ORIGINAL ARTICLE

Zinc and smoking habits in the setting of hypertension in a Spanish populations

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The objective of this study was to evaluate the relationship between trace and toxic amounts of zinc (Zn) in biological samples (blood and urine) and the smoking habits of hypertensive patients and healthy control subjects in Valladolid (Spain). In order to compare biological samples, the concentrations of these samples were measured using inductively coupled plasma mass spectrometry. The limits of detection for Zn in blood plasma ranged between 4.22 and 17.34 $\mu\text{mol l}^{-1}$ and were $<0.08 \mu\text{mol g}^{-1}$ creatinine in urine. The results of this study indicate that the highest mean values of serum Zn were found in non-hypertensive nonsmokers ($13.39 \pm 4.35 \mu\text{mol l}^{-1}$), whereas the highest urine Zn values were observed in hypertensive nonsmokers ($2.78 \pm 2.13 \mu\text{mol l}^{-1}$). Higher Zn serum/urine quotient levels were observed in non-hypertensive and nonsmoking women, whereas lower levels were noted in non-hypertensive and smoking women ($P=0.012$). This study identified a correlation between Zn serum/urine quotients and cotinine levels (a marker of smoking), a correlation that suggests that smoking lowers the Zn serum/urine quotient, which was lower in hypertensive subjects than in control subjects.

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INTRODUCTION

Hypertension is an important medical and public health issue. Normal systolic and diastolic values are $\leq 120/80$ mm Hg, and values $\geq 140/90$ mm Hg indicate hypertension.¹

In Spain, 68 deaths per 100 000 people are due to cardiovascular disease and diabetes. The prevalence of high blood pressure in adults older than 25 years of age is 27.7% in men and 18.6% in women. In Europe, the aforementioned numbers increase to 238 deaths per 100 000 people, and the prevalence of high blood pressure is 33.1% in men and 25.6% in women.² Paradoxically, smoking has been associated with a 48% decreased risk of death 1 year following acute myocardial infarction.³

Smoking, obesity, immoderate alcohol consumption, sedentary lifestyle and high salt and fat intake increase the risk of developing hypertension.⁴ There exists a considerable number of literature⁵ articles linking smoking to hypertension, a relationship attributed to components of tobacco smoke, including carbon monoxide, a toxic gas with an affinity for hemoglobin ~ 200 times higher than that of oxygen. When bound to carbon monoxide, hemoglobin loses its

capacity to transport oxygen to cells and tissues. The circulatory system responds to the resulting hypoxia by increasing the frequency at which blood is pumped throughout the body, causing hypertension.

Zinc is an essential nutrient required for the normal functions of >300 enzymes, including alcohol dehydrogenase, alkaline phosphatase, carbonic anhydrase, leucine aminopeptidase, superoxide dismutase and DNA and RNA polymerase. It participates in growth and cell division, as well as in the metabolism of nucleic acids, proteins, carbohydrates, lipids and membranes.^{6,7} Zinc also maintains the structure of nucleic acids using so-called zinc (Zn) fingers.⁶

Eating is the primary means by which living organisms take in Zn.⁶ Zinc deficiency may have a wide range of effects.⁸ The levels of daily Zn intake and systolic blood pressure values in obese patients appear to be related,⁹ which illustrates the importance of regulating the levels of Zn intake as a means of controlling blood pressure. Studies conducted with humans and animals have demonstrated that hypertension causes changes in Zn metabolism that affect its distribution between the intracellular compartments.¹⁰

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Smoking is an important source of toxic substances, including Cd, Ni, Pb, free radicals and other organic carcinogenic substances. Heavy metals such as Cd, Ni and Pb may cause hypertension and atherosclerosis by increasing oxidative stress in tissues^{11,12} (as a result of either excessive free radical production or the inhibition of antioxidants), particularly if antioxidant elements such as Zn are deficient. Deficiencies in essential nutrients, the loss of homeostasis or the presence of excess toxic elements may trigger chronic physiological disorders such as hypertension and cardiovascular disease.¹¹

