

The G⁸⁹⁴-T⁸⁹⁴ Polymorphism in the Gene for Endothelial Nitric Oxide Synthase and Blood Pressure in Lead-Exposed Workers From Korea

Mark E. Lustberg
Brian S. Schwartz
Byung-Kook Lee
Andrew C. Todd
Ellen K. Silbergeld

We evaluated whether the G⁸⁹⁴-T⁸⁹⁴ polymorphism in exon 7 of the endothelial nitric oxide synthase (eNOS) gene is associated with blood pressure or modifies the relation between lead dose and blood pressure in 803 lead workers in Korea. A total of 84.9% of individuals were homozygous GG, 14.4% heterozygous GT, and 0.8% homozygous TT. The T⁸⁹⁴ allele was not significantly associated with systolic or diastolic blood pressure. The prevalence of hypertension did not differ by T⁸⁹⁴ status (OR = 0.82; 95% CI = 0.50–1.37). There was no evidence of effect modification by eNOS genotype on relations of lead dose with blood pressure. These data provide no evidence that the T⁸⁹⁴ allele is associated with higher blood pressure or modifies the association of lead dose with blood pressure. (J Occup Environ Med. 2004;46:584–590)

From the Department of Epidemiology and Preventive Medicine, University of Maryland Baltimore, Baltimore, Maryland (Dr Lustberg); the Departments of Environmental Health Sciences (Drs Schwartz and Silbergeld) and Epidemiology (Dr Schwartz), Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland; the Department of Medicine (Dr Schwartz), Johns Hopkins School of Medicine, Baltimore, Maryland; the Institute of Industrial Medicine, Soonchunhyang University, Chonan, Korea (Dr Lee); and the Department of Community and Preventive Medicine, The Mount Sinai School of Medicine, New York, NY (Dr Todd).

Address correspondence to: Brian S. Schwartz, MD, MS, Division of Occupational and Environmental Health, Johns Hopkins Bloomberg School of Public Health, Room W7041, 615 North Wolfe Street, Baltimore, MD 21205; E-mail: bschwartz@jhsph.edu.

Copyright © by American College of Occupational and Environmental Medicine

DOI: 10.1097/01.jom.0000128158.32391.85

Lead exposure has been associated with increased blood pressure in some, but not all, studies in humans.^{1–9} Overall, the body of epidemiologic literature would support the inference that lead causes elevations in blood pressure, even at relatively low levels. In addition to the epidemiologic evidence, there is evidence from animal models, because animals fed lead have elevations in blood pressure.¹⁰ Some evidence suggests that lead could increase blood pressure by lowering steady-state levels of nitric oxide (NO),^{11–22} a principal endothelium-derived relaxing factor and one of the body's key modulators of vascular resistance.^{23–26} Constitutively produced in the endothelium by endothelial nitric oxide synthase (eNOS), NO diffuses into the vascular wall to relax the vascular smooth muscle. Animal and human studies unequivocally demonstrate that reduced steady-state NO levels substantially increase blood pressure.^{27–30} Lead could increase blood pressure either through decreasing production of NO and/or increasing NO inactivation.

Polymorphisms in the gene for eNOS have been suggested to be susceptibility factors for hypertension, with a G⁸⁹⁴-T⁸⁹⁴ nucleotide substitution polymorphism in exon 7 receiving much recent attention. The T⁸⁹⁴ allele, the minor allele, is prevalent at 10% to 40% (depending on the population sampled)³¹; studies in

Asian populations, mainly from Japan, have reported T⁸⁹⁴ allele prevalences of 5% to 9%.^{31,33,37,39} Some studies indicate that the T⁸⁹⁴ allele is associated with increased blood pressure and decreased production of NO; other studies provide conflicting evidence.^{32–53}

In the current study, we describe the association of the T⁸⁹⁴ allele with blood pressure and evaluate whether the allele modifies the association of lead with blood pressure in a cohort of 803 lead-exposed workers from Korea. The associations of lead with systolic and diastolic blood pressure and hypertension have been previously reported in this population.⁵⁴ Many mechanisms have been proposed for how lead could increase blood pressure; it is important to evaluate gene–environment interaction, like we have here, because if eNOS genotype does not modify the relation of lead dose with blood pressure, this provides evidence that lead is unlikely to act through the eNOS gene product to influence blood pressure. Such studies thus have relevance for understanding the mechanism of lead toxicity.

