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The Relationship of Blood Cadmium Level to Hypertension and Plasma Norepinephrine Level: A Romanian Study[1,2] (41159)

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Abstract. The associations of blood cadmium levels with hypertension and plasma norepinephrine concentrations were determined in normotensive and hypertensive nonsmokers and smokers. Statistical analysis showed that after adjustment for age alone, the estimated mean values of blood cadmium and plasma norepinephrine in nonsmokers were significantly ($P < 0.001$) lower than in smokers. However, after adjustment for age and blood cadmium, the estimated mean values for plasma norepinephrine were not significantly different between nonsmokers and smokers or normotensives and hypertensives. In contrast the estimated mean value for blood cadmium as a function of blood pressure (i.e., normotensive versus hypertensive) and smoking habit was still significant after adjustment for age and plasma norepinephrine ($P < 0.01$). We suggest that smoking and blood pressure affect the level of blood cadmium, and through this change in blood cadmium the level of plasma norepinephrine is affected.

In animal studies cadmium (Cd^{2+}) has been shown to induce hypertension (1–3). Based on these findings several investigators have attempted to determine if Cd^{2+} is related to human hypertension. Some studies have shown that the concentration of tissue Cd^{2+} is significantly higher in hypertensive than in normotensive patients (4–6), whereas other studies do not confirm these associations (7, 8). These conflicting reports might be explained by confounding variables such as age, smoking habits, and environmental exposure. The present studies were performed to determine the effects of age, smoking, and hypertension of blood Cd^{2+} concentration.

The relationship between plasma norepinephrine (PNE) and blood Cd^{2+} levels in normotensive and hypertensive patients was also determined, because in recent reports several investigators have shown that Cd^{2+} inhibits the neuronal uptake of noradrenaline [(9), N. W. Revis, and T. Majors, unpublished observations]. Furthermore, in human studies significant increases in PNE have been observed in patients with hypertension (10). Thus blood Cd^{2+} and PNE levels may be interrelated, and this interrelationship may have relevance to essential hypertension.

The subjects for the study were selected from Brasov, Romania. This area was chosen because it has been suggested that the high incidence of endemic nephropathy observed in Brasov may be related to the intake of Cd^{2+} (11). If this suggestion is true one may expect to find significantly elevated levels of blood Cd^{2+} which would help in establishing the relationship between blood Cd^{2+} and PNE.

Materials and Methods. *Subjects.* Two groups of subjects were selected, controls and hypertensives. These groups were further divided into nonsmokers and smokers. This latter division was made because previous investigators have shown that tobacco smoke significantly contributes to total body Cd^{2+} (12). The control subjects were randomly selected and divided into 11 nonsmokers and 17 smokers. Subjects were free from symptoms and prior evidence of cardiac and renal disease and hypertension. Their age and mean systolic pressure

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ranged from 34 to 50 and 114 to 124, respectively.

The hypertensive subjects were randomly selected and divided into 14 nonsmokers and 16 smokers. Both groups were between the ages of 36 and 51, and mean systolic pressures in these groups ranged from 158 to 166. The hypertensive groups had not received medication prior to these studies.

Venous blood samples were collected from the cephalic vein into heparinized tubes from controls and hypertensive subjects between 8:30 and 10:00 AM. Prior to blood sample collection, subjects were fasted from food, drink, and smoking for 8 hr and were recumbent for 1 hr.

PNE. Blood samples were collected in tubes containing EGTA and dithiothreitol. Plasma was separated by low-speed centrifugation; to this isolated plasma was added 2 *N* perchloric acid in a ratio of 9 parts plasma to 1 part acid (v/v). After being vortexed and incubated in ice, the acidified plasma was centrifuged (40,000*g*), and 50 μl the isolated supernatant was used to measure the level of PNE by a radioenzymatic method (13).

Blood cadmium. From each of the patients 10 ml of blood was drawn, and 1 ml was added to vials containing 1 ml concentrated nitric acid (High Pure, PA). After each sample was sealed, it was heated at 50° for 12 hr, diluted, and then assayed for Cd^{2+} by flameless atomic absorption in a Perkin–Elmer Model 603 spectrophotometer equipped with deuterium arc for background correction. A hollow cathode lamp was used for Cd^{2+} (222.8 nm) at the recommended lamp current and spectral bandwidth.

