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Determinants of Serum Zinc in a Random Population Sample of Four Belgian Towns with Different Degrees of Environmental Exposure to Cadmium

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This report investigated the distribution of serum zinc and the factors determining serum zinc concentration in a large random population sample. The 1977 participants (959 men and 1018 women), 20–80 years old, constituted a stratified random sample of the population of four Belgian districts, representing two areas with low and two with high environmental exposure to cadmium. For each exposure level, a rural and an urban area were selected. The serum concentration of zinc, frequently used as an index for zinc status in human subjects, was higher in men (13.1 µmole/L, range 6.5–23.0 µmole/L) than in women (12.6 µmole/L, range 6.3–23.2 µmole/L). In men, 20% of the variance of serum zinc was explained by age (linear and squared term, $R = 0.29$), diurnal variation ($r = 0.29$), and total cholesterol ($r = 0.16$). After adjustment for these covariates, a negative relationship was observed between serum zinc and both blood ($r = -0.10$) and urinary cadmium ($r = -0.14$). In women, 11% of the variance could be explained by age (linear and squared term, $R = 0.15$), diurnal variation in serum zinc ($r = 0.27$), creatinine clearance ($r = -0.11$), log γ-glutamyltranspeptidase ($r = 0.08$), cholesterol ($r = 0.07$), contraceptive pill intake ($r = -0.07$), and log serum ferritin ($r = 0.06$). Before and after adjustment for significant covariates, serum zinc was, on average, lowest in the two districts where the body burden of cadmium, as assessed by urinary cadmium excretion, was highest. These results were not altered when subjects exposed to heavy metals at work were excluded from analysis.

Introduction

Zinc is an essential component in many biological enzymes. Zinc plays an important role in protein synthesis, bone formation, cell-mediated immunity, endocrine function, tissue growth, and wound healing. Among the trace elements, the concentration of zinc in the body is second only to iron (*1,2*).

Serum zinc is the most frequently used index for zinc status in humans (*3*). Only few studies have described the description of serum zinc in a random sample of the general population (*4,5*). The influence of environmental exposure to heavy metals on the levels of zinc in serum has not yet been investigated. The present study was conducted in a random sample of the adult inhabitants of four Belgian districts. The objectives of this paper are *a)* to report the concentration of zinc in the serum and the factors determining serum zinc in the general population and *b)* to investigate whether environmental exposure to cadmium affects the level of zinc in serum.

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Methods

Study Population

As described in detail elsewhere (*6*), the study was conducted in four Belgian districts, representing two areas with high and two with lower environmental exposure to cadmium. For each exposure level, a rural and an urban district were selected. In Liège (polluted town), the 95th percentile of the airborne cadmium concentration during operation of the local zinc- and/or cadmium-producing plants amounted to 1.47 nmole/m^3 and in Noorderkempen (polluted rural district) to 0.36 nmole/m^3, whereas in Charleroi (control town) and Hechtel-Eksel (control rural area), the 95th percentiles of airborne cadmium never exceeded 0.27 and 0.09 nmole/m^3, respectively (*6*). In the Liège area, the cadmium concentration in the soil ranged from 36 to 320 μmole/kg (dry weight), and in grass from 4 to 222 μmole/kg (dry weight); in Noorderkempen these concentrations ranged from 4 to 213 and from 1 to 258 μmole/kg, whereas in both nonpolluted districts they did not exceed 9 and 18 μmole/kg, respectively (*6*). In each district a random sample of the households was identified and subjects with a minimum age of 20 years were invited to participate (*6*).

In the 4 districts, a total of 4,532 subjects were eligible for the study, of whom 2327 took part. The participation rate was 78% in the two rural and 39% in the two urban districts. Subjects were excluded from the present analysis when their participation did not include all data relevant to the present analysis ($n = 256$), when external contamination of the serum samples could not be excluded ($n = 8$), when 24-hr urine samples were judged to be under- or overcollected ($n = 41$), or when occupational exposure to heavy metals ($n = 39$) or smoking habits ($n = 6$) could not be ascertained from the self-administered questionnaire.

