5.65–6.04		6.04–6.63		6.63–40.38			
Age (years)	62.4	0.3†	61.8	0.3	60.4	0.3	59.4	0.3	57.6	0.3	< 0.0001	< 0.0001
BMI (kg/m ²)	23.7	0.1	23.9	0.1	23.9	0.1	23.9	0.1	23.9	0.1	0.573	0.299
Waist circumference (cm)	82.5	0.3	83.0	0.3	83.7	0.3	83.5	0.3	83.5	0.3	0.033	0.015
Blood pressure (mmHg)												
Systolic	122.2	0.6	121.7	0.6	122.4	0.6	123.0	0.6	122.1	0.6	0.568	0.751
Diastolic	78.2	0.3	78.3	0.3	78.1	0.3	78.8	0.3	78.9	0.3	0.386	0.078
Total cholesterol (mg/l)	1980	12	1983	12	1964	12	1965	12	1971	12	0.713	0.424
LDL-cholesterol (mg/l)	1259	11	1253	11	1242	11	1236	11	1240	11	0.527	0.153
HDL-cholesterol (mg/l)	445	4	447	4	444	4	458	4	464	4	0.0001	< 0.0001
TAG (mg/l)	1412	31	1494	31	1418	31	1417	31	1388	31	0.164	0.192
Fasting blood glucose (mg/l)	956	5	962	5	951	5	952	5	960	5	0.446	0.909
Alcohol intake (g)	8.8	1.4	12.6	1.4	11.8	1.4	14.1	1.4	14.4	1.4	0.046	0.008
Dietary intake‡												
Energy (kJ/d)	7051.0	61.9	6663.8	61.5	6554.5	61.5	6608.1	61.5	6785.5	61.9	< 0.0001	0.029
Protein (g/d)	37.6	0.2	41.9	0.2	45.6	0.2	48.9	0.2	54.3	0.2	< 0.0001	< 0.0001
Fat (g/d)	14.3	0.2	14.9	0.2	17.2	0.2	19.2	0.2	23.0	0.2	< 0.0001	< 0.0001
Carbohydrate (g/d)	288.5	1.0	302.5	1.0	298.4	1.0	293.4	1.0	278.9	1.0	< 0.0001	< 0.0001
β-Carotene (μg/d)	1228.2	41.9	1369.9	41.8	1712.6	41.7	2013.7	41.7	2561.2	42.0	< 0.0001	< 0.0001
Vitamin C (mg/d)	63.3	1.4	69.9	1.4	81.4	1.4	92.0	1.4	111.2	1.4	< 0.0001	< 0.0001
Folate (μg/d)	130.2	2.2	139.9	2.1	164.1	2.1	180.2	2.1	215.6	2.2	< 0.0001	< 0.0001
Vitamin E (mg/d)	5.04	0.07	5.19	0.07	5.74	0.07	6.24	0.07	7.32	0.07	< 0.0001	< 0.0001
Fibre (g/d)	3.85	0.06	4.32	0.06	4.88	0.06	5.24	0.06	5.81	0.06	< 0.0001	< 0.0001
Cholesterol (mg/d)	66.7	2.8	79.8	2.8	102.2	2.8	121.4	2.8	148.9	2.8	< 0.0001	< 0.0001
Men (%)	43.1		37.9		36.6		41.3		45.3		0.006	0.137
Menopausal women (%)§	80.9		79.2		80.2		80.0		80.3		0.900	0.928
Higher education (%)	14.9		15.5		18.2		18.9		28.7		< 0.0001	< 0.0001
Current smoker (%)	22.5		16.7		15.0		17.1		16.9		< 0.0001	0.001
Current drinker (%)	44.0		41.1		48.2		48.2		50.4		0.0001	< 0.0001
Regular exercise (%)	17.0		17.2		24.8		29.1		36.3		< 0.0001	< 0.0001

Q, quintile.

