Environmental Cardiology
Studying Mechanistic Links Between Pollution and Heart Disease
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Abstract—Environmental factors are considered key determinants of cardiovascular disease. Although lifestyle choices such as smoking, diet, and exercise are viewed as major environmental influences, the contribution of pollutants and environmental chemicals is less clear. Accumulating evidence suggests that exposure to pollutants and chemicals could elevate the risk of cardiovascular disease. Many epidemiological studies report that exposure to fine particles present in ambient air is associated with an increase in cardiovascular mortality. Statistically significant relationships between particulate air pollution and ischemic heart disease, arrhythmias, and heart failure have been reported. Animal studies show that exposure to ambient air particles increases peripheral thrombosis and atherosclerotic lesion formation. Exposures to arsenic, lead, cadmium, pollutant gases, solvents, and pesticides have also been linked to increased incidence of cardiovascular disease. Mechanistically, these effects have been attributed to changes in the synthesis or reactivity of nitric oxide that may be caused by environmental oxidants or increased endogenous production of reactive oxygen species. Additional studies are urgently needed to: identify the contribution of individual pollutants to specific aspects of cardiovascular disease; establish causality; elucidate the underlying physiological and molecular mechanisms; estimate the relative susceptibility of diseased and healthy individuals and that of specific population groups; and determine whether pollutant exposure are risk correlates, that is, whether they influence major risk factors, such as hypertension, cholesterol, or diabetes, or whether they contribute to the absolute risk of heart disease. Collectively, these investigations could contribute to the emergent field of environmental cardiology. (Circ Res. 2006;99:692-705.)

Key Words: particulate matter ■ arsenic ■ cadmium ■ carbon monoxide ■ lead ■ ozone ■ cigarette smoke ■ aldehydes ■ atherosclerosis ■ ischemic heart disease ■ stroke

Heart disease develops as a result of complex interactions between genes and environment. In the last 50 years, we have made impressive progress in understanding its varied causes and manifestations. Multiple pharmaceutical and surgical approaches have been devised to prevent, treat, or otherwise manage heart disease, yet it remains the leading cause of death both in United States and Europe. Persistent prevalence of cardiovascular disease (CVD) and its high morbidity and mortality suggest that there are significant gaps in our understanding of the mechanisms that impair cardiovascular health and that better treatment strategies are needed to manage CVD and its clinical manifestations. Because prevention is likely to provide the most effective gains against highly unpredictable events such as acute myocardial infarction (MI), stroke or arrhythmia, we need to identify more clearly the preventable and the modifiable causes of heart disease.

Causes of heart disease are best understood in epidemiological terms. The seminal Framingham Heart Study framed determinants of heart disease as “risk factors” that can quantitatively predict cardiovascular disease.1,2 These risk factors account for a major portion of CVD risk,3,4 but, in most cases, the cellular and the molecular mechanisms underlying these associations are incompletely understood. Nonetheless, the realization that some risk factors such as smoking are modifiable fosters the perception that the environment significantly influences cardiovascular health. This view is further reinforced by studies showing that CVD rates differ 5- to 100-fold among population groups of similar genetic background. These rates change quickly within the same ethnic group, and they increase when populations migrate from low to high-risk environments.5,6 Despite these studies, our understanding of environmental influences has been limited to lifestyle choices such as diet, smoking, and exercise, and it is only in the last few years that disparate lines of evidences have congealed into a coherent idea that environmental exposure to pollutants and chemicals contribute to CVD risk.7,8

In hindsight, the realization that pollutants affect cardiovascular health seems obvious. It has been known for many years that smoking is among the strongest independent predictors of premature heart disease, and, even though secondhand smoke (SHS) has only recently been classified as a pollutant, extant data show that 70% to 80% of deaths
attributable to SHS are caused by heart disease (<5% resulting from lung cancer), suggesting that cardiovascular tissues, more than any other, are extremely sensitive to environmental chemicals and pollutants. The nonpareil cardiovascular burden of smoking, however, and the extraordinary strength of the evidence linking smoking to heart disease, as well as the forgone nature of the conclusion that smoking is bad, have discouraged wide-spread attempts to understand the molecular mechanisms by which smoking causes heart disease or to link specific constituents of tobacco smoke to individual manifestations of cardiovascular toxicity. Nevertheless, this seems to be particularly important now because recently emerged epidemiological evidence suggests that several constituents of cigarette smoke, if present in ambient air, could affect CVD incidence, progression, and severity. These findings compel us to reexamine not only the cardiovascular effects of smoking but also the very nature of heart disease and the mechanisms by which it is triggered, sustained, and amplified.

**Ambient Air Particles**

Ambient air contains an aerosolized mixture of particles suspended in gas. Mass analysis of ambient aerosols reveals a bimodal distribution. In typical urban aerosols, coarse particles display peak distribution approximately 10 to 20 μm, and the fine particle size varies from 0.1 to 1.0 μm with a saddle point between 1 to 3 μm. Within the fine category, a small volume fraction consists of the ultrafine or nuclei mode particles. Despite its modest contribution to overall volume, the ultrafine fraction represents the largest number of particles and, therefore, presents the largest surface area. The fine-particle mode contains from one-third to two-thirds of total PM mass. Properties and sources of size-defined particles are listed in the Table. Additional particles in the environment may arise from the manufacture and use of nanomaterials.