Field Work

Each household was visited by an observer who measured body weight and height. The participants were asked to complete a self-administered questionnaire and to collect a 24-hr urine sample. The questionnaire inquired about the participants' medical history, their current and past occupations, exposure to heavy metals at work, smoking habits, consumption of alcohol, and intake of medications. The questionnaire classified women with respect to their menstrual status and use of birth control pills. The subjects were subdivided according to their current occupations into a high (academic and executive professions, teachers with a university degree), a medium (the self-employed, small undertakers, employees, teachers, shopkeepers), and a low (farmers, laborers, subjects without profession) employment grade subgroup. For analysis, subjects with medium and high employment grades were pooled because of the low number of subjects in the high employment grade group.

Usually within 2 weeks after the urine collection, a physician or nurse visited the households to withdraw 20 mL of venous blood.

Biochemical Measurements

The biochemical techniques and procedures for quality control have been described in detail elsewhere (*6*). Blood samples were collected in propylene metal-free tubes and were analyzed for serum total zinc (*7*), magnesium (*8*), total calcium (*9*), total and HDL-cholesterol (*10,11*), ferritin (*12*), alkaline phosphatase (*13*), and γ-glutamyltranspeptidase activity (*14*) and blood cadmium (*6*). The concentration of zinc in serum was determined using the method of deproteinization with trichloroacetic acid combined with flame atomic absorption spectometry (Perkin-Elmer Model 305) (*7*). Twenty-four-hour urine samples were analyzed for cadmium (*6*) and creatinine (*15*).

Most of the biochemical analyses were performed in duplicate, and certified reference standards were run along each series of study samples. A series of measurements was repeated when the precision of duplicate determinations fell outside previously published limits (*6*). Furthermore, 10% of the zinc, cadmium, and ferritin determinations were performed in two different laboratories, both of which participated in an external quality control program. When the results of one sample deviated more than 10%, the whole series was reanalyzed in both laboratories.

Statistical Analysis

The statistical analysis was performed using various SAS procedures (SAS Institute, Cary, North Carolina). Means were compared by Student's *t*-tests and one-way analysis of variance. Serum zinc was correlated with other measurements using Pearson's correlation coefficients. The independent covariates of serum zinc were determined by stepwise multiple regression analysis, terminating when all the partial regression coefficients were significant at the 5% level (*16*).

The distributions of serum zinc, ferritin, γ-glutamyltranspeptidase, alkaline phosphatase, and blood and urinary cadmium were normalized by a logarithmic transformation. The completeness of the 24-hr urine samples was evaluated by criteria published elsewhere (*17*).

Results

Subjects

The characteristics of the 1977 subjects included in the present analysis are summarized in Table 1.

Serum Zinc Levels

The geometric mean of serum zinc was higher ($p < 0.001$) in men than in women (13.1 vs. 12.6 μmole/L) and was lower ($p = 0.03$) in women using the contraceptive pill compared with women not using birth control pills (12.3 versus 12.7 μmole/L). Pre- and post-menopausal women had similar serum zinc levels.

Serum zinc was significantly higher ($p < 0.001$) when blood was collected in the morning, i.e., before 12:00 A.M., compared with levels when blood was withdrawn in the afternoon (14.8 versus 12.6 μmole/L; Fig. 1).

Table 1. Characteristics of the participants.[a]