To confirm the accuracy of the Cd^{2+} procedure employed in these studies, we used several methods. From the 10-ml sample of blood obtained from each patient, 1 ml was added to vials containing 2 ppb Cd^{2+} and 1 ml nitric acid, this mixture was vortexed, and blood Cd^{2+} was determined. The percentage recovery per sample was 96–99%. The National Bureau of Standards reports 27 μg Cd^{2+}/g dry wt for a liver sample standard, and we observed, using this standard, 25 μg Cd^{2+}/g dry wt. Results of these two studies support the use of this procedure for measuring blood Cd^{2+}.

Summary of methods of analysis. The information obtained in this study from individuals selected on the basis of smoking status and hypertension was analyzed initially in the form of a multiway contingency table. In this approach a class of log–linear models for the expected number of observations in each cell defined by the cross-classification of the variables age, smoking status, tension, level of blood Cd^{2+}, and level of PNE were investigated and judged with respect to providing an adequate fit to the data [e.g., Ref. (14), Chaps. 2 and 4]. In view of these findings the effects of smoking and hypertension on levels of blood Cd^{2+} and PNE were investigated via an analysis of covariance. Adjustments for differences between groups of smokers and nonsmokers and hypertensives and normotensives with respect to age and one of the response variables (i.e., Cd^{2+} or PNE) were made before the effects of smoking and tension on the remaining response variables were gauged.

Results. The mean ± SE values for blood Cd^{2+} and PNE levels from the four groups of patients are shown in Table I. As observed by other investigators (10), recumbent PNE levels were significantly ($P < 0.01$) greater in the hypertensive patients than in the corresponding normotensive controls. However, when hypertensive smokers (PNE = 411.4 pg/ml plasma) were compared with the normotensive control nonsmokers (PNE = 337.3 pg/ml plasma), a greater increase in PNE was observed, which suggests that smoking should be considered as a variable when PNE levels are measured. A similar change was observed for blood Cd^{2+} content.

Figure 1 and Table II summarize the results of a log–linear analysis of observed frequencies based on categories defined by age group (≤ 40, ≥ 41), smoking status, tension, level of blood Cd^{2+} (≤ 37, ≥ 38 ng/ml), and level of PNE (≤ 375, ≥ 376 pg/ml). One aim in this exploratory analysis of the data was to determine whether blood Cd^{2+} and PNE could be considered separately with respect to the effects of age, smoking status, and tension. The break points for

TABLE I. RELATIONSHIP OF SMOKING TO BLOOD PRESSURE, PNE, AND BLOOD Cd^{2+}

Group	Age (years)	Mean blood pressure (mm Hg)	NE (pg/ml plasma)	Cd^{2+} (ng/ml blood)
Normotensives				
Nonsmokers	41.4 ± 1.5 (11)	114/74 ± 1.9/1.4 (11)	337.3 ± 7.8 (11)	25.7 ± 3.0 (9)
Smokers	41.2 ± 1.1 (17)	124/82 ± 2.4/1.5 (17)	377.1 ± 9.5 (17)	41.7 ± 3.8 (12)
Hypertensives				
Nonsmokers	40.4 ± 1.6 NS (14)	158/91 ± 3.2***/1.1*** (14)	379.0 ± 11.8** (14)	37.7 ± 3.3** (14)
Smokers	42.3 ± 1.5 NS (16)	166/110 ± 2.3***/3*** (16)	411.4 ± 11.0** (16)	50.6 ± 2.7* (12)

Note. Results are expressed as mean ± SE. Number of patients is shown in parentheses. NS, Not significant, $^{***}P < 0.001$, $^{**}P < 0.01$, and $^{*}P < 0.05$.