Coarse and fine particles originate from different sources. They have different chemical and physical properties, and there is little mass exchange between them. Hence, it appears practically efficacious to study them as 2 distinct aerosols. Using this distinction many studies show that an increase in the concentration of fine particles is associated with increased mortality. The association between fine PM and all-cause mortality has been found to be robust and unaffected by the inclusion of individual risk factors and does not appear to be attributable to inadequately controlled regional or other spatial differences. Most data are derived from time-series studies looking at acute increases in mortality within 1 to 2 days of exposure. Other studies have reported that acute PM elevation increases emergency room visits and hospital admissions. Data collected from more than 100 million people in 119 cities in the United States and Europe show that for each 10 μg/m³ increase in PM₁₀ there is a 0.2% to 0.6% increase in all-cause and 0.3% to 0.7% increase in cardiovascular mortality. Within the range of fine PM found in US cities, the concentration response relationship appears to be nearly linear for PM₁₀ and PM₂.₅, suggesting that there is no significant threshold effect and the risk increases monotonically with exposure. In most cities in the United States, the 24-hour PM₁₀ concentration varies from 26 to 534 μg/m³, suggesting that the contribution of PM to daily mortality may be significant and substantial (Figure 1).

In contrast to the large number of time-series reports, only a few studies on the chronic effects of PM have been published. In the landmark Harvard Six Cities Study of more than 8000 subjects followed for 14 to 16 years, Dockery et al reported that the adjusted mortality-rate ratio (RR) for the most polluted cities compared with the least polluted was 1.26. This study presented evidence for the first time that
chronic PM exposure contributes to long-term increases in mortality. Since then, persistent effects of air pollution have been confirmed by several cohort studies in the United States\textsuperscript{16} and Europe.\textsuperscript{19,20} Based on analysis of data collected by the American Cancer Society for 500,000 adults living in several metropolitan areas in the United States, Pope et al\textsuperscript{16} reported a 4\% increased risk of all-cause mortality for each 10 \(\mu g/m^3\) increase in PM. Notably, the effect estimates from prospective studies are significantly greater than those indicated by daily time-series studies, suggesting that the effects of PM are not restricted to only very frail individuals with an increased risk of mortality (“harvesting” hypothesis) but that PM exposure leads to cumulative changes resulting in a decrease in life expectancy in highly polluted areas. That exposure to PM induces cumulative changes that may be causatively linked with mortality is further supported by studies showing that reduction in air pollution leads to short (weeks to months) and long-term (years) decreases in mortality. For instance, a 15 \(\mu g/m^3\) decrease in the PM\textsubscript{10} concentration in Utah Valley (during a 13-month strike at a local steel mill) was found to be associated with a 3.2\% decrease in total mortality.\textsuperscript{21} Similarly, a 35 \(\mu g/m^3\) decrease in mean black smoke concentration attributable to a ban on coal sale in the city of Dublin was accompanied by a 5.7\% decrease in nontrauma deaths in 72 months after the ban compared with the same period before the ban.\textsuperscript{22} Additionally, a recent extended follow-up of the Harvard Six Cities Study reports that a city-specific reduction in PM\textsubscript{2.5} was associated with a reduction in mortality rates.\textsuperscript{23} In this study, a 1 \(\mu g/m^3\) reduction in PM\textsubscript{2.5} was associated with a 3\% decrease in overall mortality, suggesting that the effects of air pollution, as those of smoking, are partly reversible. Moreover, because the effect size in short-term exposures or withdrawals is similar to that reported in longer-term studies, it appears that
the effects of air pollution develop and abate rather quickly. These data suggest that the major portion of the observed morbidity/mortality caused by PM exposure in the epidemiological studies can be explained by acute (hours-to-days) biological responses rather than chronic health effects (e.g., sensitizing people by promoting atherosclerosis). The rapid reduction in clinical cardiovascular events (within months to 2 to 3 years) after smoking cessation is in accord with this hypothesis.

The most surprising aspect of the association between mortality and PM is the observation that pollution is positively and selectively associated with deaths from cardiopulmonary diseases. In most studies, statistical associations between air pollution and death are stronger for cardiovascular deaths than for all-cause mortality. Recent analysis of the American Cancer Society data indicates that the largest specific cause of death associated with PM exposure was ischemic heart disease, which accounted for one-quarter of all such deaths. Statistically significant associations were also observed for arrhythmias, heart failure, and cardiac arrest, but no positive correlations were observed with other cardiovascular or respiratory diseases or chronic obstructive pulmonary disease (COPD). The study estimated that for ischemic heart disease, heart failure, and cardiac arrest a 10 μg/m³ increase in fine PM is associated with an 8% to 18% increase in mortality, suggesting that the risk of death from cardiac causes was much higher than from all other causes and significantly greater than from lung disease, which has been traditionally considered to be the major adverse outcome of pollutant exposure.

A recent study looking specifically at PM2.5 also found consistent association between short-term exposure and hospital admissions for CVD. The largest association was for heart failure, which had a 1.28% increase in risk per 10 μg/m³ increase in same-day PM2.5. The risk for admissions for ischemic heart disease, cerebrovascular disease, and heart rhythm abnormalities was also increased. Other studies have shown that transient increases in PM2.5 are associated with an increase in the incidence of acute MI within a few hours and then again 1 day after exposure. Acute PM exposures have also been linked to changes in heart rate variability, increases in the risk of ST-segment depression during stress testing among patients with stable coronary disease, and increases in implantable cardioverter defibrillator discharges, suggesting that particulate pollution could increase the frequency of life-threatening arrhythmias and susceptibility to myocardial ischemia. Given that 80% of sudden cardiac deaths (SCDs) are caused by ischemic events, it is likely that air pollution may be a contributor to SCD risk. Indeed, the National Resources Defense Council estimates that 60,000 cases of SCD (of a total of 350,000) per year in United States could be attributed to particulate air pollution.