Materials and Methods

Parent Study

The study population and design have been previously described.^{54–57} The study was reviewed and approved by Institutional Review Boards at The Johns Hopkins Bloomberg School of Public Health and Soonchunhyang University School of Medicine. In brief, individuals from this cohort were recruited as part of a 3-year longitudinal study of lead-exposed workers and nonexposed control subjects without occupational lead exposure. Enrollment for the longitudinal study began in October 1997 and ended in August 1999. A total of 803 lead-exposed workers were recruited. Participation in the study was voluntary and subjects were paid approximately \$30 US for their time and effort. Participants in this study pro-

vided written informed consent. Participation rates by plant generally exceeded 80%. No medical exclusionary criteria (eg, blood pressure, renal disease) were applied. Government-mandated industrial hygiene surveillance indicated that the lead-exposed workers were not exposed to significant levels of other heavy metals such as cadmium, which affect health.

Several assessments were made for individuals in this cohort, including: 1) a standardized questionnaire for information such as age, gender, education, smoking history, alcohol consumption, health history, and occupational history; 2) measurement of blood pressure, height, and weight; 3) measurement of tibia lead in micrograms of lead per gram of bone mineral by ¹⁰⁹Cd-based K-shell x-ray fluorescence (XRF); and 4) measurement of whole blood lead (μg/dL) with a Zeeman background-corrected atomic absorption spectrophotometer (Hitachi Z-8100 model). Details of the data collection procedures have been previously published.^{54–57} Blood pressure, systolic and fifth Korotkoff diastolic, was measured using a Hawksley random zero sphygmomanometer using The Johns Hopkins Welch Center for Prevention, Epidemiology, and Clinical Trials protocol. Three measurements, using an appropriately sized cuff, were taken by a physician trained in the method 5 minutes apart with the subject sitting.

Genotyping Assays

Genomic DNA for the assay was isolated from whole blood samples provided by the study participants, as previously described.⁵⁴ A polymerase chain reaction (PCR)-based assay was adapted from Hibi et al. to genotype individuals for the polymorphism.⁵⁸ The primer sequences were 5'-TCCCTGAGGAGGGCATGAGGC T-3' and 5'-TGAGGGT-CACACAGGTTCT-3'. The PCR resulted in a 457-bp product. *Ban* II restriction enzyme digest was used to distinguish genotypes. Digest with

Ban II cleaved the G⁸⁹⁴ variant PCR product into 2 fragments (of size 137 bp and 320 bp). The T⁸⁹⁴ variant PCR product did not have a *Ban* II restriction site. These fragments were resolved on a 1.5% agarose gel and stained with ethidium bromide. Individuals could be homozygous GG (in which case a 137-bp band and a 320-bp band appeared on the gel), homozygous TT (only a single 457-bp band appeared on the gel), or heterozygous TG (all 3 bands appeared on the gel). In 803 samples, we were able to successfully genotype 793 individuals. Control samples were sequenced to verify genotypes. All TG heterozygotes and TT homozygotes were repeated to confirm genotype. Samples were evaluated blinded to blood pressure or lead levels.

Statistical Analysis

The purpose of the statistical analysis was to model the association of the T⁸⁹⁴ allele with blood pressure and to evaluate whether the allele (TG heterozygotes or TT homozygotes) modified associations of lead dose with blood pressure. Linear regression was used to model systolic blood pressure and diastolic blood pressure. Logistic regression was used to model hypertension, which was defined by a systolic blood pressure ≥140 mm Hg, a diastolic blood pressure ≥90 mm Hg, or reported use of medication for hypertension.

The following covariates were included in the models: age, gender, body mass index (BMI; kg/m²), smoking, alcohol consumption, high school education, tibia lead, blood lead, and job duration. Because lead has a clearance half-time of approximately 30 days in blood, blood lead is more reflective of recent exposures, although bone lead stores can also contribute to blood levels. In contrast, lead has a clearance half-time of approximately 25 to 30 years in tibia, so tibia lead is a measure of retained cumulative dose. All covariates were retained in the final model. In the regression models, smoking

was represented as lifetime non-smoking, quit smoking, smoking ≥ 1 pack/day, and smoking < 1 pack/day. Alcohol consumption was represented as no current consumption, < 1 drink/day, or ≥ 1 drink/day. In models of systolic blood pressure, age was represented as a spline, with a change point at 45 years, because of the nonlinear relation with age.

Tibia lead and blood lead were represented by percentiles (rank/total N) in the regression models to allow direct comparability of the 2 biomarkers of lead exposure. This approach mitigated concerns about influential points, resulting from very high blood lead or tibia lead values. Only points with missing values were excluded from the regression analyses. Regression coefficients (\pm standard error) are expressed as mm Hg/decile increase in lead. The interaction of lead and genotype in models of blood pressure was evaluated as the product of main effects (lead * genotype). To allow for the nonlinear association of lead and blood pressure, second-order interaction terms were evaluated (lead² * genotype). The multiple partial F test was used to evaluate the combination of the first- and second-order interaction terms.

