Inhalation of Concentrated Ambient Air Particles Exacerbates Myocardial Ischemia in Conscious Dogs

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Short-term increases in ambient air pollution have been associated with an increased incidence of acute cardiac events. We assessed the effect of inhalation exposure to concentrated ambient particles (CAPs) on myocardial ischemia in a canine model of coronary artery occlusion. Six mongrel dogs underwent thoracotomy for implantation of a vascular occluder around the left anterior descending coronary artery and tracheostomy to facilitate particulate exposure. After recovery (5–13 weeks), pairs of subjects were exposed for 6 hr/day on 3 or 4 consecutive days. Within each pair, one subject was randomly assigned to breathe CAPs on the second exposure day and filtered air at other times. The second subject breathed CAPs on the third exposure day and filtered air at other times. Immediately after each exposure, subjects underwent 5-min coronary artery occlusion. We determined ST-segment elevation, a measure of myocardial ischemia heart rate, and arrhythmia incidence during occlusion from continuous electrocardiograms. Exposure to CAPs (median, 285.7; range, 161.3–957.3 μg/m^3) significantly (p = 0.007) enhanced occlusion-induced peak ST-segment elevation in precordial leads V_4 (9.4 ± 1.7 vs. 6.2 ± 0.9 mm, CAPs vs. filtered air, respectively) and V_5 (9.2 ± 1.3 vs. 7.5 ± 0.9 mm). ST-segment elevation was significantly correlated with the silicon concentration of the particles and other crustal elements possibly associated with urban street dust (p = 0.003 for Si). No associations were found with CAPs mass or number concentrations. Heart rate was not affected by CAPs exposure. These results suggest that exacerbation of myocardial ischemia during coronary artery occlusion may be an important mechanism of environmentally related acute cardiac events. *Key words:* air particles, air pollution, cardiovascular disease, ECG, myocardial ischemia. *Environ Health Perspect* 111:402–408 (2003). doi:10.1289/ehp.5775 available via *http://dx.doi.org/* [Online 30 October 2002]

There is growing epidemiologic evidence that ambient air pollution can precipitate acute cardiac events such as angina pectoris (Poloniecki et al. 1997), cardiac arrhythmias (Peters et al. 2000; Poloniecki et al. 1997; Santos et al. 2001), and myocardial infarction (Peters et al. 2001; Poloniecki et al. 1997). Increased cardiac arrhythmias have also been observed in animal models of vascular (Watkinson et al. 1998) and myocardial (Wellenius et al. 2002) injury after exposure to residual oil fly ash, a surrogate for particulate air pollution. However, the mechanisms by which inhaled particulates trigger acute cardiac events remain unknown.

To test the effects of particulate pollution on the heart, we exposed conscious animals to real-world ambient air particles in a controlled setting. Experimental coronary artery occlusion has been used in canine models for decades to explore the myriad factors that determine the extent of ischemic injury (e.g., Wégria et al. 1949). This model is particularly suitable if the interruption of blood flow is of sufficiently short duration to avoid irreversible myocardial damage. For instance, a transient 15-min coronary artery occlusion is not associated with myocardial necrosis or vascular dysfunction 24 hr later (Patterson et al. 1993). Strengths of the model include the reproducibility of the ischemic insult and the opportunity for use in conscious animals. The Harvard Ambient Particle Concentrator (HAPC) was developed for use in experimental exposure studies to concentrate ambient fine particles (0.1–2.5 μm) by about 30 times without changing their physical properties or chemical composition (Sioutas et al. 1995, 1997). We have adapted this system for use in studies with awake normal and compromised canine subjects (Godleski et al. 2000). Thus, the current study was designed to investigate and quantify the extent to which inhaled real-world ambient particles affect myocardial ischemia in a clinically relevant canine model of coronary artery occlusion.

Methods

Subject preparation. We used retired mongrel breeder dogs (female, 14–17 kg; Butler Farms, Clyde, NY) according to the principles and regulations of the National Institutes of Health under protocols approved by the Harvard Medical Area Standing Committee on Animals. Animals underwent thoracic surgery to chronically implant a balloon occluder around the left anterior descending coronary artery (LAD) as described previously (Godleski et al. 2000). Briefly, under inhalation anesthesia with isofluorane, a left lateral thoracotomy was performed through the fourth intercostal space, and a small vertical incision centered over the ventral border of the left atrial appendage was made through the pericardium. The LAD was bluntly dissected for 1–2 cm at a point ventral to the tip of the left atrial appendage with care to keep the adventitia intact. A vascular occluder (2.5–3.5 mm inner diameter; In Vivo Metrics, Healdsburg, CA) was placed around the dissected portion of the LAD and secured. The occluder was tested, the volume of normal saline needed to occlude the LAD completely was recorded, and the vascular occluder was deflated. The pericardium and thorax were closed and the occluder tubing was exteriorized via a subcutaneous tunnel to the dorsal aspect of the thorax. Each dog then underwent further surgery to create a permanent tracheostomy by the method of Orton (1995) to facilitate particulate exposure. On recovery from surgery, a period of gradual acclimatization to the laboratory and exposure chamber was used for training and to minimize any stress associated with the experiments.