Why should exposure to fine air particles increase the risk of dying from heart disease? Although the field is rife with hypotheses, no clear mechanism has emerged. A combination of PM-induced changes in blood pressure, inflammation, autonomic balance, and blood coagulation have been postulated to be the result of the many manifestations of particle toxicity, but the contribution of each of these mechanisms to individual clinical states remains to be assessed. In a laboratory setting, healthy humans respond to concentrated ambient particles with small changes in acute brachial artery vasoconstriction and increased diastolic blood pressure, suggesting that PM exposure acutely affects conduit artery flow perhaps by decreasing basal NO levels or increasing the generation of vasoconstrictors such as endothelin. Exposure to more-reactive particles, such as diesel exhaust, blunts the increase in blood flow in response to bradykinin, acetylcholine, and sodium nitroprusside, indicating that both endothelium-dependent and -independent effects are decreased, potentially because of a combination of smooth muscle changes and a decrease in NO bioavailability. Other investigators have reported that persistent increases in PM are associated with higher resting blood pressure and that high levels of PM persisting more than 1 to 2 days are associated with an increase in blood pressure in patients with preexisting cardiac disease or diabetes. Collectively, these studies support the notion that changes in vasomotor tone may be a mechanism by which PM exposure could alter hemostatic regulation and trigger acute clinical events.

Changes in vasomediators may also be related to the thrombogenic effects of PM exposure and its ability to induce systemic inflammation. It has been reported that PM10 levels are negatively correlated with hemoglobin concentration, packed cell volume, and red cell count, features suggestive of increased red cell adhesion and sequestration in capillaries. Although fibrinogen was negatively correlated with PM10 in one study, later studies have reported a positive association between PM10 and fibrinogen and an increase in blood viscosity. Exposure to diesel exhaust has been shown to decrease bradykinin-induced increase in plasma tissue plasminogen activator (t-PA), indicating impairment in acute endogenous fibrinolytic capacity. A procoagulant effect of PM exposure is also consistent with animal studies showing that intratracheal instillation of diesel exhaust particles results in rapid activation of circulating blood platelets, suggesting that PM exposure increases peripheral thrombosis. Although the concordance between animal and human data validates the phenomenology of PM toxicity, the mechanisms by which PM exposure increases thrombosis remain obscure, and it is unclear whether changes in blood pressure and coagulation are 2 facets of the same response (ie, decrease in NO) or independent effects derived from different sources of injury. In addition, it is also unclear whether acute events translate into chronic injury. In a study reminiscent of findings with smokers, Kunzli et al report a 12% increase in carotid intima/media ratio (IMT) for a PM2.5 concentration-range difference of 20 μg/m³. The increase was particular strong in women >60 years of age. Because IMT is reflective of CVD risk, these data fuel the suspicion that exposure to fine PM accelerates atherogenesis. Hence, excess CVD risk imposed by PM exposure may be distributed between a chronic long-term effect on atherosclerotic lesion formation and acute effects triggering MI, arrhythmias, or SCD.

The idea that exposure to particulate pollution has long-term effects on CVD is supported by animal studies. Two such studies have been reported. In a study involving Wa-
Intratracheal instillation with PM₁₀ twice a week for 4 weeks was found to accelerate the progression of coronary lesions to more advanced phenotypes. Increases in plaque turnover and extracellular lipid pools in coronary and aortic lesions of PM₁₀-instilled rabbits were also observed. In the other study, apolipoprotein E (apoE)-null mice were exposed to concentrated air particles for 6 months at a normalized value of 15.2 μg/m³, which is close to the National Ambient Air Quality Standard and within the concentration range measured in metropolitan areas in the United States. Although conventional measures of lesion formation (plaque formation in the aortic root or in en face preparations of aorta), were not reported, transverse sections of abdominal aorta revealed a 1.58-fold increase in percentage of plaque area in mice maintained on high-fat, but not regular, chow. The atherosclerotic plaques of exposed mice displayed more intense staining with anti-inducible NO synthase (anti-iNOS), anti-nitrotyrosine, and anti-CD68 antibodies, indicating that PM exposure elevates vascular inflammation. This is consistent with the observation that in aortic rings prepared from exposed animals, vasoconstrictor responses to phenylephrine with the observation that in aortic rings prepared from exposed animals, vasoconstrictor responses to phenylephrine were exaggerated, whereas acetylcholine-exposed animals, vasoconstrictor responses to phenylephrine were not reported, transverse sections of abdominal aorta revealed a 1.58-fold increase in percentage of plaque area in mice maintained on high-fat, but not regular, chow. The atherosclerotic plaques of exposed mice displayed more intense staining with anti-inducible NO synthase (anti-iNOS), anti-nitrotyrosine, and anti-CD68 antibodies, indicating that PM exposure elevates vascular inflammation. This is consistent with the observation that in aortic rings prepared from exposed animals, vasoconstrictor responses to phenylephrine were exaggerated, whereas acetylcholine-induced relaxation was attenuated. Whether ambient PM and serotonin were exaggerated, whereas acetylcholine-exposed animals, vasoconstrictor responses to phenylephrine were not reported, transverse sections of abdominal aorta revealed a 1.58-fold increase in percentage of plaque area in mice maintained on high-fat, but not regular, chow. The atherosclerotic plaques of exposed mice displayed more intense staining with anti-inducible NO synthase (anti-iNOS), anti-nitrotyrosine, and anti-CD68 antibodies, indicating that PM exposure elevates vascular inflammation. This is consistent with the observation that in aortic rings prepared from exposed animals, vasoconstrictor responses to phenylephrine were exaggerated, whereas acetylcholine-induced relaxation was attenuated. Whether ambient PM has similar effects on atherogenesis in humans remains unclear and is the subject of ongoing investigations. Nevertheless, data from hyperlipidemic rabbits and apoE-null mice provide empirical support to the notion that persistent PM exposure could affect atherogenesis by chronically increasing inflammation, even though the mechanism, extent, and location of this inflammatory response remain incompletely understood.