	Men	Women
Number	959	1018
Home measurements		
Age, years	48 ± 16	48 ± 16
Body weight, kg	76.5 ± 12.2[*]	65.3 ± 12.9
Height, cm	173 ± 7[*]	161 ± 6
Body mass index, kg/m^2	25.5 ± 3.6	25.3 ± 5.1
Questionnaire data		
Contraceptive pill intake, %	–	20.5
Post-menopausal women, %	–	46.0
Current smokers, %	48.8	34.8
Current alcohol intake, %	37.0	13.9
Occupationally exposed, %	31.5[*]	2.6
Biochemical measurements		
Serum zinc, μmole/L	13.1 (6.5–23.0)[*]	12.6 (6.3–23.2)
Serum calcium, mmole/L	2.37 ± 0.10	2.37 ± 0.11
Serum magnesium, mmole/L	1.01 ± 0.08[†]	1.00 ± 0.08
Serum total cholesterol, mmole/L	5.99 ± 1.35	6.09 ± 1.45
Blood cadmium, nmole/L	10.1 (0.9–129.9)	10.0 (0.9–81.0)
γ-Glutamyltranspeptidase, U/L	14.3 (2–252)[*]	9.5 (2–335)
Alkaline phosphatase, U/L	121 (33–857)[*]	109 (22–527)
Serum ferritin, μmole/L	397 (20–8417)[*]	177 (16–5032)
Urinary cadmium, nmole/24 h	9.27 (0.4–325.2)[*]	7.16 (0.1–70.8)
Creatinine clearance, mL/min	98.6 ± 29.9[*]	79.6 ± 25.4

[a]Values are means ± SD. For logarithmically transformed variables the geometric mean and range are presented.
[*]Significant sex difference, $p < 0.001$.
[†]Significant sex difference, $p < 0.05$.

Male subjects reporting occupational exposure to heavy metals had lower (13.1 versus 12.7, $p < 0.001$) serum zinc levels than those not exposed at work. This finding could not be confirmed in female subjects. In men, the concentration of zinc in serum was lower ($p < 0.001$) in the low employment grade group ($n = 649$) compared with those with medium ($n = 278$) or high ($n = 32$) employment grade. The same tendency ($p = 0.06$) was observed in women of whom 789, 220, and 9 had low, medium, and high employment grade (Fig. 1).

The geometric mean of serum zinc was comparable in smokers and nonsmokers. By contrast, serum zinc was higher ($p = 0.001$) in subjects who reported current intake of alcohol as compared with nondrinkers (13.2 versus 12.7 μmole/L). In addition, men and women consuming more than 24 g of alcohol per day had similar serum zinc levels. There were, however, only 33 such women (Fig. 1).

Determinants of Serum Zinc

The Pearson correlation coefficients between serum zinc and various other measurements are shown in Table 2. Serum zinc was significantly ($p < 0.001$) correlated with serum calcium ($r = 0.27$, $p < 0.001$) and serum magnesium ($r = 0.21$, $p < 0.001$). The three ions showed comparable trends with age. In men, the concentrations of serum zinc ($r = -0.28$, $p < 0.001$) and serum calcium ($r = -0.28$, $p < 0.001$) decreased with age, whereas in women the concentrations of the three ions were highest in the 50- to 69-year-old group (Fig. 2).

Multiple regression analysis showed that log serum zinc in men was significantly and independently correlated with age (linear and squared term, multiple partial