* P values for differences across groups and P values for the trends were determined using general linear model for continuous variables after adjustment for age and sex, and using the Cochran–Mantel–Haenszel test for categorical variables after adjustment for age (men and premenopausal women) or age and sex (higher education, current smoker, current drinker and regular exercise).

† All continuous variables were adjusted for age and sex.

‡ All nutrients were total energy adjusted by residual method after log transformation.

§ The proportion among women subjects.

|| ≥ High school graduation.

Table 3. Common carotid intima-media thickness (IMT) by zinc intake group
(Mean values and 95% confidence intervals)

	Zn intake										P*
	Q1		Q2		Q3		Q4		Q5		
	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI	
Total subjects											
<i>n</i>	912		913		912		913		914		
Median Zn intake (mg) (range)	5.00 (2.81–5.27)		5.48 (5.27–5.65)		5.85 (5.65–6.04)		6.29 (6.04–6.63)		7.19 (6.63–40.38)		
Adjusted IMT mean 1 (mm)†	0.733 ^a	0.725, 0.741	0.716 ^b	0.708, 0.724	0.715 ^b	0.707, 0.723	0.723 ^{a,b}	0.715, 0.731	0.710 ^b	0.702, 0.718	< 0.001
Adjusted IMT mean 2 (mm)‡	0.731 ^a	0.722, 0.741	0.715 ^b	0.706, 0.725	0.714 ^b	0.705, 0.723	0.724 ^{a,b}	0.716, 0.733	0.715 ^b	0.706, 0.723	0.007
Adjusted IMT mean 3 (mm)§	0.732 ^a	0.721, 0.743	0.717 ^{a,b}	0.707, 0.727	0.713 ^b	0.704, 0.722	0.721 ^{a,b}	0.711, 0.730	0.707 ^b	0.696, 0.717	0.006
Subjects aged < 65 years											
<i>n</i>	477		503		551		615		659		
Median Zn intake (mg) (range)	5.01 (2.81–5.27)		5.48 (5.27–5.65)		5.85 (5.65–6.04)		6.29 (6.04–6.63)		7.18 (6.63–24.00)		
Adjusted IMT mean 1 (mm)†	0.688 ^a	0.678, 0.698	0.670 ^{a,b}	0.660, 0.680	0.668 ^b	0.659, 0.678	0.678 ^{a,b}	0.669, 0.687	0.668 ^b	0.660, 0.677	0.018
Adjusted IMT mean 2 (mm)‡	0.686 ^a	0.674, 0.697	0.668 ^{a,b}	0.657, 0.679	0.666 ^b	0.656, 0.677	0.677 ^{a,b}	0.667, 0.687	0.670 ^{a,b}	0.660, 0.679	0.026
Adjusted IMT mean 3 (mm)§	0.687 ^a	0.673, 0.700	0.669 ^a	0.657, 0.680	0.665 ^a	0.655, 0.676	0.675 ^a	0.665, 0.686	0.666 ^a	0.655, 0.677	0.038
Subjects aged ≥ 65 years											
<i>n</i>	435		410		361		298		255		
Median Zn intake (mg) (range)	4.98 (2.86–5.27)		5.47 (5.27–5.65)		5.85 (5.66–6.04)		6.31 (6.05–6.62)		7.21 (6.63–40.4)		
Adjusted IMT mean 1 (mm)†	0.805 ^a	0.792, 0.818	0.789 ^{a,b}	0.776, 0.802	0.790 ^{a,b}	0.775, 0.804	0.794 ^{a,b}	0.779, 0.810	0.775 ^b	0.758, 0.792	0.084
Adjusted IMT mean 2 (mm)‡	0.794 ^a	0.776, 0.812	0.782 ^a	0.763, 0.800	0.781 ^a	0.762, 0.800	0.791 ^a	0.771, 0.810	0.775 ^a	0.756, 0.794	0.384
Adjusted IMT mean 3 (mm)§	0.795 ^a	0.775, 0.816	0.784 ^a	0.766, 0.803	0.778 ^a	0.759, 0.797	0.782 ^a	0.761, 0.803	0.759 ^a	0.737, 0.782	0.150

Zinc intake and intima-media thickness

Q, quintile.

