Air pollution is positively associated with increased daily incidence of myocardial infarction and cardiovascular mortality. We hypothesize that air pollutants, primarily vapor phase organic compounds, cause an enhancement of coronary vascular constriction. Such events may predispose susceptible individuals to anginal symptoms and/or exacerbation of infarction. To develop this hypothesis, we studied the effects of nonparticulate diesel exhaust constituents on (1) electrocardiographic traces from ApoE−/− mice exposed whole-body and (2) isolated, pressurized septal coronary arteries from ApoE−/− mice. ApoE−/− mice were implanted with radiotelemetry devices to assess electrocardiogram (ECG) waveforms continuously throughout exposures (6 h/day×3 days) to diesel exhaust (0.5 and 3.6 mg/m³) in whole-body inhalation chambers with or without particulates filtered. Significant bradycardia and T-wave depression were observed, regardless of the presence of particulates. Pulmonary inflammation was present only in the whole exhaust-exposed animals at the highest concentration. Fresh diesel exhaust or air was bubbled through the physiologic saline tissue bath prior to experiments to enable the isolated tissue exposure; exposed saline contained elevated levels of several volatile carbonyls and alkanes, but low to absent levels of polycyclic aromatic hydrocarbons. Vessels were then assayed for constrictive and dilatory function. Diesel components enhanced the vasoconstrictive effects of endothelin-1 and reduced the dilatory response to sodium nitroprusside. These data demonstrate that nonparticulate compounds in whole diesel exhaust elicit ECG changes consistent with myocardial ischemia. Furthermore, the volatile organic compounds in the vapor phase caused enhanced constriction and reduced dilatation in isolated coronary arteries caused by nonparticulate components of diesel exhaust.

Key Words: ischemia; vasospasm; air pollution; particulate matter; volatile organic compounds.

INTRODUCTION

Associations between air pollution and cardiovascular morbidity and mortality are well documented, but not well understood. Cardiovascular outcomes associated with particulate matter (PM) air pollution include acute myocardial infarction (Peters et al., 2001, 2004), heart failure, and arrhythmia (Schwartz and Morris, 1995). Recent findings highlighting enhancement of vascular tone due to air pollutants (Brook et al., 2002; Nurkiewicz et al., 2004) have not made a clear connection between physiological effects and pathological outcomes. Based on these observations, we hypothesize that a susceptible (e.g., partially occluded or endothelium impaired) coronary vessel subjected to air pollution-induced vasoconstriction may provoke anginal and/or arrhythmic outcomes.

The most clearly demonstrated vascular effect of air pollutants was that of brachial artery constriction in healthy volunteers after exposure to 150 µg/m³ PM and 0.10 ppm ozone (Brook et al., 2002). This work is supported by several articles that suggest certain chemicals commonly found in the ambient pollutant mixture can affect vascular physiology in animal models (Kumagai et al., 2001). Recent investigations by Nurkiewicz et al. (2004) went a step further by demonstrating vascular effects caused by the inflammatory response from a complex particulate model, residual oil fly ash (ROFA), in an anesthetized rodent preparation.

These studies have focused specifically on PM or other chemicals in the particulate phase, ignoring the potential effects of volatile air toxics, such as formaldehyde and acetaldehyde, which would be expected to access the circulation considerably more rapidly than particulates. The goal of the present study was, therefore, to expand upon the previous findings by investigating a complex combustion-source atmosphere (diesel exhaust) in a vessel of direct importance to adverse cardiac outcomes, the septal coronary artery. We employed a murine transgenic model of atherosclerosis, the ApoE−/− mouse on a high fat diet (Zhang et al., 1992). The genetic background strain (C57BL/6J) was used in initial comparison studies, to
establish the susceptible nature of the ApoE\(^{-/-}\)-associated arterial disease manifestation.