Genotype-stratified loess lines were generated to graphically compare plots of the adjusted blood pressure versus adjusted lead associations by genotype. Adjusted values were obtained from regression models for blood pressure and lead, adding the mean value of covariates to the regression residuals to obtain adjusted blood pressure and adjusted lead. All analyses were conducted using SAS release 6.12 (SAS Institute, Inc., Cary, NC).

Results

The genotype distributions were 85% (673 of 793) GG, 14% (114 of 793) TG, and 1% (6 of 793) TT. The overall frequency of the T⁸⁹⁴ allele was 8% (126 of 1586). The distributions of age, gender, body mass index, smoking, alcohol consumption,

education, tibia lead, blood lead, and duration of employment were similar in individuals with and without the T⁸⁹⁴ allele (Table 1).

Individuals with the T⁸⁹⁴ allele had similar mean (\pm standard deviation) systolic blood pressure (-0.77 ± 1.62 mm Hg, $P = 0.6$), mean diastolic blood pressure (-0.01 ± 1.19 mm Hg, $P = 0.9$), and odds of hypertension (OR = 0.82; 95% CI = 0.50–1.37; $P = 0.4$) compared with those without the variant allele. Adjustment for age, gender, BMI, smoking, alcohol consumption, high school education, tibia lead, blood lead, and job duration did not substantially alter the parameter estimates (Table 2).

The associations of tibia lead and systolic blood pressure were similar in individuals with and without the T⁸⁹⁴ allele (Fig. 1; Table 3). Tibia lead was associated with an adjusted 0.63 ± 0.21 mm Hg/decile increase in systolic blood pressure ($P = 0.003$) in GG homozygotes and an

adjusted 0.48 ± 0.51 mm Hg/decile increase in systolic blood pressure ($P = 0.3$) in TG/TT individuals. The interaction of genotype and tibia lead (modeled as tibia lead percentile as a continuous variable in the regression) was not statistically significant ($P = 0.8$ for the product of the main effects, genotype * tibia lead). Higher-order interaction terms (eg, genotype * tibia lead-squared) were not statistically significant ($P = 0.3$ in a model with a first-order interaction term). First-order and second-order interaction terms of genotype and tibia lead did not significantly improve the fit of the model when evaluated together ($P = 0.8$)

The association of blood lead and systolic blood pressure was similar in individuals with and without the T⁸⁹⁴ allele (Fig. 2; Table 3). Blood lead was associated with an adjusted 0.43 ± 0.23 mm Hg/decile increase in systolic blood pressure ($P = 0.06$) in GG homozygotes and an adjusted 0.40 ± 0.49 mm Hg/decile increase

TABLE 1
Characteristics of Study Subjects by Genotype

Characteristic	eNOS Genotype		P Value
	GG (mean \pm SD*)	TG/TT (mean \pm SD)	
Age (years)	40 \pm 10	41 \pm 10	0.7
Body mass index (kg/m ²)	23 \pm 3	23 \pm 3	0.2
Blood lead (μ g/dL)	32 \pm 15	32 \pm 15	0.9
Tibia lead (μ g/g)	37 \pm 42	36 \pm 34	0.9
Duration of employment (years)	8.2 \pm 6.6	8.7 \pm 6.5	0.4
Systolic blood pressure (mm Hg)	123 \pm 16	123 \pm 15	0.6
Diastolic blood pressure (mm Hg)	76 \pm 12	76 \pm 11	0.9
Creatinine clearance (mL/min)	113 \pm 33	118 \pm 37	0.2
Blood urea nitrogen (mg/dL)	14.3 \pm 3.6	14.9 \pm 3.7	0.11
	Percent	Percent	
Male	80	78	0.7
Education, 12 years	50	51	0.9
Hypertension	21	18	0.4
Smoking			0.9
Current, 1 pack/day	29	30	
Current, < 1 pack/day	28	29	
Former	11	11	
Never	32	30	
Alcohol use			0.8
Current, > 1 drink/day	31	32	
Current, 1 drink/day	33	38	
None	36	30	

SD, standard deviation.