^{a,b} Mean values with unlike superscript letters within a row were significantly different among the three groups by Tukey's multiple comparison test.

* P values for differences across groups were obtained using the general linear model.

† Adjusted for age and sex.

‡ Adjusted for age, sex, education, alcohol intake, smoking, regular exercise, waist circumference and HDL-cholesterol.

§ Adjusted for age, sex, education, alcohol intake, smoking, regular exercise, waist circumference, HDL-cholesterol and dietary factors (quintiles of total energy, protein, fat, carbohydrate, β-carotene, vitamins C and E, folate, dietary fibre and cholesterol).

Table 4. Risk of common carotid atherosclerosis by zinc intake group in multivariate models (Odds ratios and 95 % confidence intervals)

	Zn intake										P for linear trend
	Q1		Q2		Q3		Q4		Q5		
	OR		OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	
Criterion: IMT > 80th percentile (0.8232 mm)											
n	912		913		912		913		914		
Median Zn intake (mg) (range)	5.00 (2.81–5.27)		5.48 (5.27–5.65)		5.85 (5.65–6.04)		6.29 (6.04–6.63)		7.19 (6.63–40.38)		
Subjects with IMT > 80th percentiles (%)	235 (25.8)		177 (19.4)		170 (18.6)		190 (20.8)		148 (16.2)		
Multivariate-adjusted OR 1*	1.00		0.71	0.56–0.91	0.78	0.62–0.10	1.00	0.79–1.27	0.80	0.62–1.03	0.532
Multivariate-adjusted OR 2†	1.00		0.74	0.58–0.94	0.78	0.61–0.99	1.03	0.81–1.32	0.83	0.64–1.09	0.768
Multivariate-adjusted OR 3‡	1.00		0.72	0.55–0.93	0.70	0.52–0.93	0.86	0.63–1.17	0.64	0.45–0.90	0.069
Subjects aged < 65 years											
n	477		503		551		615		659		
Median Zn intake (mg) (range)	5.01 (2.81–5.27)		5.48 (5.27–5.65)		5.85 (5.65–6.04)		6.29 (6.04–6.63)		7.18 (6.63–24.00)		
Subjects with IMT > 80th percentiles (%)	69 (14.5)		45 (8.9)		46 (8.3)		74 (12.0)		58 (8.8)		
Multivariate-adjusted OR 1*	1.00		0.57	0.38–0.87	0.61	0.40–0.91	0.84	0.59–1.22	0.68	0.47–1.00	0.365
Multivariate-adjusted OR 2†	1.00		0.56	0.37–0.85	0.58	0.39–0.88	0.82	0.56–1.19	0.67	0.45–1.00	0.364
Multivariate-adjusted OR 3‡	1.00		0.49	0.32–0.77	0.46	0.28–0.75	0.59	0.36–0.96	0.44	0.26–0.76	0.048
Subjects aged ≥ 65 years											
n	435		410		361		298		255		
Median Zn intake (mg) (range)	4.98 (2.86–5.27)		5.47 (5.27–5.65)		5.85 (5.66–6.04)		6.31 (6.05–6.62)		7.21 (6.63–40.4)		
Subjects with IMT > 80th percentiles (%)	166 (38.2)		132 (32.2)		124 (34.3)		116 (38.9)		90 (35.3)		
Multivariate-adjusted OR 1*	1.00		0.80	0.60–1.07	0.91	0.67–1.22	1.10	0.80–1.50	0.88	0.64–1.23	0.992
Multivariate-adjusted OR 2†	1.00		0.85	0.63–1.14	0.91	0.67–1.24	1.18	0.86–1.63	0.95	0.67–1.34	0.657
Multivariate-adjusted OR 3‡	1.00		0.82	0.59–1.14	0.86	0.63–1.19	1.04	0.70–1.57	0.78	0.50–1.23	0.473
Criterion: IMT ≥ 1.0 mm											
n	912		913		912		913		914		
Subjects with IMT ≥ 1.0 (%)	52 (5.7)		39 (4.3)		36 (3.9)		40 (4.4)		17 (1.9)		
Multivariate-adjusted OR 1*	1.00		0.79	0.51–1.22	0.83	0.53–1.30	1.00	0.65–1.55	0.43	0.25–0.77	0.020
Multivariate-adjusted OR 2†	1.00		0.86	0.55–1.34	0.91	0.57–1.43	1.10	0.70–1.72	0.51	0.29–0.91	0.092
Multivariate-adjusted OR 3‡	1.00		0.83	0.51–1.33	0.77	0.45–1.33	0.82	0.46–1.48	0.34	0.16–0.70	0.005
Subjects aged < 65 years											
n	477		503		551		615		659		
Subjects with IMT ≥ 1.0 (%)	9 (1.9)		8 (1.6)		9 (1.6)		14 (2.3)		5 (0.8)		
Multivariate-adjusted OR 1*	1.00		0.85	0.32–2.24	1.01	0.39–2.59	1.28	0.54–3.00	0.49	0.16–1.48	0.372
Multivariate-adjusted OR 2†	1.00		0.96	0.35–2.62	1.13	0.42–3.02	1.47	0.59–3.66	0.62	0.19–1.96	0.640
Multivariate-adjusted OR 3‡	1.00		0.95	0.32–2.81	0.94	0.29–2.98	1.13	0.34–3.76	0.43	0.10–1.83	0.227
Subjects aged ≥ 65 years											
n	435		410		361		298		255		
Subjects with IMT ≥ 1.0 (%)	43 (9.9)		31 (7.6)		27 (7.5)		26 (8.7)		12 (4.7)		
Multivariate-adjusted OR 1*	1.00		0.78	0.48–1.27	0.78	0.47–1.30	0.90	0.53–1.51	0.42	0.22–0.83	0.029
Multivariate-adjusted OR 2†	1.00		0.83	0.50–1.36	0.84	0.50–1.42	0.99	0.58–1.68	0.47	0.24–0.94	0.082
Multivariate-adjusted OR 3‡	1.00		0.83	0.49–1.42	0.75	0.40–1.40	0.77	0.39–1.52	0.33	0.14–0.77	0.013