**METHODS**

**Animals.** Mice (male C57BL/6J and ApoE\(^{-/-}\), 10–12 weeks of age at the beginning of the study) were obtained from Jackson Laboratories (Bar Harbor, ME). Animals were housed in an Association for Assessment and Accreditation of Laboratory Animal Care International-approved rodent housing facility that maintained constant temperature (20°–24°C) and humidity (30–60% relative humidity) conditions. After a 2-week quarantine, mice were placed on a 16-week high fat diet (Harlan Teklad JK050814, Madison, WI). All procedures were approved by the Lovelace Respiratory Research Institute’s Animal Care and Use Committee.

After 14 weeks on a high fat diet (# TD88137 Custom Research Diet, Harlan Teklad), mice were surgically implanted with radiotelemetry devices (Data-Sciences, Inc., St. Paul, MN). Procedures were similar to those previously described in rats, except that the telemeter remained subcutaneous, rather than being placed in the peritoneal cavity (Campen et al., 2003). All procedures were conducted under sterile conditions and mice were allowed 7 days to recover prior to acquisition of control data; mice were maintained on the high fat diet throughout the recovery and exposure period.

**Whole-body exposures.** Mice were exposed to whole or particle-free exhaust for 6 h/day for 3 days, and then euthanized 18 h after the end of the last exposure. Electrocardiogram (ECG) data were recorded and analyzed continuously throughout exposures using a commercially available software package (Gould Ponemah Life Science Suite, Valley View, OH). The exposure system and characterization of exposure atmospheres have been defined in detail elsewhere (McDonald et al., 2004a). Exposure atmospheres were diluted with charcoal and high efficiency particulate air (HEPA) filtered ambient air to yield 500 and 3,600 \(\mu\)g/m\(^3\) particle mass concentrations (Table 1), or the equivalent dilution with a particle-free exposure atmosphere. Note that in the particle-free exposure atmosphere there was an increase in the mass of material measured on the Teflon-coated glass fiber filters used to define exposure concentration (Table 1). However, this increase in mass was not due to particles but, rather, the mass was attributed to adsorption of gases at the high exposure concentration. This finding was confirmed by the lack of any discoloration on the filters used to monitor the particle-free exposures (Fig. 1) and a lack of any measurable particles in the filtered particle chamber, measured with a condensation particle counter (Model 3025 Condensation Particle Counter, TSI Inc., St. Paul, MN). The particle counter would be able to detect either solid- or liquid-based particles in the size range of the exhaust observed in the non-filtered exposure atmosphere.

Diesel exhaust for these exposures was produced from a single-cylinder, 5500-watt, Yanmar diesel engine generator using number 2 certification diesel fuel (Chevron-Phillips, Borger, TX). Electrical current was pulled from the engine to provide a constant load (~100% with 10 × 500-watt lights). Exposures to the nonparticulate fraction of exhaust (particle-free exposures) were accomplished with a noncatalyzed commercially available ceramic particle trap (PERMIT Filter, Clean Air Systems, Santa Fe, NM). Because the filter does not scrub gases, the vapor phase concentrations of NO\(_x\) were matched to the concentrations in the whole exhaust to ensure that two exposures were conducted at the same exhaust dilutions.

Mice were euthanized 18 h after cessation of the exposures. Lungs were lavaged similarly to previous studies (Campen et al., 2002), with volumes adjusted for the small size of the mouse. Cell counts and differentials were also conducted as previously described (Campen et al., 2002).

**Ex vivo exposures.** To expose coronary vessels to the soluble components of diesel exhaust, the perfusate, a physiologic saline solution (PSS), was bubbled with diesel exhaust through an impinger at a rate of 0.5 lpm, a concentration of 3–5 mg/m\(^3\), and a duration of 1 h per liter. These samples were filtered (5 \(\mu\)m pore) to remove large particulate material, and then the pH (7.35–7.45) and osmolarity (285–295 mOsm) were confirmed to ensure that the exposure did not render the PSS incompatible with the isolated vascular preparation. The exposed PSS was then diluted 1:1 with naïve solution prior to usage in the ex vivo preparation.