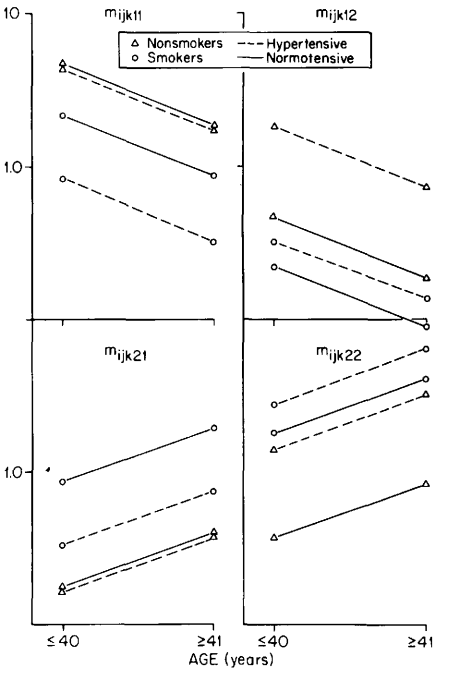

FIG. 1. This figure graphically depicts the estimated expected number of observations based on the following log–linear model for a 2^5 frequency table: $\log(m_{ijklm}) = u + u_{1(i)} + u_{2(j)} + u_{3(k)} + u_{5(m)} + u_{23(jk)} + u_{14(il)} + u_{24(jl)} + u_{35(km)} + u_{45(lm)}$, where the variables and their corresponding categories are $i = 1,2$: age (≤ 40, ≥ 41); $j = 1,2$: smoking status (NS, S); $k = 1,2$: tension (normotensive, hypertensive; $l = 1,2$: blood Cd^{2+} (≤ 37, ≥ 38); $n = 1,2$: PNE (≤ 375, ≥ 376). The value of the likelihood ratio χ^2 statistic for the goodness-of-fit test of this model was 21.4 on 21 df ($P = 0.43$). Since the individuals in this study were selected based on smoking status and tension, this configuration (margin) was fixed by sampling design and thus each model in the class of heirarchical models considered contained the u_{23} term. The symmetry apparent in this figure is essentially due to constraints imposed on the estimates of the u terms in the log–linear model. (For more details see Ref. (12).)

the "continuous variables" age, blood Cd^{2+}, and PNE were selected to approximately balance these univariate marginals and to not represent normally low or high values.

Figure 1 graphically describes the "joint response" of blood Cd^{2+} and PNE for the eight ($= 2^3$) two-way tables defined by the three classification variables (age, smoking status, and tension). Their joint response is given in terms of the estimated expected

TABLE II. STATISTICAL ANALYSIS

	PNE		
	$n = 1$	$n = 2$	
Cd^{2+}	≤ 375	≥ 376	
$l = 1$			
≤ 37	m_{ijk11}	m_{ijk12}	Estimated value of Yule's measure of association, $Q(\pm$ an approximate SE), is
$l = 2$			
≥ 38	m_{ijk21}	m_{ijk22}	$\hat{Q} = 0.91 \pm 0.03$

m_{ijkln} = expected number of observations for ith age group, jth smoking status, kth tension group, lth level of blood Cd^{2+}, and nth level of PNE.

$$\text{For } r_1(i,j,k) = m_{ijk11}/m_{ijk12},\ \log\left[r_1(i,j,k)\right] = \mu_1 + w_{3(k)},$$
$$\text{for } r_2(i,j,k) = m_{ijk11}/m_{ijk21},\ \log\left[r_2(i,j,k)\right] = \mu_2 + w_{1(i)} + w_{2(j)},$$
$$\text{for } r_3(i,j,k) = m_{ijk11}/m_{ijk22},\ \log\left[r_3(i,j,k)\right] = \mu_3 + w_{2(j)} + w_{3(k)},$$

where

$$\hat{\mu}_1 = 1.60,\ \hat{\mu}_2 = 1.56,\ \hat{\mu}_3 = 0.36,$$

and

$\hat{w}_{1(i)} =$ 0.86, age ≤ 40,	$\hat{w}_{2(j)} =$ 1.18, nonsmokers,	$\hat{w}_{3(k)} =$ 0.71, normotensive,
-0.86, age ≥ 40,	-1.18, smokers,	-0.71, hypertensive
(approx SE = 0.32)	(approx SE = 0.39)	(approx SE = 0.41)

cell counts (m_{ijkln}) based on the fitted model. Table II describes the response for a general 2×2 table of cell frequencies, and the corresponding estimated values of the m_{ijkln} values are graphed on a log scale in Fig. 1.

The log–linear analysis of observed frequencies suggested no evidence of third- or higher-order effects (a simultaneous test that three-, four-, and five-factor interactions were zero yielded a likelihood ratio χ^2 statistic of 16.11 on 16 *df*, indicating support of this hypothesis), although the overall number of observations ($N = 47$) may have been insufficient for third- or fourth-order interactions to be detected. The estimated value of Yule's measure of association, Q, between blood Cd^{2+} and PNE [e.g., Ref. (14), p. 378] is given in Table II, based on the fitted model. A consequence of the absence of any third- and higher-order effects in the fitted model was that Q had the same value in each of the eight two-way response tables. Thus, although blood Cd^{2+} and PNE were not judged to be conditionally independent given the "levels" of age, smoking status, and tension (i.e., a significant lack of fit was obtained for models without a blood $Cd^{2+} \times$ PNE interaction, the u_{45} term), their relationship (i.e., dependence) was found to be essentially the same over various combinations of age, smoking status, and tension in this set of data. The strong positive association between blood Cd^{2+} and PNE indicated that investigation of the differences between groups of individuals with respect to either blood Cd^{2+} or PNE level should involve adjustment for the other.

