UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
Environmental Criteria and Assessment Office (MD-52)
Research Triangle Park, North Carolina 27711

August 6, 1985

Dr. Richard Royall
Dept. of Biostatistics
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Dear Dr. Royall:

My letter of July 31 to you contained a memo from Joel Schwartz to Jim Ware that mentioned a revised version of the paper that he presented to the Canadian Royal Society. Enclosed, please find a copy of that revised paper; perhaps you will find it useful in preparing yor opinion for us.

Looking forward to hearing from you soon , I remain

Sincerely yours,

David E. Weil, Ph.D.
Project Manger

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

THE RELATIONSHIP BETWEEN BLOOD LEAD LEVELS AND BLOOD PRESSURE

Presented to the Royal Society of Canada
Commission on Lead in the Environment
Revised July 1985

Joel Schwartz
U.S. Environmental Protection Agency

Concerns about the health effects of ambient exposure to lead traditionally have focused on children. Although lead has a variety of adverse effects on the health of adults, most of them appear not to be of substantial concern except at very high blood-lead levels. Recently, however, two new and extensive analyses of the NHANES II data set have shown a strong and robust relationship between blood lead levels and blood pressure. That finding has important implications for the benefits of reducing lead in gasoline, because high blood pressure, in turn, is linked to a variety of cardiovascular diseases.

This paper analyzes the experimental and statistical relationship between blood lead and blood pressure. The first part provides a brief overview of the experimental and epidemiological studies previously done, while the second part provides a detailed discussion of the recently completed analysis of the NHANES II data.

A.1. Earlier Studies

A.1.a Epidemiological Studies

Lead has long been associated with effects on blood pressure and the cardiovascular system, including a paper in the British Medical Journal by Lorimer in 1886 that found that higher blood-

lead levels increased the risk of hypertension. Most of the studies have focused only on hypertension and relatively high lead-exposure levels, and have not looked for a continuous effect of lead on blood pressure. Investigators reporting such an effect include Beevers et al. (1980), Morgan (1976), Richet et al. (1966), and Dingwall-Fordyce and Lane (1963). Others have failed to find effects of lead on hypertension that were significant at the 95 percent confidence level, although most of them did find a positive association. These include Ramirez-Cervantes et al. (1978) and Fouts and Page (1942).

More recently, Batuman et al. (1983) found an association between chelatable body-lead levels and hypertension in veterans, and several recent general population studies and lower lead-exposure studies (Beevers et al., 1976; Kromhout and Coulande, 1984) have found a significant association with blood lead. Khera et al.(1980) compared blood lead levels of hypertensive and cardiovascular patients with hospital controls in Birmingham, England and found blood lead levels were 55% higher in the patients with hypertension and ischaemic heart disease. Moreau et al. (1982) also found a significant relationship ($p < 0.001$) between blood lead levels and a continuous measure of blood pressure in 431 French policemen, after controlling for age, body mass index, smoking, and drinking.

An even more recent British study (Pocock et al., 1984) found blood lead significantly related to blood pressure at the 99 percent confidence level, but the authors felt that the small size of their correlation coefficient suggested no noticeable

effect. However, that conclusion appears to reflect a misunderstanding of statistics. It is the regression coefficient that indicates the size of an effect. A correlation coefficient confounds that measurement with the variances of the dependent and independent variables. While their full data set was not available to us, Pocock et al. presented their grouped data, and we were able to perform a regression of blood pressure versus the log of blood lead on their group averages, both before and after adjustment for confounders. The regressions were weighted by the inverse of the variance of each group, and confirmed their finding that blood lead was a significant predictor of blood pressure in their data, both before and after adjusting for covariates. Moreover, the regression coefficient indicated that the size of the effect was significant, suggesting a change of 3 mm Hg (millimeters of mercury, the standard measure of blood pressure) as blood lead goes from 5 to 15 ug/dl.

Weeden (1975) found lead associated with the vascular renal changes linked to essential hypertension, indicating a possible causal pathway. Cooper and Gaffey (1974) analyzed mortality data from 1,267 death certificates for 7,032 lead workers employed between 1900 and 1969, and found a significant excess of deaths from hypertension disease and renal disease. A later analysis of similar data from 1971 to 1975 also found an increase in cardiovascular and renal disease, but it was no longer significant at the 95 percent confidence level (Cooper, 1981). A more recent analysis by Cooper (1984) found SMR's of 128 and 203 for hypertensive heart disease in battery plant and

smelter workers respectively, and of 320 and 475 for other hypertensive disease. Voors and Shuman found tissue lead to be associated with cardiovascular deaths ($p = 0.0021$) in an autopsy study in North Carolina, after controlling for cadmium and age. Lead explained 11.8 % of the variation in the proportion of deaths due to cardiovascular disease.

The direct cardiological effects of lead are also indicated by electrocardiogram changes in lead-poisoned children, which are reversed by chelation therapy (Freeman, 1965; Silver and Rodrigues-Torres, 1968). Williams (1977, 1978, 1979) has shown persistent increased susceptibility to norepinephrine-induced arrhythmias in rats exposed to lead in the first three weeks of life.

A.1.b.I. EXPERIMENTAL EVIDENCE: IN VIVO STUDIES

A considerable body of experimental evidence indicates that low to moderate doses of lead increases blood pressure in animals, by increasing in the resting tone (tension) of the vascular smooth muscles. Victery (1982) found lead associated with a significant elevation of blood pressure in rats with blood lead levels of 41 ug/dl. Importantly,this study confirms Beevers et al.'s finding of a sex differential, with male rats showing a more pronounced rise. Webb (1981) also found that rats fed lead had significantly higher blood pressure, and then examined the vascular responsiveness of tail arteries in rats exposed to blood lead levels in the 40 ug/dl range that had suffered increases in

systolic blood pressure, and found that the arteries in exposed rats had increased contraction in response to stimulation by neurotransmitters.

Iannaccone et al. (1981) also reported increased blood pressure ($p < 0.001$) in rats at blood lead levels of 38.4 ug/dl, as well as significant increases in the blood pressure response to noradrenalin. Perry and Erlanger (1979) found that low level exposure of rats to lead produced increases of 15-20 mm Hg in systolic blood pressure. Kopp (1980) repeated those findings and found electrocardiogram changes, indicating an effect on the heart itself. Subsequent tissue analysis of the heart showed reduced levels of ATP in the heart muscle, indicating that heme synthesis inhibition by lead was affecting energy availability in the heart itself.

Carmignani et al., also measured diastolic blood pressure and found that lead increased both measurements. They also found increased reactivity to alpha adrenergic stimulation, confirming Webb and Iannacone. In addition, Revis et al. demonstrated increased blood pressure in pigeons fed 0.8 ppm lead acetate in drinking water. Thus experiments by several groups , using two different animal species, have shown that lead increases blood pressure.