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Exposure Concentrations and Pulmonary Inflammatory Markers</th>
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<tbody>
<tr>
<td></td>
<td>Filtered air ((\text{n}=10))</td>
</tr>
<tr>
<td>Particulate mass ((\text{mg/m}^3))</td>
<td>0.008</td>
</tr>
<tr>
<td>NO(_x) (ppm)</td>
<td>Not measured</td>
</tr>
<tr>
<td>Total cells ((\times 10^6)), ApoE(^{-/-})</td>
<td>33 ± 11</td>
</tr>
<tr>
<td>Total cells ((\times 10^6)), C57BL/6J</td>
<td>30 ± 21</td>
</tr>
<tr>
<td>PMNs, ApoE(^{-/-})</td>
<td>912 ± 2080</td>
</tr>
<tr>
<td>PMNs, C57BL/6J</td>
<td>3220 ± 2520</td>
</tr>
</tbody>
</table>

**Note.** Particulate mass and NO\(_x\) concentrations, along with bronchoalveolar lavage end points are shown for each exposure. Diesel caused minimal signs of pulmonary neutrophilia in ApoE\(^{-/-}\) and C57BL/6J mice fed a high fat diet (values are mean ± standard deviation). Whole exhaust had a higher incidence of elevated polymorphonuclear leukocyte (PMN) infiltration, but the inter-subject variability was substantial. Particulate-filtered exposures were not conducted (NC) in C57BL/6J mice. Asterisks (*) denote significant elevations compared to control \((p < 0.05)\).

This measurement showed mass of material on the filters because of adsorption of organic vapors. The filters showed no discoloration, and particle-counting devices showed an absence of any particles in the exposure atmosphere (Fig. 1).
Chemistry. Semivolatile organics are defined here as organics that may exist simultaneously in either (or both) the gas or particle phase, depending on compound vapor pressure, concentration, or environmental conditions. These compounds were collected in the perfusate, extracted, and analyzed directly from the exposed PSS and a naïve PSS blank. Prior to extraction, the saline was spiked with a suite of deuterated internal standards (naphthalene, phenanthrene, acenaphthene, chrysene, benzo[a]pyrene, and dibenz[a,h]anthracene) that served to mimic the behavior of the target analytes throughout the extraction process. Physiologic saline solution was placed in a glass separator funnel, and a liquid/liquid extraction was conducted with an equal amount of analytical-grade dichloromethane. The dichloromethane was separated from the saline and evaporated to −1 ml before filtration through a 0.2-μm Acrodisc filter. Extracts were then evaporated to ~100 μl under a gentle nitrogen stream and brought to 0.2 ml with acetonitrile prior to analysis. Analysis was conducted by gas chromatography/mass spectrometry as described by McDonald et al. (2004b). The analysis for this study included both polycyclic aromatic hydrocarbons and n-alkanes in the C12–C25 size range.

Volatile carbonyl compounds were collected on 2,4-dinitrophenyldiazine-impregnated cartridges and analyzed by high performance liquid chromatography as previously described (McDonald et al., 2004a). Simultaneous samples were collected: (1) directly from the exhaust dilution chamber and (2) downstream of the impingers used to collect the diesel condensate. The concentration of carbonyls trapped in the impingers was evaluated by the difference between the airborne concentration and the downstream concentration. The concentration of carbonyls in the sample after the bubblers was negligible (less than 1% of concentration), indicating very efficient trapping into the saline solution for that class of compounds.