Q, quintile; IMT, intima-media thickness.

* The OR were adjusted for age and sex.

† The OR were adjusted for age, sex, education, alcohol intake, smoking, regular exercise, waist circumference and HDL-cholesterol.

‡ The OR were adjusted for age, sex, education, alcohol intake, smoking, regular exercise, waist circumference, HDL-cholesterol and dietary factors (quintiles of total energy, protein, fat, carbohydrate, β-carotene, vitamins C and E, folate, dietary fibre and cholesterol).

did not show any significant associations, which can be attributed to the low cut-off value for subclinical atherosclerosis for the elderly subjects. Since IMT increases with age, the low cut-off for subclinical atherosclerosis was not appropriate for detecting the effect of dietary Zn among the elderly subjects. When $IMT \geq 1$ was used to determine the OR, a significant inverse relationship between Zn intake and subclinical atherosclerosis was found only among the elderly subjects aged ≥ 65 years (5th v. 1st quintile, OR 0.33, 95% CI 0.14, 0.77, P for trend=0.013). Since the number of subjects with $IMT \geq 1$ was small among the middle-aged adult subjects, any significant association was not found in the middle-aged adult subjects. Taken together, these results suggest that adequate dietary Zn intake may have a beneficial effect on the risk of atherosclerosis among the middle-aged adult and elderly populations, and different criteria for subclinical atherosclerosis may be needed between age groups.