A.1.b.II. EXPERIMENTAL EVIDENCE: IN VITRO STUDIES

Webb et al. reported that when helical strips of the smooth muscle lining the tail arteries of lead-exposed and controlled rats were compared, the lead-treated tissue manifested increased contractile response to alpha adrenergic stimulation, confirming

the in vivo results of Iannaccone and Carmigniani. Piccinini et al. and Favalli et al. placed whole arteries from rats not previously exposed to lead in a supporting media, and demonstrated that, when lead was infused into the media in concentrations ranging from 5-15 umole, there was a dose dependent contraction of the arterial muscle , restricting the flow of fluid through the artery. They also found that lead disturbed the calcium metabolism of the arterial tissue, resulting in increased intracellular calcium stores, and increasing the sensitivity of the muscle to extracellular calcium concentration, suggesting a disturbed ability to keep extracellular calcium out of the cytosol.

A.1.b.III. LEAD, CELLULAR CALCIUM METABOLISM, AND BLOOD PRESSURE

It has long been known that hypertension is linked to increased resistance to blood flow, due to reduction in the radius of the arterioles. Other research has implicated changes in the tone of the smooth muscle as a principal factor in that constriction. The obvious question is whether lead fits into this picture in a coherent way.

Because lead is a divalent cation, and interferes in calcium metabolism, interference in calcium metabolism is an obvious possibility. Indeed Aub et al. remarked in 1926 that "lead follows the calcium stream." indicating that the Pb-Ca association is a long recognized one. And examining the role of

calcium metabolism in blood pressure and the role of lead in calcium metabolism reveals a picture that is consistent with the available experimental and epidemiological data.

Calcium plays a complex role in both the responsiveness of arteriolar smooth muscle to stimuli and its steady state contractile activity (tone). The multitude of interactions between lead and calcium metabolism suggest that it may interfere with almost all of those processes.

The basic points that will be discussed are :

1. Increased intracellular calcium leads to increases in the tone of the smooth muscles lining the arterioles. This appears to be important in hypertension. Indeed the FDA's recent approval of calcium channel blockers for the treatment of hypertension demonstrates the recognized importance of intracellular calcium in regulating blood pressure.

2. While there is considerable disagreement among researchers on the relative importance of the many factors affecting intracellular calcium concentrations, among the pathways considered important by some researchers are the sodium potassium pump, the calcium pump, and uptake by the endoplasmic reticuluum and mitochondria. Cyclic AMP plays a role in stimulating the calcium pump and in modifying the calcium stimulated activity of myosin light chain kinase(MLCK).

3. Lead has been shown to increase intracellular calcium in vascular smooth muscle, as well as hepatocytes, osteocytes, and neurons.

4. Lead has been shown to inhibit Na/K ATPase activity in erythrocytes and neurons, to increase stores but reduce uptake rates for calcium in the endoplasmic reticuluum and the mitochondria, and to activate the enzyme system that produces hydrolysis of cAMP.

5. Lead induced changes in smooth muscle tone are consistent with its interference with calcium metabolism.

1. Calcium Metabolism, Smooth Muscle Tone, and Blood Pressure

A. Calcium Metabolism affects Smooth Muscle Tone and Reactivity

A somewhat simplified picture of the role of calcium in smooth muscle is described below (see Rassmussen, 1983, for more details). The contraction of the smooth muscle is mediated by an increase in cytosolic calcium, although the exact sources of the calcium are not known. This increase in free ionized calcium leads, via calcium-calmodulin to an activation of myosin light chain kinase (MLCK), which phosphorylates myosin light chain, resulting in increased cross bridging activity and increased muscle tension.

Meanwhile, the cell acts to restore cytosolic calcium to normal by promoting calcium efflux across the plasma membrane, through the calcium pump and, perhaps, via sodium-calcium exchange, through the sodium -potassium pump. Calcium is also taken up by the sarcoplasmic reticuluum, and possibly mitochondria, again reducing cytostolic free calcium stores. In addition, the contractile response is controlled and attenuated by a cAMP dependent protein kinase, which, by phosphorylating the MLCK, reduces its activity.

The steady state tone of vascular smooth muscle is due to a cytosolic calcium content above the threshold for contraction. This results from a balance between efflux and influx across the plasma membrane. This makes it extremely sensitive to anything that disturbs the ionic balance in the cell: indeed Filo et al. have shown (in vitro) that smooth muscle tension exhibits a steep, logistic- shaped dependence on free calcium concentration in the cytosol.

While the specific mechanisms implicated in essential hypertension are still in dispute, it is interesting to note in this regard that Bruschi et al. reported that cytoplasmic free calcium in the platelets and lymphocytes of people with essential hypertension was 10% higher than the control group. A similar, but somewhat larger difference was seen in the spontaneously hypertensive rat. Erne, et al. also reported increased intracellular calcium in persons with essential hypertension.

Indeed, the hypothesis that much essential hypertension is related to increased intracellular calcium stores was one of the primary motivations behind the use of calcium blockers to treat hypertension, with favorable results in the spontaneously hypertensive rat (McCarran 1985) and in preliminary human clinical experience (Guazzi et al. 1983).

2. The Sodium-Potassium Pump, Calcium Metabolism, and Blood Pressure

While the role of the sodium-potassium pump in regulating intracellular calcium in vascular smooth muscle is still very controversial, there are a number of studies suggesting it may play a part. Clough et al, found decreased Na/K ATPase activity in the myocardial tissue of the one kidney, one clip hypertensive rat. Ouabain, a potent inhibitor of Na/K ATPase , has been shown to increase contractility of smooth muscle tissue and to reduce the the effect of extracellular calcium and potassium in relaxing the tissue.

Altered sodium potassium ATPase activity has been found in

persons with essential hypertension (Postonov et al, Wambach et al, Garay et al.), and the racial difference in the activity of the sodium-potassium pump is hypothesized to be a component of the higher blood pressure levels found in blacks (Lasker, et al., Weder et al.). Hamlyn, et al. found that plasma from patients with essential hypertension inhibited the activity of canine kidney Na/K ATPase. Magnesium a cofactor for the sodium-potassium pump, and Whang et al. found that patients with low serum magnesium levels required a greater amount of anti-hypertensive medication to achieve acceptable blood pressure levels, and Dyckner found that magnesium supplements added to diuretic therapy produced greater decreases in blood pressure in some hypertensive patients.

3. Lead and Intracellular Calcium Stores

Increases in intracellular calcium concentrations have been noted by investigators of lead. Goldstein (1977) showed that 100 uM lead tripled calcium accumulation in the brain capillaries of rats, and found evidence suggesting decreased efflux, an attribute of Na/K ATPase inhibition, was a factor. Kim (1980) found evidence for increased calcium accumulation in brain cells, and Pounds (1982a,b) found clear evidence of increased calcium accumulation in all three compartments of hepatocytes. Interestingly, Pounds reported that at low doses, the lead-induced increase in calcium concentration in the three intracellular compartments appeared to be uniform across all three compartments. At high lead levels (50 and 100 uM), two of

the smaller compartments saturated out, and the greatest increase was in the deep compartment, thought to include the mitochondria. Rosen (1983) has noted a similar increase in intracellular calcium in osteoclasts.