Vessel assay. To determine the effects of diesel exhaust, vessels were bathed in either diesel-exposed or naïve saline for the duration of the protocol. The heart was harvested fresh from ApoE−/− mice (also fed the high fat chow for 16 weeks) and immediately placed in ice-cold PSS bubbled with 5% CO2 and air mixture. After dissecting away the right ventricular free wall, the septal coronary artery was identified and isolated under 25–40× magnification. The isolated vessel was then transferred to the exposure chamber (Living Systems Instrumentation, Burlington, VT) and perfused with warm (35–37°C), aerated physiologic saline. Vessels were mounted between the two opposing pipettes, secured with 12–0 monofilament silk suture, and pressurized to physiologic levels (60 mm Hg). The vessels were then allowed 1 h to acclimate to the chamber conditions while bathed in nonrecirculating air- or diesel-exposed PSS.

Myogenic tone was assessed by measuring luminal diameter over increasing pressure steps (40, 60, 80, 100, and 120 mm Hg) with and without calcium in the PSS (Earley and Walker, 2002). Before the calcium-free saline was used, exposure to increasing concentrations of human endothelin-1 (ET-1) (10–12–10–7 M; Sigma Aldrich, St. Louis, MO) was conducted to observe contractile responses. Next, vessels were preconstricted with U46619 (Sigma Aldrich), a thromboxane mimetic, to approximately 50–70% of the maximal constricted diameter from the ET-1 assay, and then dilated with increasing concentrations of sodium nitroprusside (SNP) (10–8–10–5 M; Sigma Aldrich), a nitric oxide donor. Measurements of vessel internal diameter were recorded and analyzed digitally (Windaq software, Dataq; Akron, OH).

Statistics. Group data were tested by two-way analysis of variance (ANOVA; time, concentration as factors for whole-body ECG data; exposure, assay concentration in vessel assays). Bonferroni post-hoc tests were used to determine specific differences. Probability values less than 0.05 were considered significant.

RESULTS

Whole-Body Exposures

ApoE−/− mice on a high fat diet demonstrated significantly elevated fatty deposition in the aorta (under Sudan IV stain) compared to C57BL/6J mice on the same diet, confirming the hyperlipidemic status of the ApoE−/− animals (data not shown). During exposure to diesel exhaust, ApoE−/− mice demonstrated significant electrocardiographic findings compared to a relatively insensitive C57BL/6J background strain. Heart rate consistently decreased in ApoE−/− mice during the high concentration exposures; the presence of particles in the exhaust made no difference on the acute heart rate responses (Fig. 2; data for C57BL/6J mice are not shown).

During diesel exhaust exposures, several ECG findings were observed in ApoE−/− mice exposed to the high concentration. T-wave depression was clearly observed in several mice (Fig. 3A), independent of the presence of particulates in the exhaust. Overall, significant decreases in the T-wave area, as normalized to the isoelectric line, were noted in the high concentration groups (Fig. 3B). Bradyarrhythmias, particularly atrioventricular-node block and premature ventricular contractions, were ubiquitous during the latter stages of the 6 h exposure, concomitant with the bradycardia.

Lavage fluid revealed little in the way of pulmonary inflammation for ApoE−/− (Table 1) or C57BL/6J mice (not shown). At the highest concentration of whole exhaust, polymorphonuclear leukocyte (PMN) levels in both strains were 10 times that of control, but significant intersubject variation reduced the power of these findings. Interestingly, no increasing trends in PMNs were observed in the high concentration of PM-filtered exhaust, suggesting a specific role for particles in pulmonary inflammation.

Coronary Vascular Effects

Exposures to diesel components were facilitated by first bubbling diesel exhaust through PSS via an impinger. Chemical analysis of the diesel-exposed saline revealed no changes...
in pH or osmolarity, but significant uptake of several organic species was observed (Table 2).