Another mechanism of action proposed in recent years that may explain how smoking causes hypertension is the depletion and replacement of endogenous metals by metals found in tobacco. For example, Zn may be replaced by cadmium, which is found in tobacco. The theory that cadmium replaces arterial Zn has been tested. The results of these tests indicate that the metal makes arteries both fragile and inflexible, which results in increased pressure levels within the vessels, as the body attempts to protect the arterial walls using calcium and fat that diminish the luminal diameters of the vessels in question. Likewise, Zn may be replaced by lead, which may aggravate these disease processes.¹¹

Hypertension has been linked to smoking through hemoglobin saturation, but it has also been associated with the loss of essential elements such as Zn as a result of the element's depletion by metals such as Cd, which is found in tobacco.¹¹ This hypothesis has been evaluated in working populations composed primarily of men, but occupational exposures to metals other than Zn, as well as levels of Zn intake, have not been studied in an all-male population.¹¹ In addition, very few studies have evaluated Zn intake in the general population.

The objective of the present study was to evaluate the relationship between smoking and altered Zn levels in both hypertensive and non-hypertensive patients whose levels of Zn intake fell within the recommended parameters in Spain.

MATERIALS AND METHODS

Study population

To carry out this study, we used a database containing information on ~1500 individuals from the city of Valladolid, Spain. All participants provided written

consent to participate in this study before 24-hour blood and urine samples were collected.

Several inclusion criteria were used to select our study population. First, a reference value was set to distinguish between smoking and nonsmoking individuals. This limit of separation between smokers and nonsmokers was set at 10 ng ml^{-1} of cotinine¹³ (a metabolite of tobacco) in the serum. Patients diagnosed with hypertension who were receiving treatment were excluded, as their participation may have affected the results of the study. Systolic and diastolic blood pressure values were taken using criteria established in the literature¹ pertaining to gender and age, criteria indicating that levels equal to or higher than 140 mm Hg for systolic pressure and 90 mm Hg for diastolic pressure were consistent with the diagnosis of hypertension, which enabled us to classify the participants into the following two categories: normotensive and hypertensive, leaving us with a study population of 1205 subjects. Patients between 20–59 years of age were included in order to avoid the possibility of age affecting the Zn levels, decreasing the study population to 804 individuals. Finally, patients who failed to take in the recommended amounts of Zn on a daily basis¹⁴ ($9.5\text{--}12 \text{ mg day}^{-1}$ for men and $7\text{--}12 \text{ mg day}^{-1}$ for women) were excluded; therefore, the final study population was composed of 254 individuals (Figure 1).

The combination of these inclusion criteria and patients' blood pressure levels led to the formation of the following four groups: nonsmokers without hypertension (RNS), smokers without hypertension (RS), nonsmokers with hypertension (NSHP) and smokers with hypertension (SHP). Variability was compared using the primary physical, physiological and pathological characteristics of present study for all study groups (Table 1). Finally, participants were classified by gender to allow for the performance of a more in-depth analysis (Table 2).

Measuring Zn in biological samples

The concentrations of Zn and other metals were measured using inductively coupled plasma mass spectrometry (ICP-MS) with an Agilent 7500CE ICP-MS. The limits of detection for Zn in blood plasma ranged between 4.22 and $17.34 \mu\text{mol l}^{-1}$, and the limits of detection in urine were $<0.08 \mu\text{mol g}^{-1}$ creatinine.

Study procedure

This study evaluated the effects of smoking on serum and urine Zn levels in hypertensive and non-hypertensive smokers and nonsmokers. A database containing information on ~1500 patients was used. Patients were selected from the area served by Río Hortega University Hospital (214 445 inhabitants)

Figure 1 Flowchart study patients.