Correlation between the volume of atherosclerotic lesions in PM₁₀-instilled rabbits with the number of alveolar macrophages that phagocytosed PM₁₀ prompted van Eeden and colleagues to postulate that the increase in atherogenesis was caused by a systemic inflammatory response triggered by lung cells. Their observations that repeated PM₁₀ instillations increase the number of circulating immature PMN and monocytes and expand the size of the bone marrow turnover of monocytes are consistent with systemic inflammation. Although it remains unclear to what extent stimulation of bone marrow is a reflection of acute pulmonary injury caused by a large PM load aspirated into the lung, the observed increase in monocytopoiesis favors the hypothesis that PM exposure could accelerate atherogenesis by increasing systemic inflammation. Formation of atherosclerotic lesions begins with the adhesion of monocytes to activated endothelium, and hence an increase in the release of monocytes (or PMN) from the bone marrow could fuel atherogenesis, particularly when the process is aided by an increase in cytokine production by macrophages phagocytosing fine particles deposited on the lung surface. In vitro experiments do show that ambient particles induce inflammatory responses and cytokine release in alveolar macrophages and bronchial epithelial cells, but these responses are obtained at high concentration of particles whose chemical composition may have been altered by collection or storage or both. Moreover, the ability of ambient particles to stimulate cytokine production at least in some instances appears to be related to their endotoxin content because it could be attenuated by lipopolysaccharide binding protein, poly-myxin B, or by blocking CD14. Environmental endotoxin is mostly associated with the coarse particle fraction, and PM₂.₅ does not stimulate cytokine production from macrophages. Indeed, exposure of alveolar macrophages to ultrafine PM does not affect their ability to phagocytose opsonized zymogen or to undergo an oxidative burst. From these data, it appears that coarse particles preferentially affect innate host defenses in the lung. Hence, pulmonary inflammation attributable to these particles may be a significant component of respiratory response to high levels of PM₁₀. Smaller particles, on the other hand, because of their ability to penetrate the lungs and appear in systemic circulation, may be able to induce cardiovascular toxicity without increasing pulmonary or systemic inflammation. This is consistent with studies on apoE-null mice that showed no increase in the lipid content in the aortic arch on exposure to PM₂.₅, even though macrophage infiltration and iNOS expression in the lesion were increased. A local vascular effect of PM exposure is also consistent with a recent study showing that treatment of rats with low concentrations of residual oil fly ash (ROFA), which does not cause pulmonary damage or inflammation, impairs systemic microvascular function, suggesting that in healthy rodents, overt pulmonary inflammation is not a prerequisite for PM-induced cardiovascular dysfunction.

In contrast to animal data, human studies suggest that both pulmonary and systemic changes could contribute to the cardiovascular effects of PM and that preexisting disease is a powerful modifier of the overall response. Although acute exposures of healthy adult humans to high levels of PM in forest fires or diesel exhaust can induce pulmonary and systemic inflammation, in general, both ambient air particles (20 to 30 μg/m³) and diesel exhaust (100 to 300 μg/m³) cause only mild pulmonary inflammation, with little or no change in systemic markers of inflammation. The proinflammatory affects of PM are, however, significant in patients with preexisting CVD or those that are diabetic, obese, hypertensive, or elderly, indicating that even though PM could increase inflammation in young, healthy individuals, susceptibility to the inflammatory effects of PM is in part determined by preexisting disease or dysfunction. Thus, PM has the ability to trigger inflammation that could under some conditions spread from the lung to the circulation. What is less clear, however, is whether systemic and pulmonary inflammation are interlinked and whether an increase in systemic inflammation contributes to acute or chronic clinical events observed in healthy or diseased humans exposed to high levels of particulate air pollution.