From Fig. 1, the effects of age, smoking status, and tension may be noted. For example, the expected number of observations with low values of blood Cd^{2+} and PNE ($\hat{m}_{ijk11}$) was estimated to decrease with age, smoking, and hypertension, although for nonsmokers the effect of tension on this aspect of the joint response was rather small. However, the expected number of observations with low blood Cd^{2+} and high PNE levels ($\hat{m}_{ijk12}$) was estimated to be higher for hypertensives than for normotensives. This aspect of the joint response of blood Cd^{2+} and PNE can also be described by consideration of the expected

cell counts. Table II lists the estimated model for the log of $r_1(i,j,k) = m_{ijk11}/m_{ijk12}$ based on the fitted model listed in the legend for Fig. 1. For the particular model fitted, this ratio depends only on the tension variable, and the estimated effect of tension for this particular "joint response" [i.e., log r_1 (i,j,k)] is $\hat{w}_{3(1)} = +0.71$ for the normotensives and $\hat{w}_{3(2)} = -0.71$ for hypertensives. An approximate standard error for $\hat{w}_{3(i)}$ $(i = 1, 2)$ based on an upper bound for the asymptotic variance of the corresponding u terms in the fitted model is 0.41, indicating the effect of tension to be of borderline statistical significance for this measure of joint response. A model in which the u_{35} term was specified as zero (thereby implying $w_{3(i)} = 0$) was fit. The conditional test that $u_{35} = 0$, obtained by comparison of the two log–linear models, was significant $(0.025 < P < 0.05)$, indicating the need for retention of a tension × PNE term in this model, although the overall fit for the reduced model without a tension × PNE interaction was adequate as well. The reduced model thus suggested that the effects of smoking and tension on levels of PNE were not significant after adjustment for age and levels of blood Cd^{2+}. Similarly, log–linear models for the joint response measures $r_2(i,j,k) = m_{ijk11}/m_{ijk21}$ and $r_3(i,j,k) = m_{ijk11}/m_{ijk22}$ may be written, and estimates of their parameters based on the fitted model may be obtained (see Table II). An interpretation of these joint response measures may be phrased in terms of ratios of conditional probabilities or expected counts, whichever is most convenient. They also serve to indicate possible relationships among the variables measured which should be considered in subsequent analyses of the data.

In light of the results just described, further analyses of the effects of smoking and tension on blood Cd^{2+} and PNE levels were considered. A summary of the results from an analysis of covariance is given in Tables IIIA and B. This table lists the least-squares estimated adjusted mean values of blood Cd^{2+} and PNE for smokers, nonsmokers, normotensives, and hypertensives. The effects of smoking and tension on PNE level were not significant after adjustment for differences in age and blood Cd^{2+} level (marginal estimated means). However, the effects of smoking and tension on PNE concentration were significant $(P < 0.001)$ when adjustment was only for group differences in age (i.e., essentially ignoring the concomitant levels of blood Cd^{2+}). No evidence of smoking × tension interaction was indicated with respect to the measurement of PNE. If blood Cd^{2+} is considered as the dependent variable, the effects of smoking and tension were also approximately additive, but there were significant differences between smokers and nonsmokers and between normotensives and hypertensives after adjustment for age $(P < 0.001)$ as well as after adjustment for age and PNE level $(P < 0.009)$.

Discussion. The levels of blood Cd^{2+} observed in these studies are considerably higher than previously reported (15, 16). These high levels are presumably associated with the high concentration of environmental Cd^{2+} (i.e., well water and soil). Previous investigators have observed blood Cd^{2+} levels of 6–59 ng/ml in exposed workers. In support of the suggestion that our subjects are exposed to high levels of environmental Cd^{2+}, we have observed in 30 normotensive nonsmokers and 18 hypertensive nonsmokers (all U.S. subjects) with no known exposure, blood Cd^{2+} levels of 3.1 and 8.9 ng/ml, respectively. Thus, although the amount of Cd^{2+} consumed daily is not known for the Romanian patients, it can be assumed that the amount is considerable, based on the data in Table I.