Experimental protocol. To investigate the extent to which inhaled ambient particles affect myocardial ischemia, we assessed the level of occlusion-induced ischemia by electrocardiographic criteria after inhalation exposure to either concentrated ambient particles (CAPs) or filtered air. Initially, pairs of subjects were exposed for 6 hr/day on 3 consecutive days (Table 1). Within each pair, one subject was randomly assigned to breathe CAPs on the second exposure day and filtered air at other times. The second subject breathed CAPs on the third exposure day and filtered air at other times. Immediately after each exposure, subjects were led from the exposure chamber to a flat table in a quiet room independent of each other. Each subject then underwent a 5-min

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preconditioning occlusion, followed 20 min later by a 5-min experimental study occlusion (Figure 1). Coronary artery occlusions were produced by inflating the vascular occluder with the predetermined volume of saline, maintained for 5 min, and then slowly released.

To investigate the biologic effects of CAPs 24 hr after exposure, we exposed some animals for 6 hr/day on 4 consecutive days. When this occurred, both animals were exposed to filtered air on the fourth day (Table 1). Dogs participated in experiments only if they were in good physical health and the vascular occluder was functional.

ECG acquisition and analysis. Continuous electrocardiograms (ECG) from precordial leads V_4 and V_5 were obtained with a Series 8500 ambulatory Holter monitor (GE Marquette Medical Systems, Inc., Milwaukee, WI) using pediatric surface electrodes (Conmed Corp., Utica, NY) applied over a shaved area. We analyzed recordings from the experimental study occlusion on a MARS Unity Workstation (GE Marquette Medical Systems, Inc.), which automatically labels beats for subsequent review. ST-segment elevation, an electrocardiographic marker of myocardial ischemia, was measured by a semiautomated procedure. First, cursors were manually set on a representative waveform corresponding to the isoelectric baseline and the J point, which marks the end of the QRS complex on the ECG. Second, the height of the ST-segment is automatically calculated by the MARS as the difference in the level of the ECG 40 ms past the J point and the level at the isoelectric baseline. A second investigator, experienced with the MARS system and blinded to the exposure category of each ECG, independently performed the ST-segment measures. The correlation between the peak ST-segment elevation values obtained by the two analyses was 0.978, confirming that the results do not depend on the manual portion of the analysis. Average ST-segment elevation and heart rate were automatically calculated for every 15 sec of data.

We exported electrocardiographic measures to a personal computer for plotting and further analysis under Matlab (Mathworks, Inc., Natick, MA). Peak ST-segment elevation during each experimental study occlusion, the primary outcome of interest, was defined as the maximum ST-segment change during the occlusion period minus the ST-segment elevation averaged over the minute preceding each occlusion (Figure 2). Integrated ST-segment elevation was analyzed as the area under the ST-segment elevation curve (Figure 2, shaded area), starting where ST-segment elevation exceeded the average baseline value by at least 0.5 mm and ending where the ST-segment elevation was < 0.5 mm above the average value 4–5 min after the end of the occlusion. We defined peak heart rate as the maximum heart rate during the occlusion period and change in heart rate as the peak heart rate minus the heart rate averaged over the minute preceding each occlusion. We also determined the incidence of arrhythmias during and after each occlusion.

Exposure technology and characterization. The characteristics of the HAPC and exposure chamber are well documented (Godleski et al. 2000; Sioutas et al. 1995, 1997). Briefly, the HAPC concentrates ambient fine particulate matter with an aerodynamic diameter ≤ 2.5 μm ($PM_{2.5}$) to approximately 30 times ambient levels without altering their size distribution or chemical composition. Particles with aerodynamic diameters > 2.5 μm are removed upstream of the HAPC, while ultrafine particles (≤ 0.1 μm) and ambient gases are neither enriched nor excluded.

Exposures typically took place between 0830 and 1430 hr each day. Both continuous and integrated measures were used for exposure characterization. A comprehensive description of these methods has been published previously (Godleski et al. 2000). Briefly, CAPs particle characterization included analysis of integrated samples: gravimetric determinations for particle mass, ion chromatography for sulfate (Koutrakis et al. 1988, 1993), X-ray fluorescence analysis for elemental composition (Dzubay and Stevens 1975), and thermal and optical reflectance analysis for elemental (EC) and organic carbon (OC) (Chow et al. 1993). In addition, we determined CAPs particle size distribution using a micro-orifice impactor (Marple et al. 1991). Continuous measurements (5-min averages) of black carbon (BC) mass concentrations were obtained using an aethalometer (Hansen et al. 1984). We also measured continuous particle number

Table 1. Order and timing of exposures for each of 6 experimental animals.