Piccinini et al. (1977) and Favalli et al.(1977) performed several experiments on the tail arteries of normotensive rats. Starting with non-exposed rats, they found that infusion of lead into the media in doses of 5-15 uM produced a dose- dependent contraction of the arteries, including an increase in the steady state contraction. In the absence of calcium in the media there was no effect. In the presence of calcium the contractile response was independent of the calcium concentration in the media (in the range of 0 .6-2.6mM) for the control arteries, but the lead - exposed arteries, contractile response increased with calcium concentration as well as with lead concentration (at constant calcium level) in the extracellular media; this suggests a significant lead- induced effect on the cell's ability to properly regulate calcium levels. When lead was added to the calcium-free media and then calcium was added the initial response to calcium was also exaggerated.

Piccinini analyzed the arterial tissue in the lead- exposed and control arteries and found an increase in intracellular calcium ($P < 0.01$), with the fraction of exchangeable calcium in the intracellular media remaining the same (but the absolute amount increasing).

These results are consistent with the findings of Iannacone et al., (1981), and Webb et al. (1981) , who demonstrated not

only that dietary lead increased the blood pressure of normotensive rats in vivo, but also that the rats manifested increased contractile response to stimulus (compared to controls). The increased intracellular calcium stores noted above would produce exactly the exaggerated response that was found in vivo (Iannacone) and in the tail arteries in vitro after sacrifice (Webb), particularly if the endoplasmic reticuluum saturated first, before the mitochondria began to dominate the calcium increase (viz Pounds supra). Moreover, Webb found that the period of time required to reach half maximal relaxation following alpha-adrenergic stimulation was significantly longer in the arterial muscle of the lead-exposed rats than in the controls. This fits in well with Piccinini's observation that in vitro treatment with lead increased the half life of the slow component of radioactive calcium desaturation from the rat smooth muscle. It is also in accord with Webb and Bhalla's observation that slowed ability to uptake calcium in the microsomal fraction, including the endoplasmic reticuluum, was associated with the hypertension found in the spontaneously hypertensive rat.

4. Lead and the Mechanisms of Intracellular Calcium Regulation

There are several plausible mechanisms whereby lead might effect these processes in ways that led to increased blood pressure, and some of these effects have been documented in smooth muscle tissue. First is lead's long documented interference with the sodium-potassium pump, which can affect

both the influx of ionized calcium and its efflux in excitable cells (Pounds, 1984). The inhibition was noted by Hernberg (1967) and others (e.g. Raghaven 1981, Goddard and Robinson , 1976).

The disturbances in mitochondrial sequestration of calcium noted in the spontaneously hypertensive rat's smooth muscle tissue is also associated with lead. Pounds (1982) found increased calcium stores in the hepatocyte mitochondria, and lead has been found to decrease the capacity of mitochondria to take up calcium in rat hearts (Parr and Harris, 1976) and rat brains (Goldstein 1977). Kapoor and van Rossum (1984) reported that lead inhibited the rate of mitochodrial uptake of calcium in renal cortical mitochondria both in tissue and in isolated mitochondria. The finding of both slowed mitochondrial uptake of calcium and increased mitochondrial calcium stores is somewhat surprising, but may indicate a saturation effect. Mitochondrial uptake of calcium is normally very low in smooth muscle tissue at the cytostolic calcium concentrations typical of resting tone, so lead's interference with mitochondrial uptake is more likely to be linked to the increased reactivity and slowed relaxation effects of lead than to its increase in resting tone.

Cyclic AMP is another important modulator of free cytostolic calcium and smooth muscle reactivity that may be affected by lead. Cyclic AMP may increase the activity of the sodium pump, leading to a decrease in intracellular calcium, stimulates the calcium pump that removes calcium from the cytosol into the endoplasmic reticuluum, and leads to a phosphorylation of MLCK,

reducing its sensitivity to activation by calcium. All of these activities reduce muscle tension (Blaustein). Goldstein and Ar (1983) demonstrated that lead activated calmodulin in its role of activating phosphodiesterase, the enzyme that converts cyclic AMP to AMP, thereby stopping the activity described above. Thus by disturbing the balance between calcium and cyclic AMP, lead may increase the reactivity of smooth muscle and slow the return to the resting tone in the vascular smooth muscle.

This indicates that the relationship between blood lead and blood pressure found in the community studies (Pirkle et al., Harlan et al., Moreau et al., Pocock et al.,Batuman eta al. Beevers, et al.) is supported not only by the animal studies (Perry, et al., Webb et al., Victery et al., Kopp et al., Iannacone et al., Favalli et al., Piccinini et al.) but also is consistent with what we know about the ability of lead to alter cellular calcium metabolism.

Moreover, the fact that the cytosolic calcium concentration of vascular smooth muscle is normally above that which produces tension suggests that anything that altered that concentration would have an affect on blood pressure , which is consistent with the finding of no threshold for lead's effect on blood pressure in the NHANES II data. It is interesting that the effect of lead on vitamin D metabolism, which is also thought to be caused by increased cellular calcium concentration, is also a logarithmic function of blood lead, and has thus far manifested no threshold, although the blood lead levels in the published studies do not go down as far as in the NHANES data (3ug/dl).

A.2. Analysis of NHANES II Data

In light of these indications of potential effects of lead on the cardiovascular system, the relationship between blood lead levels and blood pressure has recently been explored (Harlan et al., 1985; Pirkle et al., 1985) using the NHANES II data. The NHANES II is an excellent data base for this analysis because of the care given to accurate measurements, the great range of information on possible confounding factors, and because it is a representative sample of the U.S. population. As such it avoids the problems of selection bias, healthy-worker effect, other occupational exposures, and the choice of controls that confound many occupational studies. Harlan et al. found blood lead related to blood pressure for males aged 12 to 74 after controlling for the traditional variables associated with blood pressure (age, age-squared, body mass index, race) as well as alcohol consumption, socio-economic factors, and all nutritional variables suspected of affecting blood pressure. Moreover, this relationship held in each year of the NHANES II sample, when analyzed separately, and this relationship held for both blacks and whites.

Pirkle et al. found that blood lead levels were a statistically significant predictor of blood pressure in adult males. This relationship held not only when blood lead was evaluated in a regression with all known factors that have previously been established as correlated with blood pressure, but also when that relationship subsequently was tested against 87 additional variables representing linear and nonlinear functions of every

dietary and serologic variable in the NHANES II survey. This analysis builds on the Pirkle et al. study.

A.2.a. Blood Pressure Measurements

Three blood pressure measurements were taken during NHANES II. A seated measurement was taken as soon as the examinee entered. Later, a recumbent measurement was taken. A second seated measurement was taken just before the end of the examination. It is standard medical practice to prefer the second seated measurement, because nervousness on just entering a medical examination center makes the first seated measurement less stable. All of the results presented are for the second seated measurement. However, almost all of the regressions and robustness tests described were performed on all three measurements, and on the average of the first and third seated measurements; all of the conclusions concerning lead's significance held for all eight regressions (four diastolic, four systolic).

A.2.b. Initial Analysis

After replicating the Harlan et al. results for all adult males, the first goal was to determine if blood lead levels were related significantly to blood pressure in white males, 40 to 59 years old. This subgroup was chosen for two reasons. First, we wanted to be able to use the pooling projects multiple logistic risk factors for cardiovascular disease to determine if the changes in blood pressure associated with lead were of any significance for cardiovascular disease. These factors have only been published for 40-59 year olds, and the risk is known to be

smaller for younger adults. Secondly, we chose this age group because at lower ages both blood pressure and blood lead vary with age. This collinearity could artificially mask or enhance the correlation between blood lead and blood pressure. Between 40 and 59 years of age, however, blood pressure is essentially independent of age. Choosing this subgroup avoids any collinearity problems. We focused on whites because data relating cardiovascular disease to blood pressure are less extensive for nonwhites.