TABLE 2

Adjusted Differences in Mean Systolic and Diastolic Blood Pressure and Odds Ratio for Hypertension by eNOS Genotype (TG/TT relative to GG)*

Effects in Model	Relative Effect (TG/TT relative to GG)	P Value	Model R ²
Systolic blood pressure			
Unadjusted	-0.77 ± 1.62 mm Hg	0.6	<0.00
Adjusted	-0.75 ± 1.48 mm Hg	0.6	0.19
Diastolic blood pressure			
Unadjusted	-0.01 ± 1.19 mm Hg	0.9	<0.00
Adjusted	0.22 ± 1.06 mm Hg	0.8	0.24
Hypertension			
Unadjusted	0.82 (95% CI, 0.50–1.37)	0.4	N/A
Fully adjusted	0.81 (95% CI, 0.47–1.41)	0.5	N/A

* Full model included terms for age, gender, body mass index (ln-transformed), reported smoking (nonsmoking, = 1 pack/day, >1 pack/day, former smoking), current reported alcohol consumption (none = 1 drink/day, >1 drink/day), education (high school-educated), tibia lead, blood lead, and job duration.

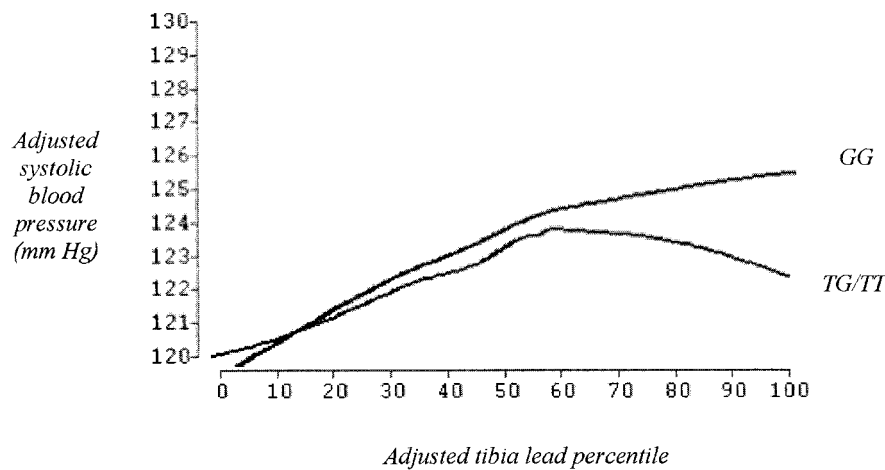


Fig. 1. Adjusted systolic blood pressure versus adjusted tibia lead percentile – loess lines by genotype.

TABLE 3

Linear Regression Models of Systolic Blood Pressure and Lead (tibia lead and blood lead divided into groups by indicated percentiles) Evaluating Effect Modification by eNOS genotype*†

Model	eNOS Genotype	
	GG	TG/TT
Tibia lead	0.63 ± 0.21 mm Hg/decile <i>P</i> = 0.003	0.48 ± 0.51 mm Hg/decile <i>P</i> = 0.3
Blood lead	0.43 ± 0.23 mm Hg/decile <i>P</i> = 0.06	0.40 ± 0.49 mm Hg/decile <i>P</i> = 0.4

* Model included terms for age, gender, body mass index (ln-transformed), reported smoking (nonsmoking, = 1 pack/day, >1 pack/day, former smoking), current reported alcohol consumption (none = 1 drink/day, >1 drink/day), education (high school-educated), and job duration.

† Regression coefficients generated from one model with interaction terms with genotype and lead.

in systolic blood pressure (*P* = 0.4) in TG/TT individuals. The interaction of genotype and blood lead (rep-

resenting blood lead percentile as a continuous variable in the regression) was not statistically significant

(*P* = 0.9 for the product of main effects, genotype * blood lead). Higher-order interaction terms (eg, genotype * blood lead-squared) were not statistically significant (*P* = 0.9 in a model with a first-order interaction term). First-order and second-order interaction terms of genotype and blood lead did not significantly improve the fit of the model when evaluated together (*P* = 0.9).

Tibia lead and blood lead were not associated with diastolic blood pressure in individuals with or without the T⁸⁹⁴ allele (data not shown).

Discussion

The T⁸⁹⁴ Allele and Blood Pressure

Overall, the T⁸⁹⁴ allele was not associated with systolic blood pressure, diastolic blood pressure, or odds of hypertension. The differences in mean systolic and diastolic blood pressure between the groups were small. Taking GG homozygotes as a reference, the difference in mean systolic blood pressure was -0.77 ± 1.62 mm Hg and the difference in mean diastolic blood pressure the difference was -0.01 ± 1.19 mm Hg. The prevalence of hypertension between the two groups was similar (OR = 0.82; 95% CI = 0.50–1.37 taking TG/TT relative to GG).

No clear consensus on the association of hypertension and the G⁸⁹⁴-T⁸⁹⁴ polymorphism emerges from the literature. Some groups report that the T⁸⁹⁴ allele is more frequent in individuals with hypertension; other groups report conflicting findings.^{32–41,59,60} The current study is consistent with reports indicating no association of the T⁸⁹⁴ allele with hypertension.