Table 1 Characteristics of the hypertensive and non-hypertensive patients

	RNS (n = 139)		RS (n = 79)		NSHP (n = 21)		SHP (n = 15)	
	Mean ± s.d.	95% CI	Mean ± s.d.	95% CI	Mean ± s.d.	95% CI	Mean ± s.d.	95% CI
Height (cm)	159.10 ± 32.05	155.51–162.69	164.61 ± 8.24	163.10–166.10	165.67 ± 10.68	161.31–170.09	169.40 ± 9.28	164.83–173.97
Weight (kg)	64.59 ± 17.59	62.63–66.57	66.80 ± 12.20	64.56–69.04	75.81 ± 16.35	69.07–82.53	78.53 ± 11.49	72.84–84.16
Body mass index (kg m ⁻²)	23.59 ± 5.60	22.97–24.23	24.60 ± 3.72	23.92–25.28	27.50 ± 4.68	25.57–29.43	27.56 ± 4.82	25.24–29.96
Systolic blood pressure (mm Hg)	114.69 ± 15.74	112.94–116.46	113.22 ± 17.39	110.01–116.39	143.49 ± 11.57	138.74–148.26	133.51 ± 13.78	126.71–140.29
Diastolic blood pressure (mm Hg)	73.81 ± 10.19	72.66–74.94	73.67 ± 11.54	71.59–75.81	91.16 ± 5.73	88.86–93.54	86.58 ± 9.69	81.83–91.37
Pulse pressure (mm Hg)	40.94 ± 10.22	39.76–42.04	39.6–10.53	37.67–41.53	52.31 ± 12.76	47.05–57.55	47.27 ± 14.78	40.02–54.58
Waist (cm)	81.73 ± 12.20	80.33–83.07	81.50 ± 10.48	79.57–83.43	89.72 ± 12.24	84.69–94.71	91.43 ± 8.01	87.47–95.33
Hip (cm)	99.60 ± 6.11	98.92–101.28	101.51 ± 14.93	98.77–104.23	104.06 ± 10.17	99.91–108.29	104.79 ± 8.96	100.37–109.23
Waist/hip ratio	0.83 ± 0.09	0.79–0.81	0.81 ± 0.09	0.78–0.82	0.86 ± 0.09	0.86–0.94	0.88–0.09	0.85–0.95
Number of cigarettes smoked per day	—	—	14.73 ± 9.49	12.96–16.44	—	—	18.47 ± 13.90	11.66–25.34
Years of non smoking	6.18 ± 6.41	5.48–6.92	—	—	14.10 ± 9.91	10.04–18.16	—	—

Abbreviations: CI, confidence interval; NSHP, nonsmokers with hypertension; RNS, referent nonsmokers; RS, referent smokers; SHP, smokers with hypertension.

in the province of Valladolid in northwest Spain. This study was a cross-sectional survey composed of two phases. For the first phase, questionnaires were mailed to a random sample of 20% of the population aged between 15 and 82 years, from whom information regarding cardiovascular risk factors was collected.

For the second phase, a new questionnaire was mailed, and programmed interviews and physical examinations were subsequently completed. A random subsample of 1500 people was selected. It was self-weighted and stratified based on age, gender, level of education, declared body mass index and other characteristics. Aside from the socio-demographic information, data were collected from the SF-36 Health Questionnaire and a tobacco use questionnaire, the latter of which reflected the number of cigarettes smoked and the length of the smoker's habit. There were no differences between collaborating and noncollaborating subjects based on other variables. Participants were representative of the general population. All patients provided informed consent and agreed to participate in the study.

Statistical analysis

To adequately complete the analysis, the population was stratified into the following groups: NSHP, SHP, RS and RNS. These groups were stratified by age and gender. Parametric (analysis of variance) and nonparametric (Kruskal–Wallis) tests were performed on the basis of the distributions of variables in order to determine whether any significant difference existed among the study groups. Values of $P < 0.05$ were considered statistically significant. The analyses were performed using SPSS (Statistical Package for the Social Sciences) v17 software (SPSS Inc., Chicago, IL, USA).

RESULTS

Table 3 includes the serum and urine Zn levels and the serum/urine quotient levels of all study groups. Serum Zn levels were higher in the RNS group ($13.39 \pm 4.35 \mu\text{mol l}^{-1}$) but were lower in the SHP group ($12.23 \pm 4.30 \mu\text{mol l}^{-1}$).

Urine Zn levels were higher in the NSHP group and lower in the non-hypertensive group, irrespective of whether the patients in question were smokers ($2.59 \pm 2.74 \mu\text{mol l}^{-1}$) or nonsmokers ($2.55 \pm 2.91 \mu\text{mol l}^{-1}$).