Dog[a]	Start date	Day 1	Day 2	Day 3	Day 4
FR	9/13/2000	FA	FA	CAPs	NE
SN	9/13/2000	FA	CAPs	FA	NE
BK	12/13/2000	FA	CAPs	FA	NE
SN	12/13/2000	FA	FA	CAPs	NE
BK	1/09/2001	FA	CAPs	FA	NE
SN	1/09/2001	FA	FA	CAPs	NE
BK	1/24/2001	FA	FA	CAPs	NE
SN	1/24/2001	FA	CAPs	FA	NE
BK	2/6/2001	FA	CAPs	FA	FA
SN	2/6/2001	FA	FA	CAPs	FA
FR[b]	2/13/2001	FA	FA	CAPs	NE
VR	2/13/2001	FA	CAPs	FA	NE
BK	2/20/2001	FA	FA	CAPs	FA
SN	2/20/2001	FA	CAPs	FA	FA
SY	2/27/2001	FA	CAPs	FA	FA
VT	2/27/2001	FA	FA	CAPs	FA
BK	3/7/2001	FA	CAPs	FA	NE
VR[c]	3/7/2001	FA	FA	CAPs	NE
SY	3/12/2001	FA	FA	CAPs	FA
VT	3/12/2001	FA	CAPs	FA	FA
SY[d]	3/27/2001	FA	FA	CAPs	FA
VT	3/27/2001	FA	CAPs	FA	FA

Abbreviations: FA, filtered air; NE, no exposure.
[a]Unique two-letter designations for each dog. [b]Used only as chamber mate; no occlusions performed. [c]Did not complete protocol because of respiratory distress. [d]No data available because of equipment failure.

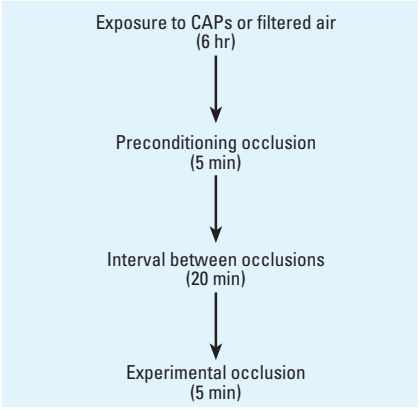

Figure 1. Diagram of the coronary occlusion protocol. Animals were exposed to either CAPs or filtered air for 6 hr and then received a preconditioning and experimental occlusion of the coronary artery.

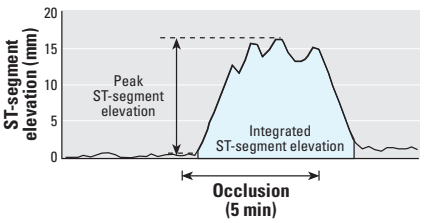

Figure 2. ST-segment elevation was quantified by the peak ST-segment elevation relative to the preocclusion value and the integrated ST-segment elevation estimated as the area under the response curve (shaded region).

concentration of CAPs (CPC Model 3022A; TSI, Inc., Shoreview, MN) and ambient levels of carbon monoxide (CO) (Model 48 CO Analyzer; Thermo Environmental Instruments, Inc., Franklin, MA).

Statistical methods. We calculated descriptive statistics for measures of CAPs mass, composition, and biological outcomes. Measures of ST-segment elevation exhibited classic log-normal–type distributions; strictly positive values with the lower bound of zero skewed the distribution to the right, making log transformation of these responses necessary. For heart rate outcomes, mixed-effects models containing CAPS exposure as fixed effects and week-within-dog as random effects were fit to each response. For the ST-segment data, which consisted of data from leads V_4 and V_5 at each time point, multivariate general linear models that simultaneously account for correlation among leads at a single time point and among repeated measures from the same lead were fit to each response (Galecki 1994; SAS Institute 1999).

For each biologic parameter, we used three analyses with exposure metrics of increasing sensitivity to detect CAPs effects. First, we used a model treating CAPS as a binary variable to assess overall differences between CAPS and filtered air responses. Second, to assess dose–response relationships, we conducted univariate analyses in which a separate repeated-measures regression model was fit using either mass, particle number, or a single elemental concentration as the exposure metric. To confirm the univariate analyses, we fit a multivariate model to each response, using multiple tracer elements of previously defined pollution sources (Clarke et al. 2000; Batalha et al. 2002) as predictors.

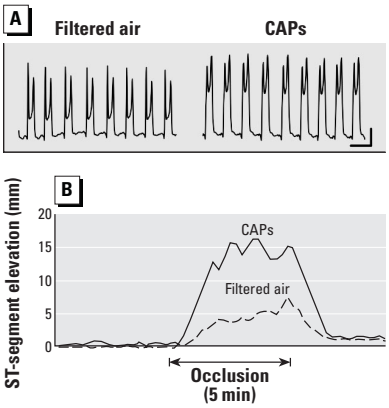

Figure 3. (*A*) ECG tracings from precordial lead V_4 exhibit a visible increase in ST-segment elevation in a representative animal during total occlusion of the LAD coronary artery after inhalation exposure to CAPs or filtered air (control). Horizontal bar = 50 ms; vertical bar = 5 mm. (*B*) Comparison of ST-segment elevation during 5-min LAD coronary artery occlusion in a representative animal after CAPs (solid line) and control (broken line) exposures.