The reasons for the different results obtained across studies are unclear, but we offer the following possible explanations: 1) The G⁸⁹⁴-T⁸⁹⁴ locus is a marker for functional polymorphisms at other genetic loci. In some populations, the T⁸⁹⁴ allele could be in linkage disequilibrium with a genetic polymorphism that

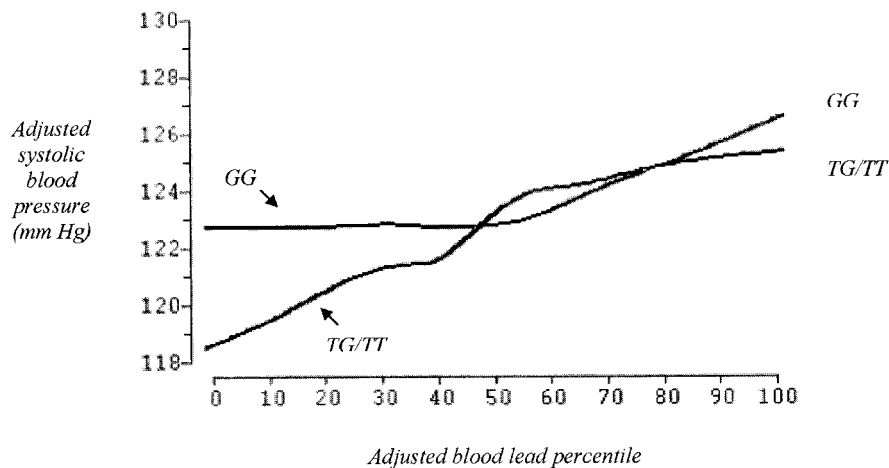


Fig. 2. Adjusted systolic blood pressure versus adjusted blood lead percentile – loess lines by genotype.

does have an effect on blood pressure, whereas in other populations, no linkage may be present. Allelic association within polymorphic loci of eNOS has been documented in several reports.^{31,39,61–63} 2) Diverse genetic, dietary, and environmental factors could substantially modify the association of the polymorphism and hypertension. In some populations, these factors could be present, and in other populations, they could be absent. 3) In some populations, decreased NO production might not be a factor in the development of hypertension; responsiveness to existing levels of NO could be decreased, NO inactivation could be increased, or processes totally unrelated to the NO system could increase blood pressure. Measures of NO production have been reported to be decreased, unchanged, and even increased in hypertension.^{64–75} If decreased NO production was not involved in the development of hypertension, then polymorphisms in the gene for eNOS would not be expected to be risk factors for hypertension.

The T⁸⁹⁴ Allele and the Lead–Blood Pressure Association

There was no evidence that the T⁸⁹⁴ allele modified associations of lead with blood pressure. A comparison of loess lines indicates a similar

association of adjusted systolic blood pressure and adjusted tibia lead in individuals with and without the T⁸⁹⁴ allele. Tibia lead was not associated with diastolic blood pressure in the cohort overall, or in individuals with or without the allele.

Individuals with the T⁸⁹⁴ allele were not more sensitive to the effects of blood lead on systolic blood pressure either. As a comparison of loess lines indicates, above the 50th percentile of blood lead, individuals with the T⁸⁹⁴ allele had a similar association of blood lead and systolic blood pressure as GG homozygotes. Below the 50th percentile blood lead, adjusted systolic blood pressure was not associated with adjusted blood lead in the cohort as a whole. The loess lines for GG homozygotes and TG/TT individuals do diverge somewhat below the 50th percentile of blood lead. The relatively small number of TG/TT individuals in this range makes it difficult to meaningfully interpret this finding.

In conclusion, in this study, no evidence for associations of the T⁸⁹⁴ allele with systolic blood pressure, diastolic blood pressure, or hypertension was observed. There also was no evidence for effect modification by the allele of the relation between lead and blood pressure. Given the uncertainties about the role of this polymorphism in NO production,

and the effect of lead on the NO system, the negative findings of this study do not rule out an effect of lead on NO production or a role for decreased NO production in hypertension.

Acknowledgments

This research was supported by grants 1-F-30 ES05922–01 (Dr Lustberg) and R01-ES07198 (Dr Schwartz) from the U.S. National Institute of Environmental Health Sciences (NIEHS), and grant HMP-97-M-4–0047 from the Ministry of Health and Welfare, Republic of Korea.