We need to overcome several conceptual and procedural hurdles to arrive at a better understanding of the recondite nature of particle toxicity and how it contributes to CVD. The most daunting of these is the extraordinary complexity of ambient aerosols. A simple orthogonal gas chromatography/mass spectrometry (GC/MS) analysis of a single sample yields more than 10,000 peaks, not including chemicals that were not adequately ionized or otherwise remained undetected. Moreover, PM composition varies from one geographic locale to another depending on atmospheric conditions and...
local sources (Figure 2). Such variations could be significant, which can confound comparisons of data from different geographic locations. Extensive efforts are underway to clearly characterize ambient aerosols and to identify in particular polyfunctional and polymeric constituents of atmospheric particles. Studies of similar magnitude and complexity are required to elucidate the health effects of these particles, specifically their cardiovascular toxicity. Multidisciplinary investigations spanning cardiology, toxicology, and atmospheric sciences are needed to provide a mechanistic basis for the epidemiological link between ambient aerosols and cardiovascular events and to establish dose-response relations necessary for testing causality. Only by simultaneously studying cardiovascular pathology and PM characteristics will it be possible to relate specific physiochemical features of atmospheric particles to individual cardiovascular events within the context of functional changes that could be rigorously attributable to well-defined molecular mechanisms. For such biological studies, however, it may be necessary to redefine atmospheric particles. Definitions based on size were developed for convenience of measurement and monitoring, but for elucidation of health effects, it may be necessary to develop alternate exposure matrices defining particles in terms of their reactivity, toxicity, and ability to participate in biologically significant redox reactions. Moreover, even if we were to identify and quantify all the chemicals in the aerosol mixture, it is not clear how we would analyze the list or link its individual components to specific health effects. Hence, it may be useful to study particles classified according to their reactive groups or classes of chemicals (eg, metals, carbonyls, poly-aromatic hydrocarbons, benzopyrenes) that could be tested independently as a subset in controlled toxicological studies.

Simpler definitions and measurement techniques may also facilitate better exposure assessments. Most studies to date have used central monitoring sites. This is a major limitation, because a central site may not accurately reflect personal exposure. This is particularly significant in the light of newer findings suggesting that the organic carbons and volatile air toxics in fresh emission may be related more strongly to cardiovascular effects than PM mass. Because particle-associated volatiles such as aldehydes and ketones are highly reactive, they change rapidly and often escape measurement. In addition, their effects may wash out at a distance. That volatile air toxics may be important is suggested by studies showing that cardiopulmonary mortality rates are higher in people living near a major road and that close exposure to traffic is associated with the onset of MI within 1 hour. The link between CVD and exposure to volatile chemicals is further strengthened by studies reporting that CVD risk is increased because of occupational exposure to butadiene, vinyl chloride, or formaldehyde or to chemicals used by embalmers and perfumery workers. Hence it is important to assess the extent to which people are exposed to volatile and nonvolatile pollutants within the microenvironment in which they live and to develop personal...
monitors that adequately and faithfully reflect individual exposures in real time.

Gaseous Copollutants
Changes in PM are part of large atmospheric changes that, in combination, may be more significant than those attributable to individual components. Several atmospheric gases, such as nitrogen oxides, carbon monoxide, sulfur dioxide, and ozone have been linked to cardiovascular toxicity.8 Recent analysis of the link between ambient air pollution and the risk of hospital cardiac readmissions of MI survivors suggests that the strength of associations with same-day CO, O₃, or NO₂ was similar to that for PM₁₀,₈₃ suggesting a significant contribution of gaseous copollutants. In particular, the association between acute MI and short-term ozone exposure (within 1 to 2 days) was found to be particularly strong.₈₄ No associations were observed with NO₂ or SO₂,₈₄ although the rate of hospitalization for congestive heart failure has been related to PM₁₀, CO, NO₂, and SO₂ but not ozone.₈₅ In contrast, changes in heart rate variability in elderly subjects were found to be associated with traffic-related particles and ozone but not SO₂ or NO₂.₈₆ Clearly, individual gases affect cardiovascular tissues differently. Ozone exposure impairs pulmonary gas exchange and increases myocardial work,₈₇ whereas SO₂ exposure reduces cardiac vagal control that could contribute to increased susceptibility to ventricular arrhythmias.₈₈

In contrast, acute cardiovascular effects of CO are mild and, at concentrations present in cigarette smoke, do not acutely affect cardiac function. Carbon monoxide poisoning, however, often incites reversible cardiac injury.₈₉ Interestingly, myocardial injury caused by acute CO poisoning has been reported to be positively associated with long-term mortality,₉₀ but the underlying mechanisms remain unknown. Evidently, additional studies at ambient concentration of these gases are much needed. In addition, more refined epidemiological studies are required to account for selection bias and uncertain comparability between exposed and unexposed participants and to develop standardized outcome definitions adjusted for CVD risk factors. At present, it is unclear whether some of the reported correlations are attributable to residual confounding rather than causation and extensive investigations are required to uncover the mechanistic link between exposures to individual components of air pollution and specific aspects of CVD.

Epidemiological studies, by their very nature, provide only a partial, often tantalizing, view of causative mechanisms. Biological plausibility and causation are best addressed in animal models, and, although some recent studies support the epidemiological link between PM and atherosclerosis, additional work is required to establish how ambient aerosols affect myocardial ischemia and arrhythmias and whether susceptibility-enhancing states (high cholesterol, smoking, and hypertension) also increase PM toxicity. Results of such studies may also suggest mechanisms that determine CVD vulnerability and risk that could be used to design urgently needed epidemiological studies to determine which specific subpopulations may be more sensitive to the effects of PM.