The particle concentration parameters used in the univariate analyses included mass, particle number, nickel, sulfur, silicon, lead, BC, and CO. To ensure that results were not sensitive to the element representing a particular source, we carried out sensitivity analyses in which models with alternative tracer elements were fit to the data. For example, aluminum, calcium, and iron were used in lieu of silicon as road-dust tracers, vanadium was used instead of nickel, and EC and OC were used instead of BC. For comparability across biologic responses and elemental concentrations, estimated regression coefficients are reported as the change in standardized response for one unit standard deviation change in concentration (Zar 1996). Statistical significance for all models was based on $\alpha = 0.05$.

Statistical analyses were performed using PROC MIXED in SAS version 8 (SAS Institute, Cary, NC). Graphical diagnostics of model adequacy were carried out using the S-Plus statistical package (Venables and Ripley 1994).

Results

Six dogs completed the experimental protocol multiple times to yield a total of 21 exposure cycles. The time between surgery and first exposure cycle of each dog ranged from 5 to 13 weeks, with a median of 6.5 weeks. This time was necessary for surgical recovery and for acclimatization of the animals to the experimental setup and protocol. Cardiac data from two of the 21 cycles were excluded, one due to equipment failure and the other due to respiratory distress of the animal. In the remaining 19 exposure cycles, 11 CAPs exposures occurred on day 2 of the protocol, and 8 CAPs exposures occurred on day 3 (Table 1).

ST-segment elevation. In a first analysis, CAPs exposure was treated as a dichotomous variable without consideration of exposure dose or composition. ST-segment elevation during a 5-min LAD occlusion was visibly increased after CAPs exposure compared with control exposure to filtered air (Figure 3). Individual animal data for lead V_4 are shown in Table 2. An increase in peak ST-segment elevation was evident on both precordial lead V_4 (9.4 ± 1.7 vs. 6.2 ± 0.9 mm; mean ± SE for CAPs vs. filtered air, respectively) and lead V_5 (9.2 ± 1.3 vs. 7.5 ± 0.9 mm; Figure 4A). When leads V_4 and V_5 were considered together in a repeated-measures mixed-effects model that accounted for dog-to-dog and week-to-week variability, a 1.24-fold [95% confidence interval (CI), 1.06–1.45; p = 0.007] mean within-dog increase in peak ST-segment elevation attributable to CAPs exposure was observed. Peak ST-segment elevation during coronary artery occlusion remained elevated 24 hr after CAPs exposure (8.8 ± 1.6 and 9.1 ± 1.5 mm, in leads V_4 and V_5, respectively; p = 0.033). After controlling for multiple comparisons, a p-value ≤ 0.022 was considered statistically significant, so the residual effect of CAPs exposure was only marginally significant.

Data for 12 of the 19 exposure cycles were provided by two of the six dogs (Table 1). The above analysis was repeated using only data from these two dogs, and the effect estimate was slightly smaller than the effect estimated by the full data set. Thus, the observed enhancement of the ST-response was not attributable to these two dogs alone.

Table 2. Peak (mm) ST-segment elevation and integrated (mm/min) ST-segment elevation during LAD occlusion measured on lead V_4.

		Day 1		Day 2		Day 3		Day 4	
Dog[a]	Start date	Peak	Integrated	Peak	Integrated	Peak	Integrated	Peak	Integrated
FR	9/13/2000	6.0	23.1	5.9	26.8	5.6	27.9	NE	NE
SN	9/13/2000	7.5	22.9	15.8	72.2	10.4	36.9	NE	NE
BK	12/13/2000	4.5	23.3	5.2	22.5	6.0	25.5	NE	NE
SN	12/13/2000	2.2	8.6	4.5	20.4	3.7	NA	NE	NE
BK	1/09/2001	8.8	40.8	NA	NA	10.6	42.9	NE	NE
SN	1/09/2001	2.9	13.0	2.9	11.2	2.1	7.8	NE	NE
BK	1/24/2001	4.3	27.0	6.3	24.8	12.1	56.6	NE	NE
SN	1/24/2001	3.2	14.6	2.2	9.0	4.5	20.3	NE	NE
BK	2/6/2001	7.5	33.2	7.2	33.3	7.8	38.1	7.3	39.0
SN	2/6/2001	1.9	10.1	4.3	21.7	3.4	15.1	3.5	15.5
VR	2/13/2001	24.3	131.8	27.9	147.3	27.5	133.3	NE	NE
BK	2/20/2001	4.4	19.1	5.6	20.3	5.0	18.5	5.2	21.8
SN	2/20/2001	1.1	4.8	2.8	16.4	2.3	13.6	1.9	9.4
SY	2/27/2001	4.0	20.3	10.1	45.2	5.2	27.0	5.0	23.9
VT	2/27/2001	10.4	51.6	6.9	30.8	8.9	41.8	7.4	43.1
BK	3/7/2001	7.3	40.6	9.1	46.1	8.8	40.9	NE	NE
SY	3/12/2001	3.7	15.0	7.4	32.5	9.8	47.8	7.6	24.3
VT	3/12/2001	9.7	44.7	25.3	111.3	13.1	60.8	17.1	73.4
VT	3/27/2001	9.9	39.7	12.6	60.8	11.9	56.9	NA	NA

Abbreviations: NA, data not available due to poor signal quality; NE, no exposure.
[a]Unique two-letter designations for each dog.