The RNS group demonstrated the highest Zn serum/urine quotients ($43.74 \pm 67.19 \mu\text{mol l}^{-1}$), followed closely by the RS group ($43.13 \pm 69.24 \mu\text{mol l}^{-1}$). The SHP group demonstrated the lowest values ($15.40 \pm 17.36 \mu\text{mol l}^{-1}$), although no statistically significant differences were noted.

In order to perform a more in-depth analysis, the proportions of genders included in each of the study groups and their possible influences on the responses of the target parameters used in this study were considered. Table 4 includes serum and urine Zn levels, as well as serum/urine quotients for the non-hypertensive population, and includes gender as a classification criterion. When gender was considered, a statistically significant difference was noted ($P < 0.05$) between men and women regarding serum Zn levels in the RNS group ($P = 0.037$). Another statistically significant difference was noted between the women in the RNS and RS groups ($P = 0.012$) based on comparisons of Zn levels of the serum/urine quotient.

Table 4 also includes serum and urine Zn values and serum/urine quotients for the hypertensive population based on gender. Although no statistical significance was observed, the women in the SHP group demonstrated the lowest levels of serum Zn ($9.26 \pm 1.99 \mu\text{mol l}^{-1}$) but also demonstrated the highest levels of urine Zn ($3.33 \pm 2.54 \mu\text{mol l}^{-1}$). Comparisons of serum/urine quotients among hypertensive patients revealed that the men in the NSHP group demonstrated the lowest values ($4.14 \pm 2.67 \mu\text{mol l}^{-1}$), whereas the men in the SHP group demonstrated the highest values ($19.45 \pm 18.77 \mu\text{mol l}^{-1}$).

Table 2 Gender distribution of control and hypertensive patients

	Control group (n = 218)				Hypertensive group (n = 36)			
	Nonsmoker (63.8%)		Smokers (36.3%)		Nonsmokers (58.3%)		Smokers (41.7%)	
	Frequency	%	Frequency	%	Frequency	%	Frequency	%
Men	48	34.5	22	27.8	8	38.1	11	73.3
Women	91	65.5	57	72.1	13	61.9	4	26.7
Total	139	100	79	100	21	100	15	100

Table 3 Serum and urine Zn levels and serum/urine quotients of all study groups

	Non-hypertensive					Hypertensive					P-value ^a	P-value ^b
	Nonsmokers		Smokers		P-value	Nonsmokers		Smokers		P-value		
	Mean ± s.d.	95% CI	Mean ± s.d.	95% CI		Mean ± s.d.	95% CI	Mean ± s.d.	95% CI			
Zn serum (μmol l ⁻¹)	13.39 ± 4.35	12.92–13.88	12.80 ± 3.77	12.10–13.50	0.314	12.32 ± 3.19	10.99–13.61	12.23 ± 4.30	10.08–14.32	0.942	0.281	0.601
Zn urine (μmol l ⁻¹)	2.55 ± 2.91	2.27–2.93	2.59 ± 2.74	2.10–3.10	0.921	2.78 ± 2.13	1.94–3.66	2.69 ± 3.57	0.93–4.47	0.771	0.728	0.902
Zn serum/urine	43.74 ± 67.19	36.17–51.23	43.13 ± 69.24	30.41–55.79	0.949	28.79 ± 60.36	4.01–53.59	15.40 ± 17.36	6.84–23.96	0.336	0.337	0.082

^aP-value between the nonsmokers of the non-hypertensive group and the hypertensive group.^bP-value between the smokers of the non-hypertensive group and the hypertensive group.

A statistically insignificant correlation was noted between serum cotinine concentrations and serum/urine quotients, as elevated cotinine concentrations correlated with lower serum/urine quotients among RS individuals. The correlation observed in the SHP groups demonstrated that an elevated serum cotinine concentration correlated with increased serum/urine quotients; however, this correlation was not statistically significant.

DISCUSSION

This study evaluated Zn levels in a Spanish population (Valladolid) and has provided researchers with valuable information regarding the behavior of an essential metal relative to patients' smoking habits and histories of hypertension. Serum Zn levels are higher in the non-hypertensive population, whereas urine Zn levels are elevated in the hypertensive population, and non-hypertensive subjects demonstrate higher Zn serum/urine quotients.