References

1. Lee WH, et al. Genetic factors associated with endothelial dysfunction affect the early onset of coronary artery disease in Korean males. *Vasc Med*. 2001;6:103–108.
2. Kirkby H, Gyntelberg F. Blood pressure and other cardiovascular risk factors of long-term exposure to lead. *Scand J Work Environ Health*. 1985;11:15–19.
3. de Kort W, et al. Occupational exposure to lead and blood pressure: a study in 105 workers. *Am J Ind Med*. 1987;11:145–156.
4. Parkinson DK, et al. Occupational lead exposure and blood pressure. *Br J Ind Med*. 1987;44:744–748.
5. dos Santos AC, et al. Occupational exposure to lead, kidney function tests, and blood pressure. *Am J Ind Med*. 1994;26:635–643.
6. Hertz-Picciotto I, Croft J. Review of the relation between blood lead and blood pressure. *Epidemiol Rev*. 1993;15:352–373.
7. Schwartz J. Lead, blood pressure, and cardiovascular disease in men. *Arch Environ Health*. 1995;50:31–37.
8. Schwartz J. The relationship between blood lead and blood pressure in the NHANES II survey. *Environ Health Perspect*. 1988;78:15–22.
9. Hu H, et al. The relationship of bone and blood lead to hypertension. The Normative Aging Study. *JAMA*. 1996;275:1171–1176.
10. Victory W. Evidence for effects of chronic lead exposure on blood pressure in experimental animals: an overview. *Environ Health Perspect*. 1988;78:71–76.
11. Gonick HC, et al. Lead-induced hypertension: interplay of nitric oxide and reactive oxygen species. *Hypertension*. 1997;30:1487–1492.
12. Ding Y, Vaziri ND. Calcium channel