Particle size distribution varied little over the 19 CAPs exposure days [mass median aerodynamic diameter, 0.26 ± 0.04 μm; geometric standard deviation (SD) 2.77 ± 0.42, mean ± SD]. In contrast, daily CAPs mass concentration varied widely, ranging from 161.3 to 957.3 μg/m^3 (Table 3). Particle composition also exhibited substantial variability (Table 3). Based on experience from previous CAPs source apportionment studies conducted by our group, we selected four particle elements (silicon, sulfur, nickel, and BC) as tracers of sources that affect Boston. These four tracers explain a large fraction of the total CAPs mass concentration variability in the data set (R^2 = 0.96; Table 4). Similarly, these tracers were good predictors of the concentrations for most of the analyzed elements. For some elements, such as chromium and arsenic, the source tracers explained only a fraction of their concentration variance (Table 4). This may be due to the high analytical uncertainties associated with these elements and the limited number of samples.

In a series of univariate regression analyses, the peak occlusion-induced ST-segment elevation was not related to either CAPs mass concentration or number concentration (Table 5). Neither was the response correlated with ambient levels of CO [p = 0.47 in a model controlling for CAPs mass concentration]. However, peak ST-segment elevation was significantly correlated with the aerosol elemental concentrations of silicon (p = 0.0018) and lead (p = 0.043). No association was found between peak ST-segment elevation and levels of nickel, sulfur, or BC (Table 5). To explore further the relationship between peak ST-segment elevation and specific constituents of CAPs, we applied a multivariate regression model that included terms for each of the four tracer elements (Table 6). In this analysis, only silicon was significantly associated with peak ST-segment elevation.

ST-segment changes were also quantified using the area under the ST-segment response curve, termed "integrated ST-segment elevation." Individual animal data for lead V_4 are shown in Table 2. An increase in integrated ST-segment elevation was evident on both lead V_4 (45.9 ± 9.0 vs. 28.6 ± 4.6 mm/min, mean ± SE for CAPs vs. filtered air, respectively) and lead V_5 (45.6 ± 6.8 vs. 37.3 ± 4.7 mm/min; Figure 4B). When leads V_4 and V_5 were considered together in a repeated-measures mixed-effects model, a 1.28-fold (95% CI, 1.07–1.54; p = 0.008) increase in integrated ST-segment elevation attributable to CAPs exposure was observed. Integrated ST-segment elevation was not related to CAPs mass or number concentration (Table 5) but was significantly associated with the mass concentration of silicon (Tables 5 and 6).

Heart rate and ventricular arrhythmias. Coronary artery occlusion induced a progressive increase in heart rate from an average pre-occlusion value of 87.3 ± 1.5 beats/min to a peak of 120.7 ± 1.7 beats/min. CAPs exposure as a dichotomous variable had no effect on either the peak heart rate during occlusion (122.4 ± 3.0 vs. 121.9 ± 4.2 beats/min for CAPs vs. filtered air, respectively; p = 0.70) nor on the maximum occlusion-induced increase in heart rate (34.1 ± 2.0 vs. 30.9 ± 2.0 beats/min; p = 0.27; Figure 4B). The heart-rate response to LAD occlusion was also not significantly associated with CAPs mass concentration, number concentration, CO levels, nor with individual elements in a series of univariate analyses (Table 5). In the multivariate analysis, neither the occlusion-induced change in heart rate nor the peak rate was significantly

Table 3. Summary of integrated measures of CAPs characteristics for 19 days of exposure.

Exposure parameter[a]	Analytical method	Mean ± SD	Median	Minimum	Maximum
Mass concentration	Gravimetric analysis	345.25 ± 194.30	285.71	161.34	957.32
Number concentration	CPC Model 3022A	52.76 ± 10.57	51.05	35.90	71.54
Sulfate	Ion chromatography	77.90 ± 58.34	46.80	25.30	259.90
BC	Aethalometer	9.78 ± 6.22	7.43	2.76	24.73
EC	TOR	21.48 ± 11.73	16.16	9.32	44.15
OC	TOR	66.71 ± 28.39	55.93	34.86	125.99
Al	XRF	2.13 ± 1.77	1.41	BD	5.50
As	XRF	0.028 ± 0.015	0.025	BD	0.06
Br	XRF	0.09 ± 0.05	0.08	0.04	0.18
Ca	XRF	4.31 ± 1.89	3.60	1.93	8.19
Cr	XRF	0.03 ± 0.01	0.03	0.01	0.05
Cu	XRF	0.19 ± 0.07	0.16	0.09	0.38
Fe	XRF	8.26 ± 3.11	8.55	3.60	16.74
K	XRF	2.15 ± 0.74	2.24	0.92	3.25
Mn	XRF	0.18 ± 0.07	0.18	0.06	0.31
Ni	XRF	0.16 ± 0.15	0.11	0.04	0.59
Pb	XRF	0.15 ± 0.07	0.13	0.04	0.32
S	XRF	27.41 ± 18.62	17.30	10.16	82.88
Se	XRF	0.02 ± 0.02	0.01	BD	0.08
Si	XRF	8.17 ± 3.94	7.89	2.31	13.93
Ti	XRF	0.41 ± 0.18	0.38	0.19	0.94
V	XRF	0.16 ± 0.12	0.14	0.05	0.43
Zn	XRF	0.58 ± 0.25	0.48	0.29	1.00