Arsenic, Metals, and CVD
In addition to smoking and PM, other chemical exposures have also been linked with CVD. The most extensive evidence has been obtained with arsenic and metals. Exposure to inorganic arsenic mainly in drinking water has been associated with increased mortality from cardiovascular and cerebrovascular disease,₉¹ hypertension,₉₂ ischemic heart disease,₉₃ and carotid atherosclerosis.₉₄ One of the most significant manifestations of arsenic toxicity is blackfoot disease, which has been attributed to arteriosclerosis obliterans and thromboangiitis obliterans caused by peripheral artery disease (PAD) and leads to spontaneous loss of extremities.₉₁ Although the association is strongest in hyperendemic villages of Southeast Asia,₉₅ a correlation between arsenic exposure and increased mortality from hypertensive and atherosclerotic heart disease has also been reported in the United States.₉₆,₉₇ The mechanisms by which arsenic causes vascular disease are not well known, but animal studies suggest that the chemical propensity of arsenic to oxidize vicinal thiols could potentially affect a number of cellular proteins with reactive thioles including endothelial NO synthase (eNOS).₁₀₁ In cell culture studies, arsenic has been shown to inactivate eNOS and decrease protein levels of Akt and eNOS. In isolated arteries, arsenic suppresses acetylcholine-induced vascular relaxation and increases peroxynitrite formation.₁₀₂ These results correlate with in vivo studies showing that arsenic increases peripheral resistance and that pretreatment with arsenic blunts acetylcholine-induced drop in blood pressure.₁₀₂ Additionally, arsenic has been shown to reduce plasma NOX levels and decrease BH₄ levels, changes that could increase reactive oxygen species (ROS) generation by uncoupling eNOS. Systemically, arsenic-induced disruption of NO signaling has been linked to an increase in thrombosis and accelerated formation of atherosclerotic lesions in apoE/LDL-R–null mice and apoE-null mice. Significantly, in apoE/LDL-R–null mice, arsenic exposure–induced increase in leukotriene E₄ was partially abolished by inhibiting NO synthase, indicating that inflammation was, in part, mediated by dysregulation of NO synthesis and presumably attributable to increased radical generation from uncoupled eNOS or increased expression of iNOS. These results are consistent with data showing a decrease in NOx levels in humans chronically exposed to high levels of arsenic in drinking water.¹₀₈ Notably, neither animal nor human studies show that arsenic affects blood lipids, indicating that low-grade vascular inflammation, often associated with mild dyslipidemia, is less important in arsenic toxicity than vascular changes caused by disturbances in NO production or reactivity.

Similar mechanisms of toxicity have been invoked for other metals as well. Increase in blood pressure in rats has been linked to the ability of the metal to increase ROS generation, leading to NOS inactivation. This could be one mechanism underlying the link between low-level lead exposure and hypertension. Although the link between lead and hypertension has been intensively studied for many years, metaanalysis of more than 30 studies suggests that there is only a weak association between blood pressure and blood lead levels.₁₀₉ A similar weak association has also been
reported for blood lead levels and all-cause circulatory and cardiovascular mortality.\textsuperscript{110} Nonetheless, at levels below the current US occupational exposure limit guidelines, Nash et al\textsuperscript{111} found a positive association between blood lead levels and both systolic and diastolic blood pressures and the risk of hypertension in postmenopausal women, in whom the blood lead levels were suggested to reflect lead liberated from the bone resulting from postmenopausal bone loss.\textsuperscript{111} In a recent community-based cohort of randomly sampled urban adults, blood lead level was associated with systolic and diastolic blood pressure, and tibia lead levels were correlated with hypertension,\textsuperscript{112} suggesting that even though individual risk may be small, population risk attributable to lead exposure may be considerable.

Animal studies support the possibility that lead exposure promotes hypertension and accelerates CVD. Chronic treatment of rats with low levels of lead in the drinking water elevates blood pressure.\textsuperscript{113-117} This increase in blood pressure is associated with an increase in nitrotyrosine formation and a decrease in NOx levels in the urine, with little or no change in the expression of NO synthases,\textsuperscript{116} indicating that chronic lead exposure decreases NO bioavailability in part by increasing ROS production. Indeed treatment with antioxidants such as ascorbate,\textsuperscript{114} vitamin E,\textsuperscript{116} or tempol\textsuperscript{117} decreases lead-mediated hypertension. This increase in ROS generation and changes in cytokine production\textsuperscript{115} could also be related to the ability of lead to increase atherogenesis,\textsuperscript{119} although these mechanisms remain to be fully explored. Nonetheless, data from human smokers suggest that exposure to metals such as lead and cadmium in smoke may be linked to the high prevalence of PAD in smokers.\textsuperscript{120} Cadmium in particular appears to be a significant mediator of smoking-induced PAD because adjustment for cadmium decreased the association of smoking with PAD.\textsuperscript{120} Given the association of arsenic with PAD, it appears that environmental pollutants that either diminish antioxidant defenses or increase radical production could increase atherogenesis, particularly PAD, by sequestering NO or targeting NO synthesis and bioactivity. Notably, like lead, cadmium has also been shown to increase atherosclerosis in animal models,\textsuperscript{119,121} and environmental exposure to cadmium has been suggested to be particularly toxic to heart and blood vessels,\textsuperscript{122,123} in part, because of the ability of cadmium to increase free radical generation.\textsuperscript{124}