The established correlates of blood pressure are age, sex, race, and one of the indices of relative height-to-weight. Body mass index (BMI = weight/height^2)was used in this analysis. By limiting attention to 40 to 59 year old white males, there was no need to control for race, sex, or, to a large degree, age. Although age was only occasionally significant in the stepwise analysis, both age and age-squared were forced into each multiple regression model to be certain any effect of lead was independent of age.

The natural log of blood lead was more normally distributed, was more statistically significant, and gave a higher R^2 than untransformed blood lead, blood-lead-squared, blood lead plus blood-lead-squared, the square root of blood lead, or blood lead to other fractional powers (0.15, 0.2, 0.3, 0.4). All of the results reported here are for the natural log of blood lead, but regressions using lead on the untransformed scale gave very similar results.

The initial regressions analyzed systolic and diastolic

blood pressures for white males, 40 to 59 years old, with a model consisting of age, age-squared, BMI, and blood lead. These regressions were done to determine whether blood lead levels were significantly associated with systolic and diastolic blood pressures after controlling for age, sex, race, and BMI, which are well-documented correlates of blood pressure. Lead was statistially significant ($p < 0.01$) for both systolic and diastolic blood pressures in all the regressions (unweighted, weighted, and weighted with design effects). The regressions also tested whether this relationship held up when other potentially confounding variables were considered.

A.3. Tests of Robustness

The regression models were expanded to incorporate additional variables, with particular attention directed to the stability and significance of the lead coefficient in the presence of nutritional factors and blood biochemistries.

A large set of nutritional and biochemical variables from NHANES II was included in the stepwise regressions. Additional regression analyses considered potential problems of interaction terms. To ensure the robustness of the relationships, further analyses were done to address marginally insignificant variables and nonnutrition variables. Although our analysis focused on males aged 40 to 59, additional regressions were also performed considering all males over age 20.

A.3.a. Nutritional and Biochemical Variables

To provide an unusually rigorous test of the independent

significance of blood lead, almost all of the nutritional and biochemical variables in the NHANES II were included in stepwise regressions. In addition, to account for possible curvilinear relationships, squared and natural logarithmic transformations of almost all of these variables were also included. The variables are listed in Table 1. The objective was not to evaluate the possible association of nutritional or biochemical measurements with blood pressure, but rather to conservatively estimate the strength and independence of the relationship between blood pressure and blood lead.

Including these additional 87 variables increases the probability of variables being found statistically significant due to chance alone. This complicates the interpretation of nutritional and biochemical factors, but not the interpretation of the lead variable; it only makes it more difficult for lead to maintain its significance.

The general procedure for variable selection was as follows. First, weighted stepwise multiple linear regression was used to determine which variables were significantly related ($p < 0.05$) to blood pressure (using the Stepwise and MAXR options of the SAS procedure, STEPWISE). The MAXR procedure was the principle one used; it determines for any given model size (i.e., number of variables) the variables that explain the greatest amount of the variance (i.e., maximize R^2). We chose the largest model with

TABLE 1 Variables Included in the Stepwise Regression Analyses

age *	dietary iron +
age-squared *	dietary vitamin A +
body mass index	dietary vitamin C +
dietary sodium +	dietary thiamine +
salt shaker sodium	dietary riboflavin +
dietary sodium X salt shaker sodium	dietary niacin +
	serum cholesterol+
dietary potassium +	serum vitamin C +
dietary sodium - potassium ratio	serum iron +
	serum transferrin saturation
dietary calcium +	serum zinc +
dietary phosphorus +	serum copper +
dietary protein +	serum albumin +
dietary fat +	hemoglobin +
dietary carbohydrate +	red blood cell count
dietary cholesterol +	ethanol consumption / week +
dietary saturated fatty acids +	cigarettes smoked / day
	total dietary grams +
dietary oleic acid +	total dietary calories +
dietary linoleic acid +	cigar or pipe smoking
blood lead+	

* forced into each regression to remove any possible age effects on blood pressure.

+ the natural log and squared transformation of these variables were also included in the stepwise regression.

all variables significantly related to blood pressure ($p < 0.05$). The Stepwise option, which uses forward selection with backwards elimination, chose very similar models, and also always chose blood lead. From the 87 nutritional and biochemical variables, the weighted stepwise regression selected five additional variables for diastolic pressure and six additional variables for systolic pressure using a 5 percent significance test. These were used as the starting model for the SAS procedure SURREGR, which additionally incorporated the survey design effects. For both systolic and diastolic blood pressures, one variable from the weighted stepwise regression failed to maintain significance at the 5 percent level after the design effects were incorporated. The final regression results for systolic and diastolic pressures, after accounting for the weighting and design effects, are given in Table 2.

The multiple logistic regressions (of the probability of hypertension) were also performed using programs from SAS. The procedure LOGIST was used for the stepwise unweighted multiple logistic regression, and the procedure NLIN (nonlinear regression) was used for the weighted logistic regression calculations. The selection process again chose the largest significant model that explained the greatest amount of the variance. Calculations of threshold levels for effects were made using the procedure NLIN on segmented regression models, which finds the threshold point that minimizes the sum of the squares of the error terms. The results of the logistic regression on hypertension are shown in

Table 3. Note that the logistic regressions included blacks as well as whites, because these regressions were used only to predict the effect of lead on the probability of having hypertension and were not used to estimate the number of cardiovascular diseases and deaths. As noted earlier, blacks were not included in the linear regressions because the best available coefficient for predicting cardiovascular risks included insufficient numbers of blacks.

TABLE 2 Regression of Diastolic and Systolic Blood Pressures in White Males Aged 40 to 59

Variable	Coefficient	t-Statistic	Probability
Diastolic			
Age	0.2768	0.17	0.8636
Age2	-0.0014	0.10	0.9321
Body Mass Index	1.131	8.55	0.0001
Log(blood lead)	3.954	2.85	0.0080
Dietary Potassium	-0.0018	4.92	0.0001
Hemoglobin	1.548	3.90	0.0005
Albumin	3.587	2.50	0.0179
Log(dietary vitamin C)	1.838	4.65	0.0001
Systolic			
Age	1.311	0.57	0.5720
Age2	-0.0068	0.30	0.7706
Body Mass Index	1.736	9.42	0.0001
Log(blood lead)	8.436	3.24	0.0028
Albumin	7.088	2.50	0.0178
Log(dietary Vitamin C)	2.411	3.84	0.0005
Log (dietary riboflavin)	-5.509	3.07	0.0044
Log(dietary oleic acid)	3.992	2.49	0.0183
Log(serum vitamin C)	-3.472	2.47	0.0184

TABLE 3 Weighted Logistic Regression on Probability of Diastolic Blood Pressure Greater Than or Equal to 90 mm Hg in Men Aged 40 to 59

Variable	Coefficient	t-statistic	p-Value
Constant	-16.41	10.13	0.0000
Log(Blood Lead)	0.693	3.96	0.0000
Albumin	0.0873	3.70	0.0001
Body Mass Index	1.700	9.34	0.0000
Hemoglobin	0.0329	5.25	0.0000
Log(Vitamin C)	0.3585	5.98	0.0000
Dietary Potassium	-0.00058	7.47	0.0000
Total Carbohydrates	0.00246	3.09	0.0010

After including the nutritional variables, the blood analytes, and their curvilinear transformations, lead remained significantly associated ($p < 0.01$) with both systolic and diastolic blood pressures. The magnitude of this relationship, adjusted for the other significant variables, is shown graphically in Figures 1 and 2. Furthermore, segmented regression analyses indicated there was no threshold blood lead level in the data.