The concentrations of PNE observed in these studies are similar to those in previous reports (10, 17–19). The concentrations of PNE in hypertensive subjects is reported to be significantly higher than in normotensive subjects (19, 20). However, other investigators (10) have not observed significant differences between hypertensives and normotensives when both groups are adjusted for age (10). This latter finding has been challenged by a recent report which showed a significant difference between these groups after adjusting for age (18). Thus, this issue remains controversial. Results from this study show that when adjustments are made for age and blood

TABLE III. ESTIMATED ADJUSTED MEAN ± SE OF BLOOD Cd^{2+} AND PNE LEVELS[a]

	(A) Within-cell means	
	Nonsmokers	Smokers
Normotensives		
Cd^{2+} (age)	25.6 ± 2.62	41.9 ± 2.27
Cd^{2+} (age, PNE)	29.3 ± 2.63	41.9 ± 2.04 NS
PNE (age)	337.3 ± 10.11	377.6 ± 8.22
PNE (age, Cd^{2+})	372.6 ± 14.29	377.0 ± 9.61
Hypertensives		
Cd^{2+} (age)	40.2 ± 2.13	47.5 ± 2.31
Cd^{2+} (age, PNE)	39.8 ± 1.93	45.2 ± 2.2
PNE (age)	382.7 ± 9.09	407.6 ± 8.51
PNE (age, Cd^{2+})	385.2 ± 8.94	388.3 ± 10.93

	(B) Marginal estimated means[b]			
	Cd^{2+} (age)	PNE (age)	Cd^{2+} (age, PNE)	PNE (age, Cd^{2+})
Nonsmokers[c]	32.9 ± 1.69	360.0 ± 6.84	34.6 ± 1.60	378.9 ± 8.33
Smokers[c]	44.7 ± 1.62	392.6 ± 5.91	43.6 ± 1.50	382.7 ± 7.51
Normotensives[d]	33.7 ± 1.73	357.4 ± 6.55	35.6 ± 1.66	374.8 ± 8.22
Hypertensives[d]	43.9 ± 1.54	395.2 ± 6.20	42.5 ± 1.45	386.8 ± 7.02

[a] Cd^{2+}, ng/ml blood; PNE, pg/ml plasma. The variables in parentheses indicate adjustments were made for these covariants.

[b] The mean effects of smoking and hypertension are shown in Table IIIB using the least-square estimate of adjusted means values based on an analysis of covariants model. The statistical significance of these effects is discussed in the text based on the values of F statistic obtained in the analysis of covariants.

[c] Collapsed over tension status.

[d] Collapsed over smoking status.

Cd^{2+}, significant differences in PNE between normotensive and hypertensive subjects are not observed.

In the present study the relationships between blood Cd^{2+} and PNE levels and the effect of age, smoking, and hypertension were investigated. The analysis of covariance considered the effects of hypertension and smoking on the amount of blood Cd^{2+} adjusted for age and PNE content and on the level of PNE adjusted for age and blood Cd^{2+} concentration. Results showed that after adjustment for age and PNE, the effect of hypertension was significant ($P < 0.005$), with higher estimates of blood Cd^{2+} level in hypertensives than in normotensives. Similarly, the effects of smoking were significant ($P < 0.001$) after adjustment for age and PNE content, with higher estimates of blood Cd^{2+} level in smokers. The analysis also indicated that after adjustment for age and PNE level, the effects of smoking and tension were approximately additive ($P = 0.11$), so that, for example, the increase in blood Cd^{2+} level of smokers over nonsmokers was judged to be about the same in normotensives as in hypertensives. These data thus suggest that blood Cd^{2+} level is related to smoking and hypertension, with increased amounts in smokers and hypertensives after adjustment for differences in age and level of PNE.

Previous investigators have observed a decrease in neuronal uptake of NE (9) and an increase in the half-life of circulating PNE (N. W. Revis and T. Majors, unpublished observations) in experimental animals treated with Cd^{2+}. The present studies support these experimental results in that they show a significant association between blood Cd^{2+} and PNE levels after adjustment for differences in age. However, when adjustments are made for differences in age and blood Cd^{2+} level, significant differences between normotensives and hypertensives with regard to PNE were

not detected, while the effect of tension was significant after adjustment for age only. These results suggest a complex association between blood Cd^{2+} and PNE levels and hypertension; further investigation with larger sample sizes is needed.

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