**Pollutants and CVD: Is NO the Achilles Heel?** Despite paucity of mechanistic data, a recurrent theme that emerges from pollution research is the high vulnerability of NO and NO-generating systems. Changes in blood pressure are the most commonly reported outcomes of pollutant exposure and many studies invoke defects in NO signaling or generation as the main mechanism underlying the cardiovascular toxicity of cigarette smoke, chemicals or particles. The exquisite sensitivity of NO to environmental insults is underscored by the observations that cigarette smoke has acute and immediate (<30 minutes) effects on endothelium-dependent vasodilation\textsuperscript{125} and that exposure to ambient particles and ozone decreases conduit artery diameter even in healthy adults.\textsuperscript{37} Although it remains unclear whether changes in NO represent a part of the overall vascular response to vasoconstrictors and autonomic regulators, the significance of these changes cannot be entirely discounted. NO regulates vascular tone, but, at the same time, it also exerts antiinflammatory, antithrombotic, and antiatherogenic effects.\textsuperscript{126} Hence, disruption of NO may be one mechanism that could link the disparate effects of pollutants ranging from changes in blood pressure and thrombosis to vascular inflammation and increased atherogenesis.

The high pollutant vulnerability of NO echoes similar themes in other areas of CVD research. All major cardiovascular risk factors either inhibit NO synthesis or decrease NO availability, and it has been suggested that such changes contribute to essential hypertension, myocardial ischemia/reperfusion injury, atherogenesis, thrombosis,\textsuperscript{126,127} insulin resistance,\textsuperscript{4,128} and heart failure.\textsuperscript{129} NO is a reactive gas that readily combines with metals and other free radicals. The processes that generate NO or those that mediate its biological effects involve multiple redox transformations that proceed at high rates more than low-energy barriers. These reactions have precarious equilibria, low selectivity, and a steep dependence on initial conditions. Consequently, they are readily disrupted or waylaid by aberrant reactants generated endogenously (ROS or lipid peroxidation products) or those that are delivered from the environment (eg, cigarette smoke or PM). It appears that much of CVD, and our inability to control it, may stem from this vulnerability. This susceptibility may be a particularly critical determinant of injury caused by environmental pollutants and blood-borne chemicals. Many ambient particles\textsuperscript{130} as well as manufactured nanomaterials\textsuperscript{131} generate ROS such as superoxide, which could react with NO at diffusion-controlled rates and thereby decrease NO availability or increased radical generation by uncoupling eNOS. Moreover, because the endothelium serves as a barrier and an interface between the blood and mesenchymal tissues, it is likely it is to be the first site of toxicant injury once chemicals and pollutants enter the circulation. Hence cardiovascular responses local to the endothelium or those orchestrated by endothelial triggers need to be prime considerations in any systemic evaluation of pollutant toxicity. In particular, it is imperative to know how processes involved in NO synthesis and availability are affected and how these changes could be prevented or therapeutically treated. In addition, it is important to understand how changes in NO are related to other outcomes of PM exposure, such as changes in heart rate variability, autonomic disturbances, and arrhythmias, and to distinguish whether these are independent effects of PM exposure or related to disturbances in NO synthesis or production.

**Is Pollution Exposure a New Risk Factor for CVD?** From the recognition that cardiovascular tissues are highly vulnerable to pollutant exposure, it follows that the role of environmental exposures should be routinely considered in evaluating CVD risk. Smoking is necessarily considered as a major risk factor, but exposures to PM, arsenic, or metals may be important factors as well. Because exposure to endemic pollutants such as ambient aerosols and pollutant gases is positively associated with heart disease, it could be argued...
that in the general population exposure to pollutants such as PM is a significant determinant of the overall CVD risk as well. Classic CVD risk factors vary in prevalence among different populations, and, for a given set of risk factors, absolute risk varies among different population and age groups. Further studies are, therefore, required to determine whether the risk attributable to pollutant exposure is transportable across populations of different ethnic origins and age groups or whether it is predictive of risk only in a limited subset of individuals. For instance, although it has been shown that advanced age,24 diabetes,44 obesity,71 hypertension,73 and preexisting CVD43,72 accentuate the effects of PM exposure, it remains unclear how and why different disease states modify responses to PM and whether lifetime PM exposures contribute to cumulative CVD risk in healthy individuals. Additional studies are also required to examine the role of comorbid conditions in regulating pollutant toxicity. The increase in plasma lipids, for example, could affect clearance and toxicity of environmental electrophiles by forming covalent adducts with them and, thus, increasing their residence time in circulation. For instance, the environmental pollutant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), which has been linked to an increased risk of ischemic heart disease,131 partitions in low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL) particles.132 Such partitioning could potentially increase TCDD toxicity in hypercholesterolemic individuals by carrying the toxin to atherosclerotic plaques. Changes in plasma lipoproteins or cholesterol levels could also influence drug and chemical detoxification by affecting hepatic or vascular metabolism or both. Similar modulating effects may be associated with aging, hypertension, obesity, or long-term diabetes. Interestingly, the half-life of TCDD or dibenzofurans in exposed workers was positively related to percent-age of body fat,133 suggesting that retention of fat-soluble xenobiotics and pollutants in obese individuals could contribute to their greater susceptibility to pollutant toxicity and consequently CVD. Thus it appears important to delineate the contribution of comorbid conditions. Estimates of the relative susceptibility of diseased and healthy individuals may also be useful in guiding public policy by helping to distinguish whether decreasing a specific pollutant would be useful as a primary or a secondary risk prevention strategy.