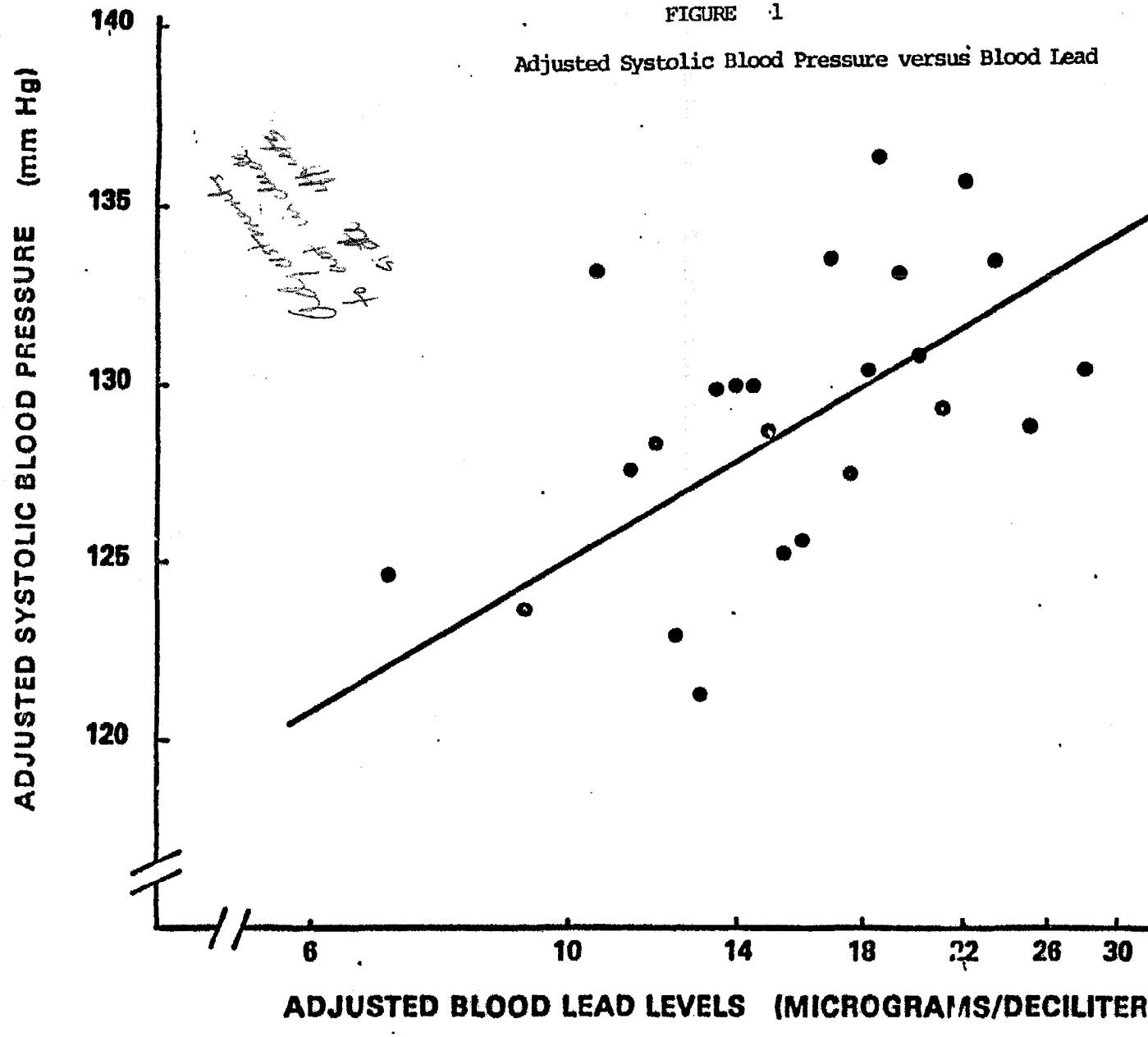
These segmented "hockey stick" regressions fit two regression lines to the data. One, below the putative blood lead threshold T, depends on all the variables except lead. The other, for blood lead levels above T, includes lead. An iterative technique is used to find the value of T that minimizes the sum of the squares of the error terms over the full range of both regression lines. In this case, the error in the regression was minimized at a threshold of zero; that is, lead was significantly related to blood pressure at all levels down to zero.

A.3.b. Interaction Terms

In multiple regression analysis, another consideration is the possibility of significant interaction terms. To evaluate this possibility, an additional weighted stepwise regression analysis was done for systolic and diastolic blood pressures. The variables consisted of the linear interaction terms between