On the basis of the literature,¹¹ when the relationship among Zn, smoking and hypertension was analyzed, the highest and lowest blood levels of Zn were noted in the RNS and SHP groups, respectively. Higher urine Zn levels were observed in the SHP group, but the RNS group exhibited lower levels. The results of the present study are consistent with those of other studies in the literature, as serum Zn levels are higher among non-hypertensive patients and lower among hypertensive individuals, with the lowest values occurring in the SHP group.

Patients suffering from hypertension have significantly lower serum Zn levels than patients who are normotensive.¹⁵ Smoking is associated with hypertension, although the mechanisms implicated in these disease processes appear to be related to the hypoxia that results from the competition between carbon monoxide and oxygen for binding site on hemoglobin.

The results of the present study pertaining to urine Zn levels are also consistent with those obtained in previous studies,¹¹ as higher and lower urine Zn levels were noted in hypertensive and non-hypertensive individuals, respectively.

When comparing the results included in Table 3 (in which the study population was processed in its entirety) with those presented in Table 4 (which distinguishes the values corresponding to each gender), the values of the study groups change. This suggests possible differences in circulating and excreted levels of Zn relative to smoking and hypertension and that the pathogenesis of these disease processes is dependent on gender.

It is necessary to undertake new studies to better determine the causes of these variations. A significant difference was noted when the zinc levels of the zinc serum/urine quotients of RNS and RS women were compared. These difference are not present in men and could be attributed to their different occupational exposure, particulate metals. That men tend to have more contact with metals at work may mask the effects that tobacco has on Zn levels.

The small number of significant values obtained by this study may be owing to its final population size (254 individuals) after applying its inclusion criteria, criteria deemed necessary to avoid information biases despite of the study's relatively large initial sample size. The study offers more sound results, as confounding factors such as those related to age-appropriate levels of Zn intake are eliminated. The application of these inclusion criteria has demonstrated that a large proportion of the study population presented with inadequate Zn intake levels for their ages: of the 804 participants, the study population decreased to 254 participants when intake levels were taken into account, a 68.41% reduction of the population's size, suggesting that correcting for possible distorting factors may be

Table 4 Serum and urine Zn levels and serum/urine quotients for the hypertensive and non-hypertensive patients based on gender

	Nonsmokers				Smokers				P-value ^a	P-value ^b	
	Men		Women		Men		Women				
	Mean ± s.d.	95% CI	Mean ± s.d.	95% CI	Mean ± s.d.	95% CI	Mean ± s.d.	95% CI			
											P-value
Non-hypertensive											
Zn serum (μmol l ⁻¹)	14.45 ± 3.66	13.55–15.25	12.84 ± 4.59	12.24–13.36	0.037	12.87 ± 5.62	10.90–14.90	12.78 ± 2.81	12.41–13.19	0.788	0.929
Zn urine (μmol l ⁻¹)	2.91 ± 3.24	2.16–3.64	2.37 ± 2.72	2.07–2.73	0.296	2.91 ± 2.70	1.94–3.86	2.46 ± 2.77	2.11–2.89	0.516	0.838
Zn serum/urine	9.80 ± 11.48	7.16–12.44	14.10 ± 20.03	11.68–16.52	0.172	11.00 ± 18.84	4.28–17.72	7.16 ± 6.14	6.36–8.04	0.369	0.012
Hypertensive											
Zn serum (μmol l ⁻¹)	12.48 ± 2.40	11.16–13.84	12.23 ± 3.69	10.30–14.16	0.867	13.30 ± 4.47	11.88–14.72	9.26 ± 1.99	7.56–11.04	0.105	0.145
Zn urine (μmol l ⁻¹)	2.52 ± 1.79	1.49–3.51	2.94 ± 2.36	2.07–3.73	0.670	2.46 ± 3.97	2.50–2.50	3.33 ± 2.54	1.13–5.47	0.691	0.779
Zn serum/urine	4.14 ± 2.67	2.59–5.61	6.37 ± 5.40	4.54–8.26	0.291	19.45 ± 18.77	13.56–25.44	4.27 ± 2.62	2.04–6.56	0.137	0.469

^aP-value between the reference nonsmokers (men) and reference smokers (men).^bP-value between the reference nonsmokers (women) and reference smokers (women).^cP-value between the hypertensive nonsmokers (men) and hypertensive smokers (men).^dP-value between the hypertensive nonsmokers (women) and hypertensive smokers (women).

necessary for future studies, although this also entails reducing the sample size substantially.