Ex vivo diesel exposure had no significant effects on the resting myogenic tone of isolated septal coronary arteries, suggesting equivalent viability and recovery from the isolation and mounting procedure between control and exposed vessels (Table 3). Control coronary arteries (in normal PSS) demonstrated consistent constrictive responses to ET-1 and dilatory responses to SNP (Fig. 4). In vessels bathed in the diesel exhaust–exposed PSS, responses to ET-1 (maximum constriction = 47.5% reduction from original diameter at $10^{-7}$ M) were enhanced compared to those bathed in normal, air-exposed PSS (maximum constriction = 34.5% reduction from original diameter at $10^{-7}$ M). Significant enhancement of constriction was observed at the $10^{-8}$ and $10^{-7}$ M levels.

Similarly, SNP-induced dilation was blunted in vessels resting in the diesel-exposed saline (Fig. 4). Naïve vessels preconstricted with U46619 generally returned to the full original resting diameter at $10^{-5}$ M SNP. Diesel-exposed, preconstricted vessels only returned to 77.4% of the baseline diameter at $10^{-5}$ M SNP.

### DISCUSSION

The findings of the present study suggest a role for the coronary artery in causing the adverse cardiovascular health...
effects of air pollutants. Secondarily, the data provide evidence suggesting that other combustion-related gases, possibly aldehydes and alkanes, may have a greater role in driving the adverse effects on coronaries than do particles. The conclusions that can be drawn from the present study must be tempered with caveats inherent in small animal studies, but the data imply a promising route for future research.

Coronary Health and Air Pollution

Previous epidemiological studies have made it clear that myocardial infarction is related to PM air pollution (Peters et al., 2001; 2004), and animal studies have provided evidence that air pollutants, primarily particulates, can alter the ECG of animals in a manner consistent with myocardial ischemia (Godleski et al., 2000; Kodavanti et al., 2000; Wellenius et al., 2004). Our data suggest that the complex mixture of soluble gaseous compounds in whole diesel exhaust can reduce dilatory responses and enhance constrictive responses of a major conduit coronary artery, which is consistent with the pathophysiological risk factors for unstable angina (Halcox et al., 2002). Data for whole-body exposed mice in the present study show electrocardiographic changes consistent with myocardial ischemia. Recent findings have suggested that the destabilization and rupture of plaques occurs a considerable period (up to 3 days) prior to the occurrence of acute coronary symptoms (Ojio et al., 2000), leading some investigators to hypothesize a crucial role of vasospasm in the onset of anginal outcomes (Shimokawa, 2000). The effects of air pollutants, if consistent with the findings of the present study, may not only predispose individuals to stable or unstable angina, but also exacerbate sequelae of plaque rupture by inhibiting collateral recruitment and blunting the efficacy of nitrate therapies. Indeed, PM-associated exacerbation of experimentally administered myocardial ischemia has been documented in dogs and rats, as noted by increased frequency of ventricular arrhythmias (Wellenius et al., 2002, 2003).

Our findings are consistent with other air pollution studies in humans (Brook et al., 2002) and animals (Nurkiewicz et al., 2004), although several differences in study design and interpretation certainly exist. The brachial artery constriction observed by Brook et al. (2002) occurred acutely, much in line with epidemiological findings of acute myocardial infarction and particle exposure (Peters et al., 2001). The studies by Nurkiewicz et al. (2004) were conducted in situ 24 h after administration of a high dose of instilled ROFA particle. Our earlier work noted the considerable solubility, high levels of transition metals, and potential for pulmonary inflammation related to instillation with a similar ROFA particle (Campen et al., 2002). The vascular effects occurring at 24 h post-instillation may be due to a combination of events, not least of which is downstream inflammatory signaling, as detailed by the investigators (Nurkiewicz et al., 2004). In addition, the soluble components of the ROFA, especially nickel, may cause prolonged toxicity up to 72 h following instillation (Campen et al., 2002).