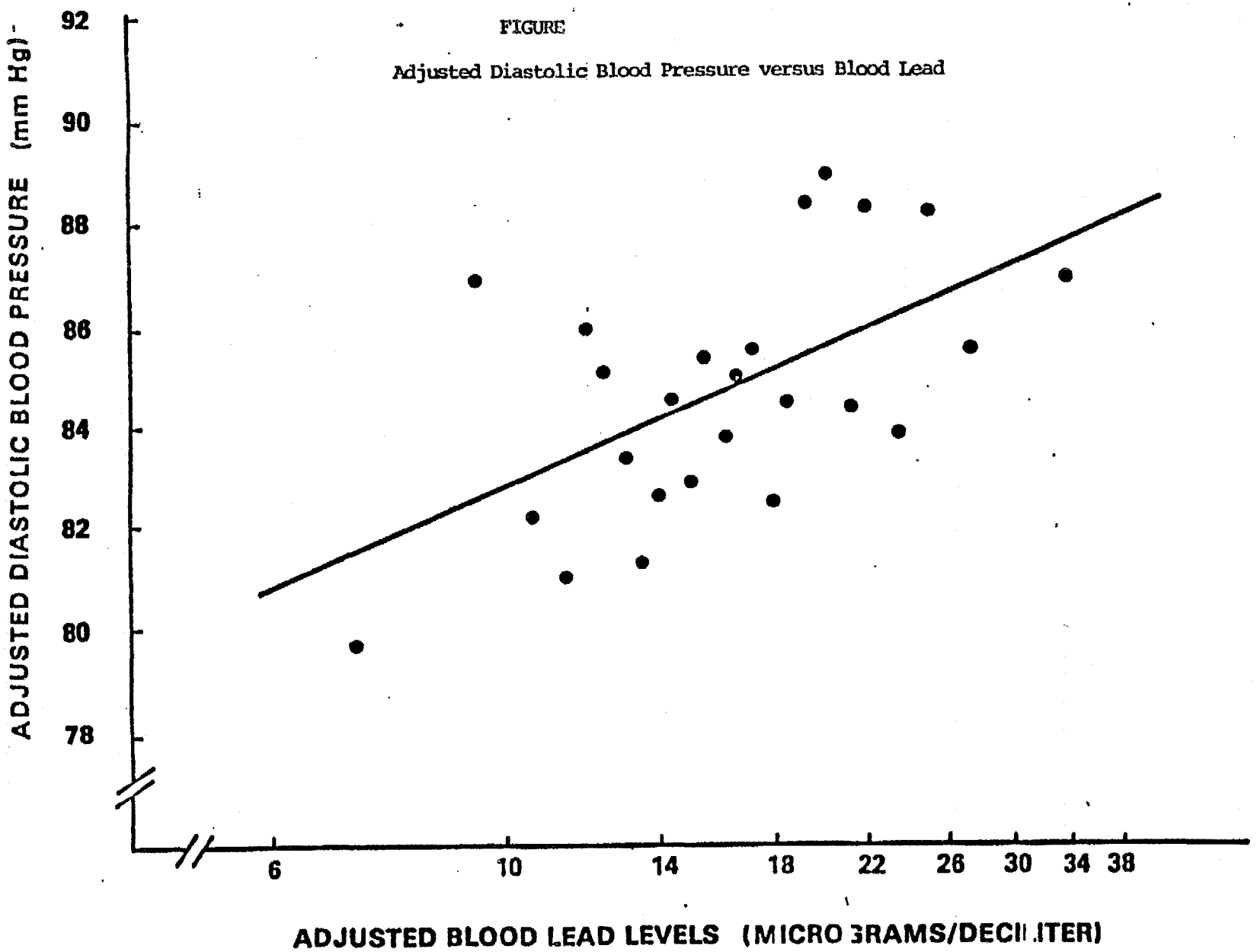
FIGURE 1

Adjusted Systolic Blood Pressure versus Blood Lead



FIGURE

Adjusted Diastolic Blood Pressure versus Blood Lead



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the final variables in the model (shown in Table 2) and the linear form of all the other variables originally selected for the initial stepwise regression, including their log and square transforms (Table 1). This meant running a stepwise regression with 162 interaction terms added to the final regression models for systolic and diastolic pressures. Using such a large set of variables gave a high probability that some variables would enter at the 5 percent level by chance. However, the purpose was not to determine if those variables were independently significant, but, rather, to further test the significance and independence of the relationship between blood pressure and blood lead. As expected, several interaction variables entered the systolic and diastolic regressions, but in each regression the lead coefficient varied less than 10 percent and remained significant ($p < 0.015$).

A.3.c. Marginally Insignificant Variables

Three other analyses were done to ensure that this relationship was robust. First, the original weighted stepwise regression was extended to include variables significant through the 15 percent level to see if marginally insignificant variables influenced the significance of lead. For both systolic and diastolic pressures, lead remained significant and there was little change in the magnitude of the coefficient.

Second, for diastolic blood pressure, all the variables were included that were significant between the $p = 0.05$ and the 0.15 levels, and every possible combination of those variables was

considered. All 255 combinations were added to the variables that were statistically significant, and a regression was performed on each one. The coefficient of the log of blood lead varied by only plus or minus 10 percent from the value we obtained when we included only significant variables, and the highest p-value for lead was still less than 0.01.

The last analysis was the most demanding test of the independence of the relationship between blood pressure and blood lead. Models for diastolic and systolic blood pressures were fit by weighted stepwise regression to the original model variables (Table 1), excluding lead. This gave all of the other variables and their curvilinear transformations the maximum opportunity to explain variation that could also be explained by lead. After obtaining this new final model without lead, a single regression was run adding the lead variable to the variables of this new final model. For both systolic and diastolic pressures, lead was still statistically significant ($p < 0.016$) and the magnitude of the lead coefficient changed less than 10 percent from those obtained in the original analysis. The results of all these analyses indicated that the strength and independence of the relationship between blood pressure and blood lead were remarkably stable.

Because some people have found small amounts of ethanol associated with reduced blood pressure, ethanol was also modeled as a quadratic function of consumption, and with two dummy variables for light and heavy drinking. The stepwise regression was repeated, with no change.

A.3.d. Nonnutrition Variables

Pirkle et al. then considered nonnutrition variables that might be associated with blood pressure. In additional runs completed since then, we have added several other variables. The complete set is shown in Table 4. Socio-economic and demographic factors as well as additional medical history variables were included.

Hypertension medication and low salt diet were tested -- not for inclusion in a final model, as they are essentially indicators of high blood pressure, but rather to see if the response to lead differed in those groups. The coefficient of lead did not change appreciably, and lead interaction terms with the two variables were insignificant.

The other variables in Table 4 were tested in two ways. First, the stepwise regression procedure was repeated with them using all nutritional and serum measurements that were significant at the $p = 0.15$ level. The nutritional factors were limited to those significant at the 0.15 level to give the nonnutritional factors a greater chance to enter the model. Again, lead was selected ($p < 0.005$) and its coefficient changed by less than 10 percent from the original model that included only age, age2, and body mass index.

TABLE 4 Nonnutrition Variables Tested in the Stepwise Regression

Demographic Variables	Other Personal-History Variables
Family Income	Tricep Skinfold
Poverty Index	Subscapular Skinfold
Region of the Country	Recreational Exercise
Season of the Year	Work-Related Exercise
Degree of Urbanization	Recent Weight Loss
Residence Inside Central City	Family History of Hypertension
Educational Level	Kidney Disease
	Serum Creatinine

Hypertension Variables
Hypertensive Medication
Low Salt Diet

The variables in Table 4 were then added to all of those on Table 1 (including their nonlinear transforms) and the stepwise process was repeated -- with the same results. Finally, the stepwise procedure was rerun using all of the variables in Tables 1 and 4 except lead; lead was then inserted into the model resulting from this procedure. It was still significant ($p < 0.006$), with less than a 10 percent change in its coefficient. Because the presence of two terms to describe the curvilinear dependence of blood pressure on age might reduce the chances of variable correlated with age achieving significance, age was modeled as a single curvilinear function (sine of age), and the stepwise regression repeated; the results were the same.

In addition, smoking and drinking were forced into the regression, and lead was still significant ($p < 0.01$), with only a 3 percent change in its coefficient.

Our previous studies have shown that about half of the lead in people in the NHANES II sample came from gasoline. Tetraethyl lead has very little cadmium in it, so confounding with cadmium (which is also suspected of affecting blood pressure) is unlikely. However, we repeated the regression excluding occupationally exposed workers, who may also have cadmium exposure. Lead remained significant ($p < 0.01$), and its coefficient increased somewhat. We also regressed gasoline lead directly on blood pressure, and it was significant.

Although all of these analyses make it clear that collinearity is not a problem in these regressions, variance inflation factors were computed; no significant variable had a

variance inflation factor above 1.4. (Variance inflation factors below 4 are considered acceptable in multiple regression analyses.)