Correlations were observed between cotinine concentrations and Zn serum/urine quotients for a portion of the study groups, although these correlations were not significant. This may be owing to the study's final population size, which supports the hypothesis that tobacco components act by displacing Zn within cells. This finding is significant enough to warrant an analysis of the effects of tobacco substitute treatments, as these treatments incorporate nicotine and cotinine; therefore, the effects on Zn levels may persist.

The correlations noted by our study indicate that when serum cotinine levels rose in the RS group owing to smoking, Zn serum/urine quotients decreased. These findings are consistent with those of the literature,¹¹ as these studies demonstrated that Zn is replaced by other tobacco-derived metals and that their excretion in urine increases. Therefore, the pathways in which Zn participates become nonfunctional, including the enzyme-driven metabolic pathways in which Zn is an essential participant, as Zn affects the activity of catalase, alkaline phosphatase, carboxypeptidases and alcohol dehydrogenase. An analysis of the correlation between serum cotinine levels and the Zn serum/urine quotients of the SHP group is not consistent with previous hypotheses. This study determined that higher cotinine concentrations correlate with increased Zn serum/urine quotients, the opposite of what the above hypotheses allege. These findings may be attributed to the displacement of Zn from cells into the circulatory system.

The existence of a process that depletes Zn via the utilization of other metals has been studied by other research groups, one of which¹⁶ studied Zn, Cd and Pb levels in patients with rheumatoid arthritis as well as a control group, in both Pakistan and Ireland, based on subjects' smoking habits. The study reported that as Cd and Pb concentrations increased, Zn levels also increased, which may be indicative of a depletion process. In addition, this study noted very high values of all metals among the Pakistani study population groups, which may indicate different metal exposures among Pakistan's working population.

Another study¹⁷ analyzed the levels of Zn and Cd for a study population stratified based on smoking habits and a history of cardiovascular disease, a study that obtained results similar to those of our study, as it determined that smoking and cardiovascular disease decrease serum Zn levels and increase urine Zn levels. This study was also conducted in Pakistan. Compared with the above studies, our study has the advantage of having included both male and female participants, as well as ensuring that patients' Zn intake levels were appropriate for their ages.

Clinical and laboratory data indicate that Zn participates in the regulation of blood pressure and in the pathogenesis of hypertension. Serum Zn levels decrease in lymphocytes and bones, but they increase in the heart, erythrocytes, kidneys, liver, suprarenal glands and spleen.^{10,18} These changes appear to be defense mechanisms that lower blood pressure. Loss of Zn homeostasis may be both a cause and an effect of high blood pressure.¹⁸ Another factor that affects both Zn metabolism and hypertension is age; serum Zn levels decrease, whereas Zn level increases in both erythrocytes and urine with age.^{19,20}

We have demonstrated the relationship between Zn levels and diseases such as hypertension. There are examples in the literature²¹ of studies that have determined that dietary supplementation of Zn, as well as other metals and vitamins, resulted in statistically significant reductions in the systolic and diastolic blood pressures of the patients who were treated, findings consistent with our results. Therefore, nutritional and pharmacological Zn supplementation may be a means

of preventing the harmful diseases resulting from its deficiency, including hypertension.

This study includes several novel elements compared with previous studies,¹¹ as its population includes women; therefore, the Zn levels are more representative of the general population. However, it also determines serum Zn levels as opposed to plasma Zn levels, which is important given the different reference ranges of each.²² Zinc values may be 9 or 10 times higher in the plasma compared with the serum. In addition, the age range used for this study was wider than those used by previous studies.¹¹

This study indicates that smoking predisposes individuals to hypertension not only as a result of hypoxia but also via smoking-induced alterations to biochemical pathways and the displacement of elements such as Zn. These alterations render Zn useless and may lead to cellular aging (oxidative stress), which favors the development of hypertension.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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