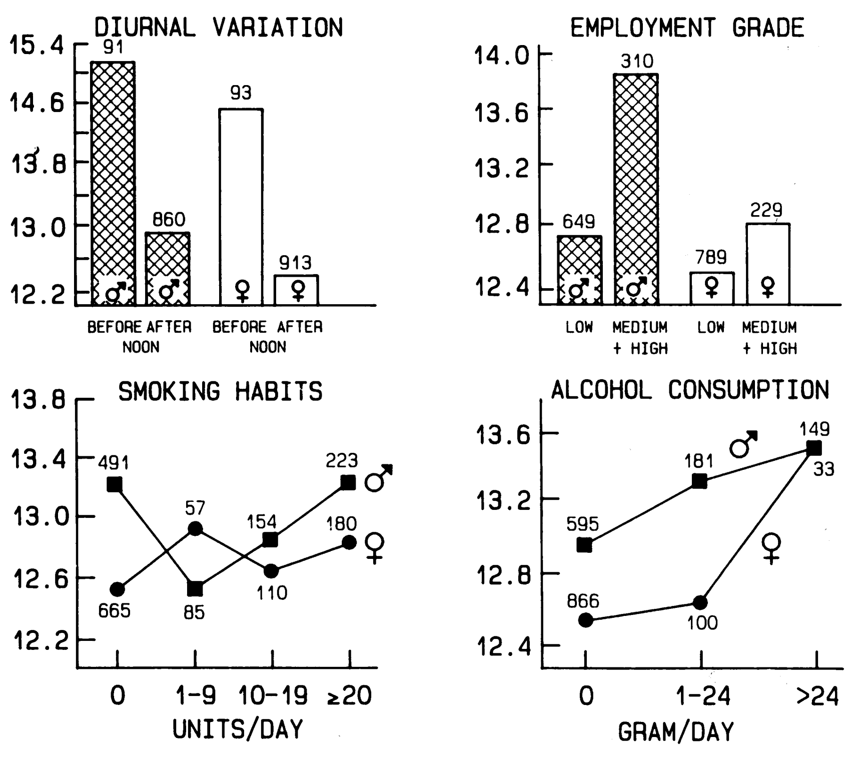


FIGURE 1. The geometric mean of serum zinc in several subgroups. Subjects were classified according to gender, diurnal time of blood sampling, employment grade, current smoking habits, and current alcohol intake. The number of subjects in each subgroup is given.

Table 2. Pearson correlation coefficients relating log serum zinc to other measurements.

	Men	Women
Age	-0.28^{*}	0.03
Age squared	-0.26^{*}	0.02
Body weight	$0.10^{\dagger}$	-0.01
Body mass index	$0.06^{\ddagger}$	-0.03
Serum magnesium	0.18^{*}	0.23^{*}
Serum calcium	0.27^{*}	0.26^{*}
Total cholesterol	0.13^{*}	0.12^{*}
HDL cholesterol	0.11^{*}	0.15^{*}
Blood lead	-0.03	0.08^{*}
Blood cadmium	-0.12^{*}	0.001
Urinary cadmium	-0.22^{*}	-0.01
Log alkaline phosphatase	$-0.09^{\dagger}$	0.07^{**}
Log γ-glutamyltranspeptidase	0.07^{**}	0.13^{*}
Log serum ferritin	0.06^{**}	$0.09^{\dagger}$
Creatinine clearance	0.08^{**}	$-0.09^{\dagger}$

$^{*}p < 0.001$.
$^{\dagger}p < 0.01$.
$^{\ddagger}p < 0.06$.
$^{**}p < 0.05$.

$R = 0.29$), diurnal time of blood sampling (partial $r = 0.29$), and serum total cholesterol ($r = 0.16$). After cumulative adjustments for these covariates, log serum zinc was negatively related to both blood cadmium ($r = -0.10$, $p = 0.002$) and urinary cadmium ($r = -0.14$, $p < 0.001$). In women, the following factors were independently associated with log serum zinc: diurnal time of blood sampling ($r = 0.27$), age (linear and squared term, $R = 0.15$), creatinine clearance ($r = -0.11$), log γ-glutamyltranspeptidase ($r = 0.08$), contraceptive pill intake ($r = -0.07$), cholesterol ($r = 0.07$), and log serum ferritin ($r = 0.06$) (Table 3). After adjustment for these variables, the partial correlation coefficients with body mass index, employment grade, and blood cadmium were not significant in either sex. The partial correlation coefficient between serum zinc and HDL-cholesterol, corrected for all the significant covariates except total cholesterol, was 0.13 ($p < 0.001$) in women and 0.05 ($p = 0.10$) in men. The results were not materially altered by excluding the subjects who were exposed to heavy metals at work (Table 3).

Serum Zinc According to Place of Residence

Serum zinc was significantly lower in the two rural as compared with the two urban districts (12.4 versus 13.1 μmole/L, $p < 0.001$). In the rural area, the levels of zinc in serum were lower in the high than in the low exposure district (12.2 versus 12.6 μmole/L, $p = 0.004$), whereas in the urban area the opposite was found (13.4 vs. 12.4 μmole/L, $p < 0.001$). The geometric mean of urinary cadmium was similar in the two urban districts (6.2 and 6.9 nmole/24 hr in the control and polluted town, respectively), but was markedly higher in the two rural districts (8.4 and 12.5 nmole/24 hr in the control and polluted area, respectively).