### TABLE 3

<table>
<thead>
<tr>
<th>Luminal pressure</th>
<th>PSS</th>
<th>Diesel-PSS</th>
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<tbody>
<tr>
<td>40</td>
<td>7.8 ± 3.6</td>
<td>6.8 ± 3.3</td>
</tr>
<tr>
<td>60</td>
<td>9.2 ± 3.2</td>
<td>9.7 ± 3.4</td>
</tr>
<tr>
<td>80</td>
<td>10.2 ± 2.8</td>
<td>9.3 ± 3.0</td>
</tr>
<tr>
<td>100</td>
<td>11.1 ± 2.0</td>
<td>9.2 ± 2.9</td>
</tr>
<tr>
<td>120</td>
<td>10.7 ± 1.1</td>
<td>9.7 ± 1.4</td>
</tr>
</tbody>
</table>

Note. Myogenic tone was determined as the difference in resting diameters at a given luminal pressure between baseline and in a calcium-free saline solution. The data presented are also normalized for vessel diameter. No significant effects of diesel were observed on myogenic tone.

### FIG. 4

Soluble diesel components cause alterations in constriction to endothelin-1 (ET-1) (upper panel) and dilation to sodium nitroprusside (SNP) (lower panel) in septal coronary arteries. Vessels were bathed in either air-exposed (n = 9) or diesel-exposed (n = 5) physiologic saline solution (PSS) throughout the assay. Analysis of variance (ANOVA) revealed significant differences between control and diesel-exposed responses for both assays; asterisks indicate specific concentrations that were different in Bonferroni post-hoc analysis (p < 0.001).
It must be noted that these various scenarios for air pollution-induced vascular pathophysiology are not mutually exclusive. Neural, inflammatory, and translocative mechanisms have been proposed as drivers for the adverse effects of air pollution on cardiac health (Brook et al., 2003; Stone and Godleski, 1999), and there is no reason to believe that all three mechanisms would not manifest as a result of exposure to a complex mixture. Our findings of pulmonary inflammation in whole exhaust-exposed, but not filtered exhaust-exposed, mice at 18 h following the last exposure suggests that particles may prolong vascular dysfunction through a combination of inflammatory and direct chemical effects. The time course generally noted in epidemiological studies is acute, often within hours of exposure, and lasting 24–48 h (Peters et al., 2004). The complexity of these findings may well reflect the complexity of the air pollutant mixture.

**Air Toxics**

As noted, many organic compounds are absorbed into the saline solution used in the coronary vascular assay. The most abundant compounds, both in the saline condensate and the whole-exhaust exposure atmosphere, are predominantly found in the gas phase (McDonald et al., 2004b), not the particulate phase. Particulate phase compounds may have been present in the saline perfusate, but after dilution they were below the detectable range for the analysis, which is ~20 ng/ml. For the most part, these compounds, including long chain alkanes and aldehydes, are infrequently studied in terms of cardiovascular health effects of air pollution. Some specific aldehydes on the air toxics list have been studied, but rarely as a component of a complex mixture such as diesel exhaust. The physicochemical complexity of whole exhaust may require an equally complex approach to studying the health outcomes. Burnett et al. (2000) found that four compounds (sulfate ion, iron, nickel, and zinc) had a stronger association with mortality than did the standard fine particulate mass measure, and they furthermore concluded that other compounds that were not measured (and thereby excluded from analysis) may be additional drivers of adverse health outcomes. Urch et al. (2004) found that organic and elemental carbon composition of particles had the closest association to the vascular effects in humans (Brook et al., 2002). As has been frequently noted (Moolgavkar and Luebeck, 1996; Phalen, 1998), particulate mass concentrations may only be surrogates for other responsible co-pollutants. Those pollutants that share the same environmental origins and fate of the particulates would be the most hidden, statistically, as opposed to photochemical pollutants (ozone, nitrogen dioxide), whose origin and fate depend more on sunlight than on combustion. The chemicals implicated in the present study, namely alkanes and volatile organics/aldehydes, would be expected to track statistically with particles, especially fines and ultrafines, that originate from the same sources and may exhibit stoichiometric variation in concentration.