- blockade enhances nitric oxide synthase expression by cultured endothelial cells. *Hypertension*. 1998;32:718–723.
13. Ding Y, et al. Lead-induced hypertension. III. Increased hydroxyl radical production. *Am J Hypertens*. 2001;14:169–173.
 14. Vaziri ND, Liang K, Ding Y. Increased nitric oxide inactivation by reactive oxygen species in lead-induced hypertension. *Kidney Int*. 1999;56:1492–1498.
 15. Vaziri ND, Ding Y, Ni Z. Nitric oxide synthase expression in the course of lead-induced hypertension. *Hypertension*. 1999;34:558–562.
 16. Vaziri ND, Ding Y, Ni Z. Compensatory up-regulation of nitric-oxide synthase isoforms in lead-induced hypertension; reversal by a superoxide dismutase-mimetic drug. *J Pharmacol Exp Ther*. 2001;298:679–685.
 17. Quinn MR, Harris CL. Lead inhibits Ca(2+)-stimulated nitric oxide synthase activity from rat cerebellum. *Neurosci Lett*. 1995;196:65–68.
 18. Khalil-Manesh F, et al. Lead-induced hypertension: possible role of endothelial factors. *Am J Hypertens*. 1993;6:723–729.
 19. Blazka ME, Harry GJ, Luster MI. Effect of lead acetate on nitrite production by murine brain endothelial cell cultures. *Toxicol Appl Pharmacol*. 1994;126:191–194.
 20. Vaziri ND, et al. Altered nitric oxide metabolism and increased oxygen free radical activity in lead-induced hypertension: effect of lazaroid therapy. *Kidney Int*. 1997;52:1042–1046.
 21. Vaziri ND, Ding Y. Effect of lead on nitric oxide synthase expression in coronary endothelial cells: role of superoxide. *Hypertension*. 2001;37:223–226.
 22. Ding Y, Gonick HC, Vaziri ND. Lead promotes hydroxyl radical generation and lipid peroxidation in cultured aortic endothelial cells. *Am J Hypertens*. 2000;13:552–555.
 23. Moncada S, Higgs A. The L-arginine-nitric oxide pathway. *N Engl J Med*. 1993;329:2002–2012.
 24. Palmer RM, Ashton DS, Moncada S. Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature*. 1988;333:664–666.
 25. Palmer RM, Ferrige AG, Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature*. 1987;327:524–526.
 26. Ignarro LJ, et al. Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. *Proc Natl Acad Sci USA*. 1987;84:9265–9269.
 27. Sander M, Chavoshan B, Victor RG. A large blood pressure-raising effect of nitric oxide synthase inhibition in humans. *Hypertension*. 1999;33:937–942.
 28. Haynes WG, et al. Inhibition of nitric oxide synthesis increases blood pressure in healthy humans. *J Hypertens*. 1993;11:1375–1380.
 29. Huang PL, et al. Hypertension in mice lacking the gene for endothelial nitric oxide synthase. *Nature*. 1995;377:239–242.
 30. Shesely EG, et al. Elevated blood pressures in mice lacking endothelial nitric oxide synthase. *Proc Natl Acad Sci USA*. 1996;93:13176–13181.
 31. Tanus-Santos JE, Desai M, Flockhart DA. Effects of ethnicity on the distribution of clinically relevant endothelial nitric oxide variants. *Pharmacogenetics*. 2001;11:719–725.
 32. Hingorani AD, et al. A common variant of the endothelial nitric oxide synthase (Glu298→Asp) is a major risk factor for coronary artery disease in the UK. *Circulation*. 1999;100:1515–1520.
 33. Miyamoto Y, et al. Endothelial nitric oxide synthase gene is positively associated with essential hypertension. *Hypertension*. 1998;32:3–8.
 34. Lacolley P, et al. Nitric oxide synthase gene polymorphisms, blood pressure and aortic stiffness in normotensive and hypertensive subjects. *J Hypertens*. 1998;16:31–35.
 35. Kato N, et al. Lack of evidence for association between the endothelial nitric oxide synthase gene and hypertension. *Hypertension*. 1999;33:933–936.
 36. Benjafield AV, Morris BJ. Association analyses of endothelial nitric oxide synthase gene polymorphisms in essential hypertension. *Am J Hypertens*. 2000;13:994–998.
 37. Shoji M, et al. Positive association of endothelial nitric oxide synthase gene polymorphism with hypertension in northern Japan. *Life Sci*. 2000;66:2557–2562.
 38. Jachymova M, et al. Association of the Glu298Asp polymorphism in the endothelial nitric oxide synthase gene with essential hypertension resistant to conventional therapy. *Biochem Biophys Res Commun*. 2001;284:426–430.
 39. Tsujita Y, et al. Association analyses between genetic polymorphisms of endothelial nitric oxide synthase gene and hypertension in Japanese: the Suita Study. *J Hypertens*. 2001;19:1941–1948.
 40. Chen W, et al. Combined effects of endothelial nitric oxide synthase gene polymorphism (G894T) and insulin resistance status on blood pressure and familial risk of hypertension in young adults: the Bogalusa Heart Study. *Am J Hypertens*. 2001;14:1046–1052.
 41. Karvonen J, et al. Endothelial nitric oxide synthase gene Glu298Asp polymorphism and blood pressure, left ventricular mass and carotid artery atherosclerosis in a population-based cohort. *J Intern Med*. 2002;251:102–110.
 42. Sofowora G, et al. In-vivo effects of Glu298Asp endothelial nitric oxide synthase polymorphism. *Pharmacogenetics*. 2001;11:809–814.
 43. Yoon Y, et al. Plasma nitric oxide concentrations and nitric oxide synthase gene polymorphisms in coronary artery disease. *Clin Chem*. 2000;46:1626–1630.
 44. Jeerooburkhan N, et al. Genetic and environmental determinants of plasma nitrogen oxides and risk of ischemic heart disease. *Hypertension*. 2001;38:1054–1061.
 45. Fairchild TA, et al. Acidic hydrolysis as a mechanism for the cleavage of the Glu(298)→Asp variant of human endothelial nitric-oxide synthase. *J Biol Chem*. 2001;276:26674–26679.
 46. Tesauro M, et al. Intracellular processing of endothelial nitric oxide synthase isoforms associated with differences in severity of cardiopulmonary diseases: cleavage of proteins with aspartate vs. glutamate at position 298. *Proc Natl Acad Sci U S A*. 2000;97:2832–2835.
 47. Persu A, et al. Modifier effect of ENOS in autosomal dominant polycystic kidney disease. *Hum Mol Genet*. 2002;11:229–241.
 48. Savvidou MD, et al. Endothelial nitric oxide synthase gene polymorphism and maternal vascular adaptation to pregnancy. *Hypertension*. 2001;38:1289–1293.
 49. Sorensen KE, et al. Non-invasive measurement of human endothelium dependent arterial responses: accuracy and reproducibility. *Br Heart J*. 1995;74:247–253.
 50. Guzik TJ, et al. Relationship between the G894T polymorphism (Glu298Asp variant) in endothelial nitric oxide synthase and nitric oxide-mediated endothelial function in human atherosclerosis. *Am J Med Genet*. 2001;100:130–137.
 51. Grossmann M, et al. Heterogeneity in hand veins responses to acetylcholine is not associated with polymorphisms in the G-protein beta3-subunit (C825T) and endothelial nitric oxide synthase (G894T) genes but with serum low density lipoprotein cholesterol. *Pharmacogenetics*. 2001;11:307–316.
 52. Philip I, et al. G894T polymorphism in the endothelial nitric oxide synthase gene