Figure 3 displays the geographical trends in serum zinc standardized for age, diurnal variation, log γ-glutamyltranspeptidase, serum cholesterol, serum ferritin, creatinine clearance, and contraceptive pill intake in women. After adjustment for these covariates, serum zinc was 5% lower in the two rural compared with the two urban districts. These findings were not different when subjects reporting occupational exposure to heavy metals were excluded from analysis.

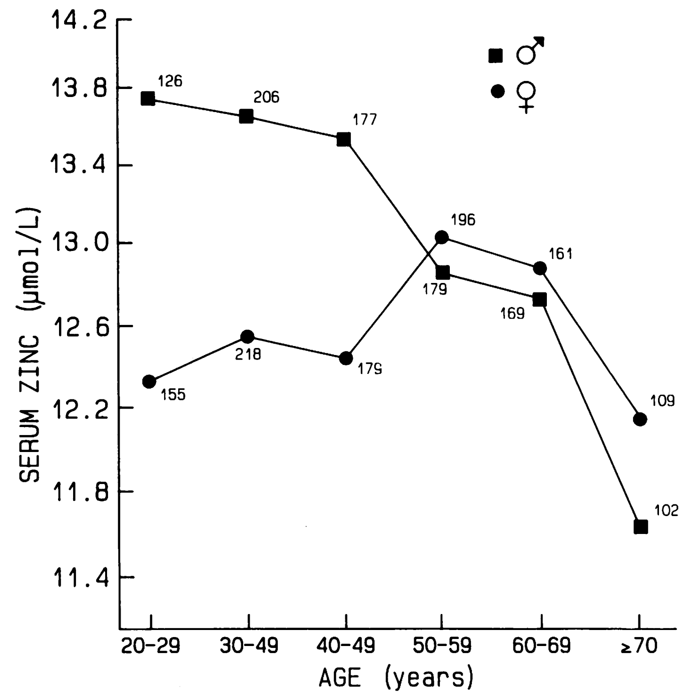
Discussion

The arithmetic mean of serum zinc in the present study was 13.1 μmole/L, which is somewhat lower than generally reported in the literature (*4*). This could be related to the relatively high level of environmental exposure to cadmium in Belgium. Indeed, during life, cadmium (*18,19*) accumulates in the body and is concentrated mainly in the liver and kidneys. The main storage protein for cadmium in these organs is metallothionein. This protein binds both cadmium and zinc, and its synthesis is stimulated by exposure to cadmium. Therefore, exposure to cadmium could lead to a redistribution of zinc from plasma to the liver and the kidneys (*1,2,20*). The inverse relationship between serum zinc and both blood and urinary cadmium observed in men of the present study may also be explained by this mechanism (Tables 2 and 3). However, serum zinc must also be influenced by other factors as a negative correlation between serum zinc and blood and urinary cadmium was not observed in women. In addition, when the areas were compared, adjustments for confounding factors being applied as necessary, a negative association between serum zinc and environmental exposure to cadmium was only observed for the contrast between the two rural versus the two urban areas and for the comparison between the two rural areas. The gradient in serum zinc expected on the basis of the environmental exposure levels in the two urban areas was not found. Roels et al. (*21*) investigated two groups of healthy, elderly women, one group living in a cadmium-polluted district and the other group in an area less contaminated by cadmium. These investigators found that plasma zinc concentrations were significantly lower in the polluted area compared with the control district (*21*).

The participation rate in the two urban districts was low (39%). Selection bias may possibly limit the generalizability of the results obtained in the two urban areas. As far as this could be assessed, there were no socioeconomic differences between respondents and nonrespondents, but nonrespondents in the two urban areas were on average 12 years older than the subjects taking part in the study. The determinants of serum zinc were not materially altered when the analysis was restricted to the rural areas, where the participation rate of 78% could be considered to be satisfactory. The regression coefficients remained similar in magnitude, although some coefficients were not significant any more because of the lower number of subjects.