Abbreviations: BD, minimum value was less than limit of detection; TOR, thermal and optical reflectance; XRF, X-ray fluorescence.
[a]All measurements are reported in micrograms per cubic meter except number concentration, which is in 10^3 particles/cm^3.

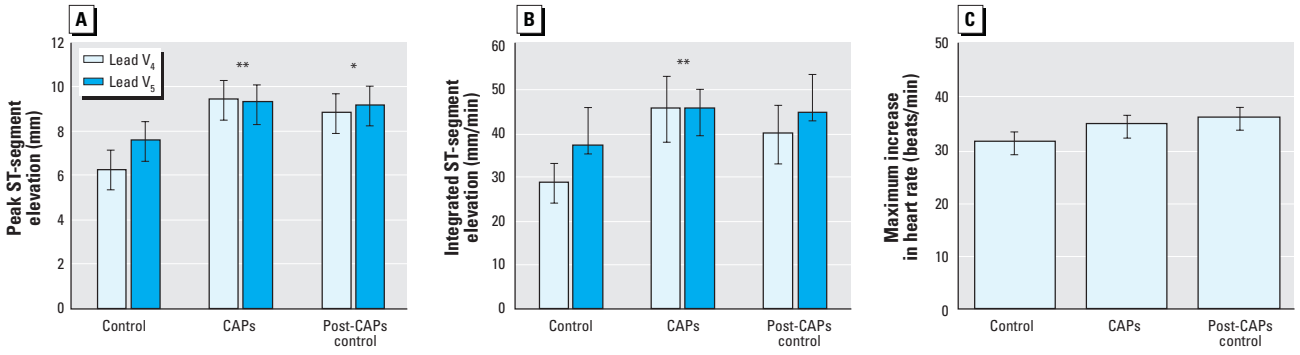

Figure 4. Peak (*A*) and integrated (*B*) ST-segment elevation in precordial leads V_4 (gray bars) and V_5 (black bars) was significantly greater after exposure to CAPs as compared to filtered air (control). (*C*) The peak occlusion-induced increase in heart rate was not affected by exposure. Each bar represents the mean ± SE response from 19 exposure cycles.
*p < 0.05; **p < 0.01 by repeated-measures analysis of variance. After controlling for multiple comparisons, p < 0.022 was considered statistically significant.

associated with the concentration of any element (Table 6).

Ventricular arrhythmias were rarely observed during all experimental occlusions, and these were unrelated to CAPs exposure.

Discussion

The goal of this study was to quantify the effect of inhaled ambient particles on myocardial ischemia. To explore this relationship, we used urban Boston outdoor air, which typically contains particles generated by vehicle exhaust, power plants, home heating, and road dust (Oh et al. 1997; Spengler and Thurston 1983). There was substantial variability in both particle mass concentration and composition. Our results show that exposure to concentrated ambient particles exacerbates coronary artery occlusion-induced ischemic insult, as quantified by the elevation of the ST-segment on the electrocardiogram.

This finding agrees with a previous study carried out on a separate set of dogs which determined that exposure to CAPs enhances ischemia-induced ECG changes in a coronary occlusion model (Godleski et al. 2000). In that study, dogs were exposed either to CAPs for 3 consecutive days or to filtered air for 3 consecutive days. ST-segment elevation increased more rapidly after the start of the occlusion and reached a higher peak value in CAPs-exposed dogs than in sham-exposed controls. However, the latency or the duration of these effects could not be established. In the current study, we observed enhanced ST-segment elevation during the 5-min experimental occlusion immediately after 6 hr of CAPs exposure, indicating a short latency between particulate exposure and cardiovascular end points. Such a short time lag is supported by recent epidiomologic evidence of an association between elevated levels of fine particles and increased risk of myocardial infarction within 2 hr of exposure (Peters et al. 2001), as well as experimental evidence of arterial vasoconstriction in subjects within 2 hr of exposure to CAPs and ozone (Brooke et al. 2002). We also observed enhancement of the ST-segment elevation on the day after CAPs exposure, indicating that, although transient, the effects of CAPs exposure persisted for at least 24 hr. This observation is consistent with reports of an association between the risk of acute myocardial infarction and particulate levels on the previous day (Peters et al. 2001; Poloniecki et al. 1997).