Limited epidemiological studies have examined the relationship between Zn intake and atherosclerosis. Dietary Zn intake was positively correlated with serum Zn levels in cross-sectional studies^(10,32), and lower dietary Zn intake and lower concentrations of serum Zn were associated with a higher prevalence of CHD^(10,12) or an increased cardiovascular mortality⁽³³⁾. Lower serum Zn concentrations have been reported in patients with various forms of coronary artery diseases in case-control studies^(13,34–36).

Several potential mechanisms may account for the cardiovascular benefits of Zn^(6,8,9,37–43). Atherogenesis involves the oxidation of LDL and its uptake into macrophages, and apoptosis of endothelial cells. Zn ions inhibited LDL oxidation by macrophages and endothelial cells⁽⁶⁾. The activity of caspase enzymes involved in apoptotic pathways was inhibited by Zn^(8,9). Zn deficiency increased apoptotic cell death induced by linoleic acid and TNF- α , and supplementation of Zn attenuated apoptosis⁽³⁷⁾.

NO is an endogenous signalling molecule produced by NO synthase. NO plays a substantial role in platelet and leucocyte function in human subjects. Several studies have suggested that NO can inhibit leucocyte adhesion through various mechanisms^(38–40). Zn plays a key role in endothelial NO synthase function by stabilising a dimer of two subunits of NO synthase⁽⁴¹⁾. Zn deficiency can reduce the activity of NO synthase, which causes a change in NO production. Changes in NO production are associated with the development of atherosclerosis⁽⁴²⁾. Indeed, NO is involved in Zn release from proteins by thiol nitrosylation⁽⁴³⁾.

However, in the case-control studies conducted by Iskra *et al.*^(14,15), serum concentrations of Zn in individuals with atherosclerosis obliterans were higher than those in the healthy control groups. Even if the present findings reflect a causal relationship between Zn in food and atherosclerosis, this would not imply that the same amount or more Zn in supplements would have the same effect. High dose of Zn supplements (≥ 50 mg) induced a decline in plasma HDL-cholesterol concentrations in healthy young males^(44–46), which demonstrates that a high dose of Zn supplementation may induce an atherogenic lipoprotein profile. The tolerable upper intake levels of Zn for Koreans and Americans are 35⁽²⁵⁾ and 40 mg/d⁽⁴⁷⁾, respectively. Therefore, the role of Zn in CHD needs to be investigated further.

There are several limitations to the present study which need to be considered when interpreting the findings. First, convenience sampling may preclude generalisation of the results in the present study. Secondly, since this was a cross-sectional study, we cannot conclude causality of Zn intake for subclinical atherosclerosis. Thirdly, there were no subjects who took Zn supplement on a regular basis, and a total of 269 subjects (5.9%) answered that 'they are taking multivitamin supplements', but most of the subjects could not remember the brand names of the multivitamins. Therefore, Zn intake from supplement was not considered. Fourthly, during the assessment of dietary Zn status, the bioavailability of Zn should be taken into account⁽⁴⁸⁾. Phytate is a major inhibitory factor for Zn absorption, and phytate is present in whole grains, cereals and legumes. The phytate:Zn molar ratio has been used in several studies to predict the inhibitory effect of phytate on the bioavailability of Zn^(26,48,49). However, due to a lack of information on the phytate content of foods in the Korean Food Composition Table, the inhibitory effect of phytate on the bioavailability of Zn was not taken into account in the present study. Finally, despite the lack of a reliable and specific index, Zn status of the subjects needs to be assessed by biomarkers such as plasma and/or erythrocyte Zn concentrations as well as dietary intake, which was not done in the present study⁽⁵⁰⁾.

To date, this is the first epidemiological study to determine the relationship between dietary Zn intake and IMT, an indicator of subclinical atherosclerosis. Among the rural middle-aged adult and elderly populations in Korea, inadequate intake of Zn was related to high IMT and high risk of subclinical atherosclerosis. The present findings suggest that adequate intake of Zn may have a beneficial effect in reducing the risk of atherosclerosis. Further studies are necessary to examine these findings in prospective studies.

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