It is well known that serum or plasma zinc levels are higher in men than in women and that they are lower in women using oral contraceptives compared with women not using birth control pills (*5,22,23*). This may be explained by the induction of hepatic metallothionein synthesis by the female sex steroids, particularly the estrogens (*24,25*).

A decline in serum or plasma zinc with age in adult men has been observed by some investigators (*4,5,26*). However, the influence of age on the concentration of serum zinc in women is less clear (*4,5,26*). This could be related to the fact that the correlation between serum zinc and age is usually described by means of simple correlation coefficients that are not able to detect a nonlinear relationship between two variables. The present results demonstrate a concave curvilinear relationship between serum zinc and age in women. The increase in serum zinc, total calcium, and magnesium in the 50- to 69-year-old female subjects may be the result of the increase in protein concentration after menopause.



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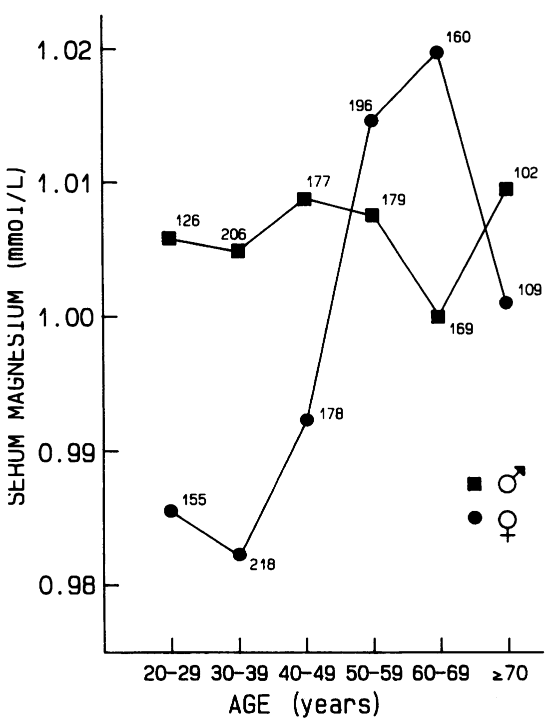

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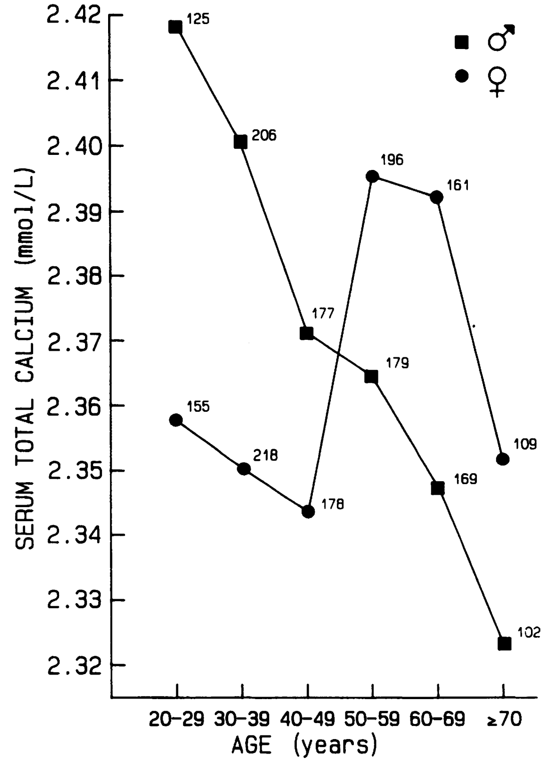

C

FIGURE 2. The geometric mean of serum zinc (*A*) and the arithmetic mean of serum magnesium (*B*) and calcium (*C*) in six age groups, for women (●) and men (■) separately.