The 4 conventional risk factors (cigarette smoking, diabetes, hyperlipidemia, and hypertension) can account for 80% to 90% of clinically significant CAD.4 Hence, for any pollutant exposure to have predictive value, inclusion of exposure assessment should add to the prognostic information beyond that of Framingham risk stratification and increase the projective power of the risk-predicting equations. It should, however, be remembered that attributable risk for complex diseases could exceed 100% because the disease could be avoided in more than one way.134 Hence, the high predictive power of known risk factors does not preclude the possibility that there may be other significant contributors and modifiers that could independently affect CVD or those that could indirectly contribute to classic risk factors. For example, it remains to be determined which pollutant exposures are risk correlates, ie, they elevate CVD risk by influencing other major risk factors, and which ones are determinants of absolute risk. The possibility that pollutant exposures may be risk correlates is consistent with the observations that exposure to PM,8 lead,109,111 or arsenic92 is associated with an increase in blood pressure, whereas exposures to ozone and TCDD132,135 are associated with an increase in LDL cholesterol. Cholesterol levels may also be elevated because of an unresolved acute-phase response established by constant exposure to environmental agents that act as ersatz microbes or microbial products (eg, pollutant-modified proteins or ambient aerosols). It has also been reported that the prevalence of diabetes is increased in subjects exposed to dioxin-like compounds136 and that there is a significant relationship between the levels of toxic air chemicals in the United States and the prevalence of diabetes.137 Collectively, these findings raise the possibility that the CVD risk factors endemic to industrialized societies are, in part, influenced by chemicals and pollutants, and, therefore, there is an urgent need to understand how environmental exposure to harmful chemicals affects cardiovascular function and disease.

Environmental Cardiology: A New Field or Discipline?

Although the effects of genetic traits and environmental factors cannot be entirely disentangled, it has been estimated that more than 70% stroke, 80% CAD, and 90% of adult-onset diabetes could be attributed to modifiable, nongenetic factors,134 suggesting that the environment is a strong determinant of CVD risk. This, however, does not discount the possibility that responses to the environment are, in part, genetically determined. Cardiac risk factors such as LDL cholesterol and blood pressure have variably hereditable,138 and familial studies have shown that CVD risk increases 5- to 7-fold in individuals who have a first degree relative with premature CVD.139 Nevertheless, it remains unclear how much of this risk is attributable to shared genes and how much of it could be attributed to shared environments. In studies involving twins or families, the contribution of the environment is always underestimated because variations in the environment are usually small.134 As a result, it becomes difficult to tease out environmental influences from genetic predispositions. Furthermore, despite vast collective efforts, most associations between polymorphic genes and CVD have been found to be only nominally significant,138 and <5% of CAD cases could be attributed to rare penetrant mutations that are responsible for familial clustering of heart disease.134 Hence, taken together, this evidence suggests that the effects of individual genes on the risk of complex traits such as CVD are likely to be weak and that large subsets of genes involved in many processes such as blood pressure regulation, cholesterol metabolism, insulin sensitivity, redox signaling, antioxidant defense, and inflammation collectively contribute to CVD risk. Therefore, looking at CVD primarily as a response to environmental insults offers one tangible starting point for systemically deconstructing complex mechanisms and etiologies. By identifying specific environmental conditions that are most closely associated with individual features of CVD, it may be possible to delineate only those processes and genes
that respond to environmental perturbations and those that contribute directly to disease development. Moreover, by studying cardiovascular responses to real-world environments, we may be able to learn how CVD develops in the real-world and how it could be prevented and alleviated, not just symptomatically treated.

From the perspective of environmental cardiology, it appears that if the environment is indeed the major player, then the environmental context within which a physiological process takes place should be studied as closely and rigorously as the biochemical content of the process. At present, most investigators address gene/environment interaction as a second-order question, one that needs to be addressed only after the biochemical mechanism has been understood in well-controlled environments and the susceptibility gene has been identified. But if disease manifestation is contingent on environmental cues, important genetic effects may be blunted, if not completely obscured.\(^{140}\) Although inclusion of environmental variables is critical to both epidemiological and mechanistic investigations, the latter in particular need to incorporate well-defined variations in the environment because only such studies can establish causality, construct dose-response relationships, and discover mechanisms linking individual cardiovascular responses to specific environmental conditions. Furthermore, if cardiovascular responses are context-dependent, it is possible that molecular and biochemical mechanisms that contribute to disease development in one environmental context may not be operative in another. For instance, deletion of a candidate gene may not affect plaque formation driven primarily by hypercholesterolemia (as in naive apoE-null mice) but may contribute to accelerated atherogenesis caused by toxic environments (eg, PM or metal exposure).

In large prospective cohort studies, the effect of the environment, particularly exposure to chemicals, pollutants, pesticides, etc, needs to be routinely considered. For this, it may be necessary first to refine exposure measurements. Most exposure assessments reported to date are either crude or superficial. Often epidemiological studies lack proper controls or exposure assessments and animal studies mechanistic detail. Nevertheless, much remains to be done because the effects of many other chemicals remain unknown. More than 80,000 chemicals are currently registered in the United States for commercial use,\(^{11}\) and the cardiovascular toxicity of only a handful of these has been examined. The use of nanomaterials is another area of emerging concern.\(^{11}\) Hence, new concepts, models, tools, and procedures are needed for creating a discrete, well-defined environmental cardiology approach. This would help not only in understanding the environmental mechanisms of heart disease but also in developing specific preventive or therapeutic strategies for minimizing the harmful influences of the environment.

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**References**


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