- is associated with an enhanced vascular responsiveness to phenylephrine. *Circulation*. 1999;99:3096–3098.
53. Schneider MP, et al. Functional gene testing of the Glu298Asp polymorphism of the endothelial NO synthase. *J Hypertens*. 2000;18:1767–1773.
 54. Lee BK, et al. Associations of blood pressure and hypertension with lead dose measures and polymorphisms in the vitamin D receptor and delta-aminolevulinic acid dehydratase genes. *Environ Health Perspect*. 2001;109:383–389.
 55. Schwartz BS, et al. Associations of blood lead, dimercaptosuccinic acid-chelatable lead, and tibia lead with polymorphisms in the vitamin D receptor and [delta]-aminolevulinic acid dehydratase genes. *Environ Health Perspect*. 2000;108:949–954.
 56. Schwartz BS, et al. Associations of blood lead, dimercaptosuccinic acid-chelatable lead, and tibia lead with neurobehavioral test scores in South Korean lead workers. *Am J Epidemiol*. 2001;153:453–464.
 57. Todd AC, et al. Predictors of DMSA chelatable lead, tibial lead, and blood lead in 802 Korean lead workers. *Occup Environ Med*. 2001;58:73–80.
 58. Hibi K, et al. Endothelial nitric oxide synthase gene polymorphism and acute myocardial infarction. *Hypertension*. 1998;32:521–526.
 59. Yoshimura T, et al. Association of the missense Glu298Asp variant of the endothelial nitric oxide synthase gene with severe preeclampsia. *J Soc Gynecol Invest*. 2000;7:238–241.
 60. Pulkkinen A, et al. Intron 4 polymorphism of the endothelial nitric oxide synthase gene is associated with elevated blood pressure in type 2 diabetic patients with coronary heart disease. *J Mol Med*. 2000;78:372–379.
 61. Yoshimura M, et al. Genetic risk factors for coronary artery spasm: significance of endothelial nitric oxide synthase gene T-786->C and missense Glu298Asp variants. *J Invest Med*. 2000;48:367–374.
 62. Poirier O, et al. Polymorphisms of the endothelial nitric oxide synthase gene—no consistent association with myocardial infarction in the ECTIM study. *Eur J Clin Invest*. 1999;29:284–290.
 63. Nakayama M, et al. T-786->C mutation in the 5'-flanking region of the endothelial nitric oxide synthase gene is associated with coronary spasm. *Circulation*. 1999;99:2864–2870.
 64. Forte P, et al. Basal nitric oxide synthesis in essential hypertension. *Lancet*. 1997;349:837–842.
 65. Sagnella GA, et al. Plasma and urinary nitrate in essential hypertension. *J Hum Hypertens*. 1997;11:587–588.
 66. Surdacki A, et al. Reduced urinary excretion of nitric oxide metabolites and increased plasma levels of asymmetric dimethylarginine in men with essential hypertension. *J Cardiovasc Pharmacol*. 1999;33:652–658.
 67. Bode-Boger SM, et al., Role of endogenous nitric oxide in circadian blood pressure regulation in healthy humans and in patients with hypertension or atherosclerosis. *J Invest Med*. 2000;48:125–132.
 68. Higashi Y, et al. Effect of L-arginine infusion on systemic and renal hemodynamics in hypertensive patients. *Am J Hypertens*. 1999;12:8–15.
 69. Node K, et al. Reduced plasma concentrations of nitrogen oxide in individuals with essential hypertension. *Hypertension*. 1997;30:405–408.
 70. Campese VM, et al. Salt intake and plasma atrial natriuretic peptide and nitric oxide in hypertension. *Hypertension*. 1996;28:335–340.
 71. Fujiwara N, et al. Study on the relationship between plasma nitrite and nitrate level and salt sensitivity in human hypertension : modulation of nitric oxide synthesis by salt intake. *Circulation*. 2000;101:856–861.
 72. Sumino H, et al. Effect of enalapril on exhaled nitric oxide in normotensive and hypertensive subjects. *Hypertension*. 2000;36:934–940.
 73. Stuhlinger MC, et al. Relationship between insulin resistance and an endogenous nitric oxide synthase inhibitor. *JAMA*. 2002;287:1420–1426.
 74. Mehta JL, et al. Alterations in nitric oxide synthase activity, superoxide anion generation, and platelet aggregation in systemic hypertension, and effects of celiprolol. *Am J Cardiol*. 1994;74:901–905.
 75. Camilletti A, et al. Decreased nitric oxide levels and increased calcium content in platelets of hypertensive patients. *Am J Hypertens*. 2001;14:382–386.