Components of ambient pollution. The importance of considering CAPs composition rather than CAPs mass concentration has been highlighted in several recent reports from our laboratory (Batalha et al. 2002; Clarke et al. 2000; Saldiva et al. 2002). In the current study, the ST-segment response was significantly different in a binary comparison between CAPs and filtered air exposure, but was not well correlated with either particle mass concentration or number concentration. However, ST-segment elevation was related to a specific component of the particulate aerosol represented by the mass concentration of silicon. This finding is consistent with other reports of an association between silicon and biological outcomes. For example, in CAPs-exposed dogs, increases in brochoalveolar lavage neutrophil percentage, circulating neutrophils and lymphocytes, and total peripheral white blood cell counts were significantly associated with an aluminum/silicon factor (Clarke et al. 2000). Batalha et al. (2002) observed that in normal and bronchitic rats exposed to CAPs, the degree of pulmonary vascular vasoconstriction was strongly correlated with the elemental concentration of silicon. Finally, in a mouse allergic asthma model, Kobzik et al. (2001) found that the degree of bronchoconstriction was significantly associated with an aluminum/silicon factor.

The above observations suggest that components of ambient particles whose concentrations vary in parallel with the concentration of silicon may be highly toxic. In the current data set, silicon concentration was highly correlated with the concentration of other crustal elements such as aluminum and calcium, suggesting that silicon originates from the resuspension of soil dust, albeit of very small particle size given the size characteristics of our aerosol. Considering that the HAPC is located in an urban environment approximately 75 m from a major roadway and that road dust can contribute significantly to fine

Table 4. Elemental and total mass concentrations (micrograms per cubic meter) calculated from multivariate regressions of each species versus the four tracer elements.

Dependent variable	Ni	S	BC	Si	R^2
Total mass	37.01	254.15	52.33	115.44	0.96
EC	1.69	0.45	15.68	< 0.01	0.94
OC	1.23	13.20	29.53	11.38	0.88
Al	< 0.01	< 0.01	0.36	3.10	0.94
As	< 0.01	< 0.01	0.01	0.01	0.35
Br	0.01	0.05	< 0.01	0.02	0.42
Ca	0.58	< 0.01	1.36	2.36	0.54
Cr	< 0.01	< 0.01	0.01	0.01	0.37
Cu	0.01	< 0.01	0.06	0.10	0.65
Fe	0.28	< 0.01	2.14	5.47	0.82
K	0.19	0.25	0.24	1.51	0.88
Mn	0.04	0.01	0.01	0.08	0.41
Pb	< 0.01	0.01	0.08	0.06	0.78
Se	< 0.01	0.02	< 0.01	< 0.01	0.65
Ti	< 0.01	< 0.01	0.14	0.30	0.88
V	0.12	< 0.01	0.04	0.01	0.90
Zn	0.10	0.03	0.22	0.14	0.50

Table 5. Standardized regression coefficient estimates from univariate regression analyses of occlusion-induced change in ST-segment elevation and heart rate.

Outcome	Mass	Number	BC	Ni	S	Si	Pb
Peak ST-segment elevation[a]	0.103	0.079	0.117	0.067	0.090	0.224**	0.154*
	(0.079)[b]	(0.236)	(0.080)	(0.077)	(0.081)	(0.069)	(0.075)
Integrated ST-segment change[a]	0.101	0.131	0.125	0.097	0.083	0.218**	0.157*
	(0.079)	(0.232)	(0.081)	(0.093)	(0.081)	(0.071)	(0.077)
Change in heart rate	0.118	0.234	0.073	0.151	0.097	0.127	0.130
	(0.149)	(0.154)	(0.151)	(0.150)	(0.150)	(0.146)	(0.149)
Peak heart rate	–0.069	0.320	0.007	–0.043	–0.090	–0.008	0.014
	(0.093)	(0.212)	(0.095)	(0.100)	(0.095)	(0.093)	(0.093)

Mass, particle mass concentration; number, particle number concentration.
[a]Models fit with log-transformed responses. [b]SE in parentheses. *$p < 0.05$; **$p < 0.005$.

Table 6. Standardized regression coefficient estimates from multivariate regression analyses of occlusion-induced change in ST-segment elevation and heart rate.

Outcome	Ni	S	Si	BC
Peak ST-segment elevation[a]	–0.047	–0.135	0.334**	0.010
	(0.095)[b]	(0.144)	(0.105)	(0.166)
Integrated ST-segment change[a]	–0.045	–0.167	0.309**	0.059
	(0.115)	(0.146)	(0.107)	(0.168)
Change in heart rate	0.179	0.111	0.160	–0.251
	(0.193)	(0.261)	(0.204)	(0.301)
Peak heart rate	–0.085	–0.391	0.020	0.386
	(0.141)	(0.191)	(0.142)	(0.220)

[a]Model fit with log-transformed responses. [b]SE in parentheses. **$p < 0.005$.

particle mass (Schauer et al. 1996), silicon may be serving as a marker of particles derived from the resuspension of road dust. Although nontoxic silicon and aluminum oxides represent a large fraction of the urban road dust, combustion-derived material, organic semivolatile compounds, brake dust, tire debris (Rogge et al. 1993), and pollen and other bioaerosols (Miguel et al. 1999) can also be abundant in road dust. Children living within approximately 100 m of a main road experience increased incidence of respiratory morbidity (van Vliet et al. 1997; Venn et al. 2001), which has been attributed to vehicular emissions. However, the role of resuspended road dust in eliciting these health effects cannot be excluded. Thus, although silicon or silicate concentrations representative of pure soil particles are not expected to induce adverse health effects, silicon as a surrogate for urban road dust containing a large number of toxic components may be responsible for the effects observed in this study, as well as those observed in our previous investigations.