Several reports have described a diurnal rhythm of zinc in serum (*4,27,29*). The present study was not designed to look at diurnal variations in serum zinc concentrations. However, before and after adjustment for possible confounding factors, serum zinc was significantly lower in subjects who had their blood taken in the afternoon. These findings are in agreement with the results of the Second National Health and Nutrition Examination Survey in the United States (*5*).

The present study demonstrated in women an independent positive relationship between serum zinc and γ-glutamyltranspeptidase, an index for alcohol consumption. Some investigators observed significantly lower serum or plasma zinc concentrations in chronic alcoholics compared with controls (*29,30*). This does not contradict the present findings. Indeed, Conri et al. (*31*) studied three groups of subjects: normal individuals and alcoholics with normal and disturbed liver function. These authors found

Table 3. Multiple regression analysis.[a]

	Men	Women
N	951	1006
	(651)	(980)
R^2	0.22	0.11
	(0.23)	(0.11)
Intercept	1.07*	1.04*
	(1.07)*	(1.03)*
Age	2.2×10^{-3}†	1.1×10^{-3}†
	(2.8×10^{-3})†	(1.1×10^{-3})
Age squared	-3.4×10^{-5}*	-1.9×10^{-5}†
	(-4.2×10^{-5})*	(-2.0×10^{-5})†
Blood sampling in morning[b]	6.8×10^{-2}*	6.8×10^{-2}*
	(6.9×10^{-2})*	(6.0×10^{-2})*
Contraceptive pill intake[b]	–	-1.3×10^{-2}†
		(-1.2×10^{-2})†
Log γ-glutamyl-transpeptidase, U/L	-4.1×10^{-3},NS	2.2×10^{-2}‡
	(2.9×10^{-3},NS)	(2.2×10^{-2})‡
Log serum ferritin, μmole/L	1.2×10^{-2}*	1.3×10^{-2}†
	(9.2×10^{-3},NS)	(1.4×10^{-2})†
Serum total cholesterol, mmole/L	1.0×10^{-2}	4.4×10^{-3}†
	(8.6×10^{-3})*	(4.9×10^{-3})†
Creatinine clearance, mL/min	-8.2×10^{-5},NS	-3.5×10^{-4}*
	(-1.5×10^{-4},NS)	(-3.3×10^{-4})*
Log blood cadmium, nmole/L[c]	-2.0×10^{-2}†	-9.2×10^{-3},NS)
	(1.9×10^{-2}†	(-1.0×10^{-2},NS)
Log urinary cadmium, nmole/24 hr[c]	-3.5×10^{-2}*	-3.9×10^{-3},NS
	(-3.8×10^{-2})*	(-5.6×10^{-3},NS)

NS, not significant.

[a]Partial regression coefficients are given. The regression coefficients in parentheses were obtained after excluding the subjects who were exposed to heavy metals at work. The following factors were not selected by the stepwise multiple regression procedure: body mass index, employment grade, and blood cadmium.

[b]Coded 0 or 1 for condition absent or present.

[c]Regression coefficients were derived for a model including either blood or urinary cadmium (not both).

*$p < 0.001$.

†$p < 0.05$.

‡$p < 0.01$.