To ensure that the significance of lead in the regression was not due to the presence of a few influential observations, influence diagnostic procedures were run. Studentized residuals were plotted for all the observations, and the largest residuals were clustered near the middle of the data, where their influence is slight. Cook's D statistics also were computed for each observation. The highest Cook's D was 0.029, and the second highest was 0.023, both of which are very small. Moreover, of the 10 observations with the largest Cook's D statistics, six had positive residuals and four had negative residuals, indicating that the most influential observations split almost evenly on which way they would influence the lead regression coefficient.

Because the nutritional variables are often collinear with each other, a second approach was taken to dealing with the nutritional and serologic variables. Principal components analysis was done to find the linear combinations of the nutritional and serologic factors that explained the greatest amount of their common variation. These principal components were then entered in the stepwise regression to allow those factors to explain a greater amount of the variation in blood pressure before collinearity prevents any more variables form entering. For instance, the first 5 principal components of the variables on table 1 explain 50% of their variance. When these were used in the stepwise regression lead was still significant ($p < .01$) and the coefficient had changed by less than 10 % from its original value.

A.3.e. Other Age Groups

The 40 to 59 year old age group represents about one-third of adult males, and is the only one where the confounding of age and blood lead can be eliminated unambiguously. Additional regressions were performed, however, to confirm the Harlan et al. finding of an effect in all adult males. Tables 1 and 4 contain several variables that Harlan et al. did not consider in their analysis. Therefore, the stepwise regression analysis was repeated using all of the variables in both tables, (plus race) and their square and natural log transforms as indicated. All males over the age of 20 were considered. Lead was selected by the regression, p= 0.0008 systolic, p= 0.0039 diastolic. To assure that a spurious correlation was not occurring due to an interaction of lead levels with hypertensive medication or due to persons with kidney diseases and hypertension having elevated blood pressure and blood lead due to that kidney disease, and causing a spurious correlation between the two, we reran the regression excluding all persons with hypertension serious enough to require medication. Lead was still significant, p = 0.0015 systolic, p = 0.0018 diastolic.

To assure that the relationship was robust to specification changes the stepwise regression was repeated without lead. Lead was then reinserted, and was significant ($p < 0.01$).

To check whether the relationship might be substantially different for different age groups, dummy variables for the age groups 20-45 and 46-74 were created, and an interaction term

groups 20-45 and 46-74 were created, and an interaction term between lead and the dummy for the earlier age group was inserted in the regression. Such interaction terms check for differences in the lead/blood pressure relationship without having to sub-divide the sample. The interaction term was not significant. Two further tests were done. First, the stepwise regression was repeated separately in both age subgroups, and lead was selected as significant in each one. Secondly, a separate regression was run for the 25 age subgroups 20-49, 21-50,...45-74. Lead was significant in each one.

Because lead levels fell during the four years of the NHANES II study two tests were done to assure that lead was not picking up the effect of a time trend in some other causal variable of blood pressure. We note first that if such factor was on the two tables listed before, there would be no problem, changes in obesity, exercise, etc. will be attributed to those variables. Fortunately, the lead levels in the United States fell at a much faster rate during the last two years of the NHANES study, because gasoline lead regulations were changed. If lead were merely correlating with the change in some other factor, that correlation would be different in the last two years than in the first two years. We therefore tested whether the relationship between blood lead and blood pressure was different between the first and last half of the NHANES survey, and it was not ($p = .75$ systolic, $p = .79$ diastolic). As a further test, we inserted a term for time into our regression model, despite not knowing of

any time trend in an omitted factor related to blood pressure. Lead was still significant even with time in the model. All of the candidate counties in the United States were grouped into 32 strata on the basis of similarity in region, size and socieconomic characteristics, and sample areas were chosen from each strata. To assure that there was no confounding of lead with location, a variable for each strata was forced into the regression. Lead was still significant. Then, a variable for each individual stand was included in the stepwise regression. Only a few of the locations were selected as significant, and lead remained significant.

A.4. Summary of Blood Lead - Blood Pressure Results

The final models for blood pressure, including all statistically significant variables, are shown in Table 5. The final logistic model for the probability of hypertension is

TABLE 5. Regression of Diastolic and Systolic Blood Pressures in White Males Aged 40 to 59

Variable	Coefficient	F-Statistic	Probability
Diastolic			
Age	-0.210	0.02	0.8960
Age-squared	0.003	0.04	0.8373
Body Mass Index	1.082	67.88	0.0000
Blood lead+	4.609	12.19	0.0014
Potassium	-0.002	25.30	0.0000
Hemoglobin	0.151	16.81	0.0003
Albumin	0.354	7.42	0.0104
Dietary Vitamin C+	1.886	23.67	0.0000
Family history of hypertension	2.085	4.37	0.0446
Recreational exercise	-1.851	9.48	0.0042
Systolic			
Age	1.142	0.25	0.6226
Age-squared	-0.005	0.05	0.8208
Body Mass Index	1.710	85.90	0.0000
Blood lead+	8.510	10.54	0.0027
Albumin	0.695	6.09	0.0192
Dietary Vitamin C+	2.458	13.78	0.0008
Dietary Riboflavin+	-5.101	8.14	0.0075
Dietary Oleic Acid+	3.650	5.34	0.0275
Serum Vitamin C+	3.365	5.81	0.0218
Family history of hypertension	3.683	4.59	0.0399

+ log transform

TABLE 6. Logistic Regression on Probability of Blood Pressure Greater Than or Equal to 90 mm Hg in Men Aged 40 to 59

Variable	Coefficient	t-Statistic	p-Value
Constant	-15.40	7.0	0.0000
Log(Blood Lead)	0.793	3.20	0.0014
Albumin	0.650	2.06	0.0399
Body Mass Index	0.1571	6.57	0.0000
Hemoglobin	0.0265	3.19	0.0015
Log(Vitamin C)	0.3593	4.22	0.0000
Dietary Potassium	-0.00053	5.33	0.0000
Total Carbohydrates	0.00286	2.86	0.0080
Recreational Exercise	0.3864	0.128	0.0026

shown in Table 6.

It is noteworthy that the logarithmic form of the dose-response relationship suggests a large initial effect, leveling off at higher blood-lead levels. This may explain why only about 60 percent of the occupational studies (i.e., high lead-exposure studies) have found an effect that was significant at the 95 percent confidence level, while almost all of the studies of lower lead levels have found the relationship to be significant.

The other low-exposure studies, the animal data, and the robustness of these results suggest that the relationship is causal. Moreover, specific analyses to determine whether there is a lower threshold below which lead has no effect on blood pressure showed that the data were fit best with a threshold of zero.

References

1. Rasmussen, H Cellular calcium metabolism Ann Int Med 1983:98 (part 2) 809-816

2.Blaustein, M. Sodium ions, Calcium ions, and hypertension: a reassessment and a hypothesis Am. J. Physiol. 232(3): C165-C173

3.McCarron, D Calcium in the pathogenesis and therapy of human hypertension Am. J. Med. 78(2B): 27-34

4. Clough D.L., Pamnani, M.B., and Haddy. F.: Decreased myocardial Na+-K+ -ATPase activity in one kidney, one clip hypertensive rat. Am. J. Physiol. 245(Heart Circ. Physiol. 14): H244-H251, 1983.