Limited research has been undertaken to examine the potential for these volatile air toxics to induce vascular effects. Conklin et al. investigated coronary vascular effects of certain toxics, including acrolein and allyl amine (Conklin et al., 2001), whereas Kumagai et al. have shown that larger poly-cyclic aromatic hydrocarbons(phenanthraquinone) affect nitric oxide synthase at high concentrations (Kumagai et al., 2001). Among the compounds seen in greater quantities in the perfusate for the isolated vascular assays, acetaldehyde and acetone are reported to have dilatory properties in skeletal muscle arterioles (Altura et al., 1990) and constrictive properties in splanchic and mesenteric beds (Altura and Altura, 1982; Altura and Gebrewold, 1981). Vascular effects of long chain alkanes, such as hexadecane and octadecane, have not been reported to our knowledge.

**Limitations of Study**

There is a wide gap of information between the exposure of ex vivo coronary arteries and the actual pulmonary uptake of the chemicals described. Many of the organic compounds are highly reactive and/or easily metabolized, and as such would not build up in the circulation to any great extent (Shibata et al., 2002). However, the coronary bed is at particular risk from inhaled toxins as a first-pass effect, and many coronary vascular drugs (e.g., nitroglycerine) are only active when delivered intravenously prior to hepatic metabolism. Further work needs to be conducted to identify the compounds that drive the responses observed and then validate the relevance of the exposure concentrations, perhaps in an isolated heart-lung preparation to avoid Phase I metabolic interference. Lastly, the effect of ultrafine particles cannot be discounted, as the filtration system for the saline was not sufficient to exclude those particles; however, the vessel imaging system would have enabled observation of particles >0.5 µm, and no particles were ever noted in contact with vessels. The generally insoluble nature of diesel particulates strongly implicates the role of vapor-phase organics in causing the effects seen in the present study.

Identifying acute, reversible, nonlethal coronary vasospasm in a small animal is not readily feasible. Recent knockouts of specific potassium channels have shown fatal coronary vasospasm (Chatkow et al., 2002; Miki et al., 2002). Such models of angina may be useful to further characterize coronary vulnerability to air pollution, but the limitations of real-time assessments in small rodents would preclude anything more than a mortality study and perhaps telemetric ECG monitoring. Unfortunately, findings in the ECG of rodents are difficult to characterize, as several events (not just ischemia) can cause alteration of T-wave morphology. Without blood gas and serum electrolyte information, it is difficult to make a solid interpretation from an ECG. The observations in the present study imply ischemia and are similar to previously reported ECG changes (Kodavanti et al., 2000), but it is not known if
this is a result of systemic hypoxemia, metabolic changes or, as hypothesized, a local event in the coronary bed.

Lastly, the concentrations of diesel exhaust used in the present study are considerably greater than would be encountered anywhere but an occupational, possibly mining, scenario (McDonald et al., 2004b). Furthermore, the dosimetric comparisons between the ex vivo preparation and actual circulating levels has not been validated. Future studies will need to focus on couching the results of the present study with more precise dose measurements. The implications of high concentrations, for results of the present study, are uncertain. While obviously susceptible, the ApoE mouse, even at 16 weeks of high fat diet, is not at serious risk of spontaneous mortality. Lower concentrations of air toxics may continue to have effects on more severely diseased subjects. Also, while we have used a specific and stable diesel exhaust generation technique, the measurement of the individual air toxics should enable comparisons to different engine systems of pollution sources. We would not conclude that the negative cardiovascular impact was caused by diesel, specifically, but rather that diesel contains some amount of vasoactive compounds, which is probably true of many exhaust sources.

CONCLUSIONS

These studies implicate a pathological effect of combustion-source gases on the coronary artery. Although it is not possible to conclusively draw a connection between cardiac outcomes (such as angina) and air pollution based on these animal studies, further investigation into the vascular mechanisms and dosimetric concerns of the present study should help reveal the environmental relevance of the findings.

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