**$p < 0.08$.

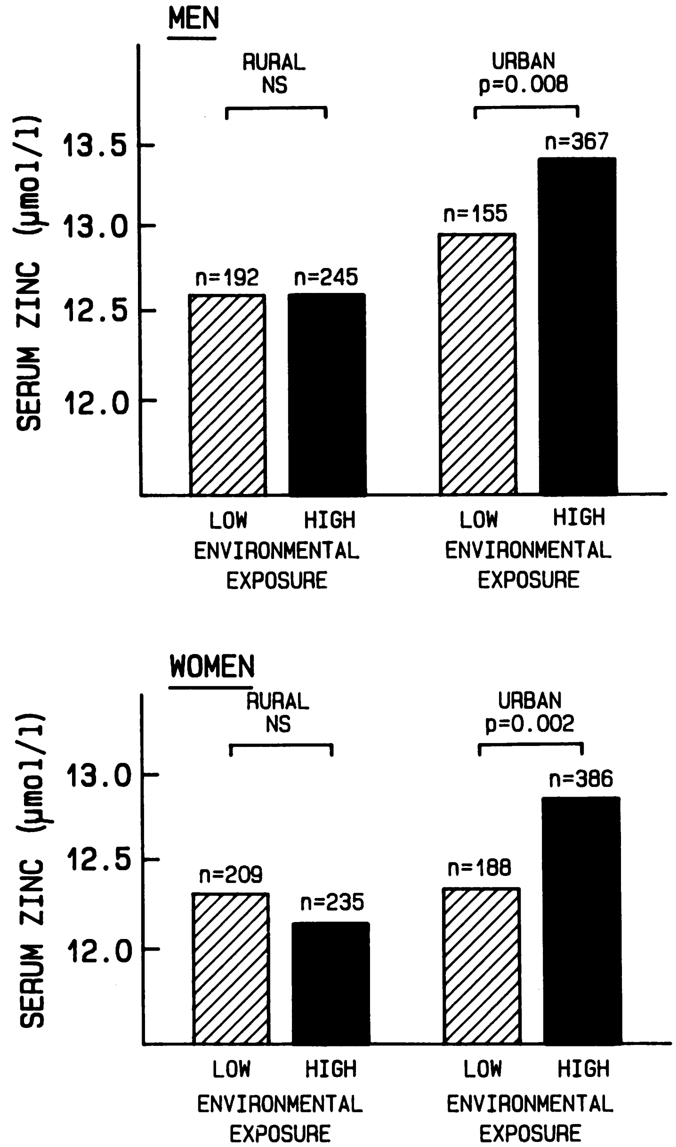

FIGURE 3. The geometric mean of serum zinc according to gender and the residence of the subjects in one of the four districts. Serum zinc levels were adjusted for age (linear and squared term), diurnal variation, serum γ-glutamyltranspeptidase, cholesterol, ferritin, creatinine clearance, and contraceptive pill intake in women.

that serum zinc levels were depressed in the latter group only, whereas alcoholics with normal hepatic function had significantly higher serum zinc concentrations compared to controls (*31*).

Some studies in subjects with experimental zinc deficiency have demonstrated a slight decrease in total cholesterol (*32,33*), whereas intake of supplementary zinc does not seem to be associated with significant changes in total cholesterol (*34–38*). The latter studies (*34,35,37,38*) reported a decrease in HDL-cholesterol. Most published observational studies have not found a significant relationship between serum or plasma zinc and total and HDL-cholesterol (*39–41*). The present results show a significant positive correlation between serum zinc and these two lipid fractions, but the explained variance was less than 3%, indicating that the observed relationships are probably biologically less important. One study in 3373 Finnish children also observed a significant positive relationship between serum zinc and total, HDL-, and LDL-cholesterol (*42*).

Experiments in animals and humans (*43,44*) have provided evidence for a competitive interaction between iron and zinc when these minerals are supplemented in amounts well in excess of the Recommended Dietary Allowances for adults (*45*). To our knowledge, the correlation between zinc and iron has not yet been described in population studies. The present study demonstrated a weak positive relationship between serum zinc and ferritin in women, and the same tendency was observed in men.

Both the positive correlation between serum zinc and ferritin and the positive relationship between serum zinc and total HDL-cholesterol may reflect the nutritional status in individual subjects of the general population. Indeed, high levels of serum zinc, ferritin, and cholesterol may be expected when the daily food intake is high.

Depressed serum and plasma zinc levels have been reported in patients with chronic renal disease (*4,46*). Several mechanisms have been suggested: decreased dietary intake (*47*), increased urinary and fecal losses (*48*), imparied intestinal absorption (*49,50*), decreased protein binding (*4*), or a shift from plasma to erythrocyte zinc (*51*). The present study could not demonstrate a positive relationship between serum zinc and renal function. In contrast, a negative correlation was found between serum zinc and creatinine clearance, suggesting that only severe renal disease is associated with decreased serum zinc levels.

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