5. McCarron, DA, Yung, NN, Ugoretz, BA, et al. : Disturbances of calcium metabolism in the spontaneously hypertensive rat. Hypertension 1981: 3:I162-I167

6. Webb, RC and Bohr, DF : Mechanism of membrane stabilization by calcium in vascular smooth muscle Am. J. Physiol. 235(5): C227-C232 1978.

7. Webb,RC and Bhalla, RC : Altered calcium sequestration by subcellular fractions of vascular smooth muscle from spontaneously hypertensive rats. J. Mol. Cell Cardiol. 1976; 8: 651-661.

8. Hinke, JA Effect of Ca++ upon contractility of small arteries from DCA-Hypertensive rats Circ. Res. (I):I23-I33 1966.

9. Greenberg, S. and Bohr, D.F. Venous smooth muscle in hypertension. Enhanced contractility of portal veins from spontaneously hypertensive rats. Circ. Res. 36,37, Suppl 1:I208-I215, 1975.

10. Bruschi, G. Bruschi, M et al. Cytoplasmic free Ca++ is increased in the platelets of spontaneously hypertensive rats and essential hypertensive patients. Clin. Sci. (1985) 68:, 179-184.

11. Erne, P. et al. NEJM (1984), 310,1084-1088.

12. Guazzi, MD et al. Treatment of hypertension with calcium antagonism (review) Hypertension 1983:5: I97-I102.

13. Postonov, et al. Altered sodium permeability, calcium binding, and Na+-K+-ATPase activity in red blood cell membrane in essential hypertension. Pfleugers Arch. 371:263-269, 1977.

14. Wambach, G et al. : Natrium-Kalium Adenosine -triphosphatase -Aktikat in erythrosytenghosts von patienten mit essentieller

hypertonie. Klin. Wochenschr. 57|:169-172, 1979.

15. Garay, RP and Meyer, P : A new test showing abnormal net Na+ and K= fluxes in erythrocytes of essential hypertension patients. Lancet 1:349-353, 1979.

16. Lasker, N et al. Racial differences in erythrocyte sodium-potassium adenosine triphosphatase Clin Res. 31:330A 1983

17. Weder, AB et al. Racial differences in erythrocyte cation transport. Hypertension 6:115-123, 1984.

18. Whang, R et al. Hypomagnesia and hypokalemia in 1,000 treated ambulatory hypertensive patients. J. Am. Col. Clin. Nut. 1: 317-322 (1982).

19. Dyckner, T and Wester, PO Effects of magnesium on blood pressure. Brit. Med. J. 286: 1847f 1983.

20. Pounds, JG Effect of lead intoxication on calcium homeostasis and calcium mediated cell function: a review. NeurToxicology 5(3):295-332 1984.

21. Hernberg, S et al. Deficient red cell membrane (Na+K+) - ATPase in lead poisoning. Arch. Env. Health 1967; 14: 313-318.

22. Raghavan SRV, Culver BD, Gonick HC, Erythrocyte lead binding protein after occupational exposure. II influence on lead inhibition of membrane (Na+,K+) -adenosinetriphosphatase./ J. Toxicol Environ Hlth 1981; 7:561-568.

23.Goddard GA, Robinson, JD Uptake and release of calcium by rat brain synaptosomes. Brain Res 1976; 110: 331-350.

24. Kim, CS et al. The effects of lead poisoning on calcium transport by brain in 30-day-old albino rabbits. Toxicol. Appl. Pharmacol. 1980; 52;491-496.

25. Pounds, JG et al. Effect of lead on calcium homeostasis in the isolated rat hepatocyte. Toxicol Appl Pharmacol 1982; 63:389-401.

26. Rosen, JF The metabolism of lead in isolated bone cell populations: interactions between lead and calcium. Toxicol. Appl. Pharmacol. 71: 101-112 1983.

27. Piccinini, et al. Experimental investigations on the contraction induced by lead in arterial smooth muscle. Toxicol. 1977; 8: 43-51.

28. Favalli, et al. Experimental investigation on the contraction induced by lead in arterial smooth muscle. Acta Pharmacol. Toxicol. 1977; 41(2) :412-420.

29. Iannaccone, et al. Cardiovascular reactivity in the rat following chronic exposure to cadmium and lead. Ann. 1st Super Sanita, vol 17 655-660 1981.

30. Webb, RC et al. In vivo and in vitro effects of lead on vascular activity in rats. Am. J. Physiol. 241 (Heart Circ. Physiol. 10): H211-H216 1981.

31. Parr DR, Harris EJ. The effect of lead on the calcium handling capacity of rat heart mitochondria. Biochem. J. 1976; 158:289-294.

32. Goldstein, GW Lead encephalopathy: the significance of lead inhibition of calcium uptake by brain mitochondria. Brain Res. 1977;136: 185-188.

33. Kapoor SC, van Rossum, GDV Effects of Pb++ added in vitro on Ca== movements in isolated mitochondria and slices of rat kidney cortex. Biochem. Pharmacol. 1984.

34. Pirkle, JL et al. The relationship between blood lead levels and blood pressure and its cardiovascular risk implications. Am. J. Epidemiol. 1985 121:246-258.

35. Harlan, et al. Blood lead and blood pressure. Relationship in the adolescent and adult U.S. population. J. Amer. Med. Assoc. 253: 530-534. 1985

36. Moreau, T et al. Blood lead levels and arterial pressure: initial results of a cross sectional study of 431 male subjects. Rev. Epedemiol. Sante Publique. 30: 395-397.

37. Pocock, S et al. Blood lead concentrations, blood pressure, and renal function. Br. Med. Journal 289: 872-874. 1984

38. Beveers, DG et al. Blood lead and hypertension. Lancet 2(1975); 1-3.

39. Victery, w et al. Lead, hypertension, and the renin-angiotension system in rats. J. Lab.Clin. Med. 99: 354-362.

40. Perry, HM and Erlanger,EJ Pressor effects of chronically feeding cadmium and lead together. in Hemphill, DD ed Trace substances in environmental health-XIIColumbia Mo. pp 268-275 1978.

41. Filo,RS et al. Glycerinated skeletal and smooth muscle: calcium and magnesium dependence. Science 147: 1581-1583 1972

42. Freeman R Reversible myocarditis due to chronic lead poisoning in childhood. Arch Dis Child 1965: 40:389-93

43. Williams et al. Effects of chronic lead treatment on some cardiovascular responses to norepinephrine in the rat. Toxicol

Appl Pharmacol 1977; 40: 407-413.

44. Williams et al. Noradronergic affects of lead on the neonatal rat. Pharmacology. 1978; 20(3): 186.

45. williams BJ Hejtmancik M Time and level of perinatal lead exposure for development of norepinephrine cardiotoxicity. Res comm CP 1979; 24(2): 367-76.

46. Carmignani et al. Effects of chronic exposure to cadmium and lead on some neurohumoral mechanisms regulating cardiovascular function in the rat. 1983 Proceedings of the fourth International Conference on Heavy metals in the Environment. CEP consultants.