Mechanisms of enhanced ischemia. Myocardial ischemia results from an imbalance between myocardial oxygen demand and supply. Coronary blood flow is an important determinant of oxygen supply to the heart (Ardehali and Ports 1990). In the setting of coronary artery occlusion, oxygen supply to the region at risk of ischemia depends primarily on the extent of collateral circulation in that region. Particulate exposure may enhance occlusion-induced ischemia by increasing the resistance or reactivity of collateral blood vessels. This notion is supported by recent evidence of particulate-related vascular changes: *a*) Increased circulating levels of the vasoactive peptide endothelin have been reported in rats (Bouthillier et al. 1998; Vincent et al. 2001a) and in healthy adults (Vincent et al. 2001b) exposed to urban particles; *b*) endothelial cell activation by ultrastructural criteria was noted in the coronary vasculature of dogs from high pollution areas but not in those from low pollution areas (Calderon-Garciduenas et al. 2001); *c*) acute brachial artery vasoconstriction has been reported in humans after exposure to CAPs and ozone (Brooke et al. 2002); and *d*) vasoconstriction of pulmonary (Batalha et al. 2002) and coronary (Godleski JJ. Unpublished observations) vessels has been observed in CAPs-exposed rats. Together, these findings identify multiple factors by which ambient air particles might contribute to coronary vasoconstriction and myocardial ischemia.

Myocardial oxygen supply also depends on the oxygen-carrying capacity of the blood, which is determined by the hemoglobin content of the blood and the systemic oxygenation (Ardehali and Ports 1990). Decreases in circulating red blood cell (RBC) count, hemoglobin concentration, and hematocrit have been correlated with particulate exposure in humans (Seaton et al. 1999) and in dogs (Clarke et al. 2000), raising the possibility that enhanced ischemia may also be attributed to a decrease in the oxygen-carrying capacity of the blood. In a hematologic study carried out on the same dogs in parallel with the current study, Savage et al. (2002) found decreased RBC and platelet counts, hemoglobin concentration, and hematocrit after CAPs exposure. As the mechanism of these changes are not presently known, it is unclear whether the changes in RBC indices are responsible for the enhanced ST-segment elevation observed in the present study.

Particulate-induced changes in myocardial oxygen consumption could conceivably explain our results. Myocardial oxygen consumption can be estimated by the product of heart rate and systolic blood pressure (Rooke and Feigl 1982). We did not measure blood pressure during these experiments, so we cannot rule out a particulate-induced increase in myocardial oxygen consumption. However, as particulate exposure did not affect the occlusion-induced change in heart rate, it seems unlikely that increased metabolic demand is the dominant mechanism responsible for the observed ST-segment changes.

Model considerations. The degree of ischemic injury produced during brief coronary artery occlusion is reflected by changes in the magnitude of ST-segment elevation as well as the overall duration. Several investigators have demonstrated that ST-segment elevation measured early after coronary artery occlusion correlates with reductions in myocardial blood flow (Heng et al. 1976; Irvin and Cobb 1977; Kjekshus et al. 1972; Wégria et al. 1949) and cellular damage as estimated by myocardial creatine phosphokinase activity (Heng et al. 1976; Maroko et al. 1971). It is now postulated that ST-segment changes are caused by intracellular electrical potential differences between normal and ischemic myocytes during different phases of the cardiac cycle. This current of injury may arise either from a change in the cellular resting potential (diastolic current) or from an unopposed current flowing from the injured area during systole (systolic current) (Fisch 1997). However, the mechanisms underlying acute ST-segment changes are complex and still not completely understood [reviewed by Kléber (2000)]. Therefore, studies that examine the effects of particulate matter using more direct measures of myocardial ischemia and cellular damage are needed. It should also be noted that the effects of particulate air pollution on ischemia may be different or may be mediated by different mechanisms in the elderly and in those with cardiovascular disease than in an animal with experimental coronary artery occlusion.

Summary and Conclusions

The present study indicates that under controlled laboratory conditions, concentrated ambient air particles significantly exacerbate myocardial ischemia during acute coronary artery occlusion in conscious canines. These observations carry important implications because they suggest a mechanism for the increased cardiovascular morbidity and mortality associated with environmental pollutants. The clinical relevance of our findings and the importance of further investigation of pathologic mechanisms is underscored by the recent investigation of Pekkanen and colleagues (2002), who demonstrated that air pollution worsens exercise-induced ST-segment changes in patients with coronary artery disease.

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