EFFECTS OF CONTROLLED OZONE EXPOSURE IN VOLUNTEERS WITH CARDIOVASCULAR DISEASE

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ABSTRACT

We hypothesized, on the basis of epidemiologic and animal toxicologic findings, that exposure to ozone (O_3) in air pollution can acutely impair cardiovascular function in susceptible human populations. We tested this hypothesis in 10 hypertensive and 6 healthy male volunteers aged 40-78 yr. Each subject, after catheterization of the right heart and a radial artery, was exposed in an environmentally controlled chamber to filtered air (FA) on one day and to 0.3 ppm O_3 on the following day, for 3-hr periods with intermittent exercise. Relative to FA, O_3 exposure induced little change in cardiac index, pressures and resistances in the central, pulmonary, or peripheral circulations, cardiac enzymes, homeostatic hormones (norepinephrine, epinephrine), or arterial oxygen saturation, as measured at rest after exposure. Stroke volume and central venous pressure showed small changes associated with O_3 exposure, which were statistically but not clinically significant. During O_3 exposure, heart rate increased relative to FA, even though ventilation rate did not increase. Post-O_3-exposure resting heart rate also increased. Ozone exposure elicited statistically and clinically significant respiratory effects, i.e., increased the mean alveolar-arterial oxygen gradient by 10 mm Hg ($P < 0.01$), increased respiratory symptoms slightly but significantly ($P < 0.05$), and decreased mean forced vital capacity and FEV_1 by >5% ($P < 0.01$). These effects did not differ significantly between hypertensive and healthy subjects. This study, although limited by a non-ideal design and small sample, suggests that direct acute effects of moderate O_3 exposure on cardiac function are unlikely in typical ambulatory healthy or hypertensive middle-aged and older men. However, O_3 exposure can impair gas exchange, thereby indirectly increasing the hemodynamic load on the cardiovascular system. This, in turn, increases oxygen demands of a stressed myocardium, to a degree that may be clinically important in some patients with preexisting cardiopulmonary impairment.

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CONCLUSIONS

We found little direct unfavorable effect of O_3 on the heart and vasculature, at an effective dose level comparable to a worst-case Southern California ambient exposure, either in representatives of a presumed "high-risk" population (hypertensives) or in healthy volunteers. Thus, our results do not strongly support the hypothesis that direct O_3 effects previously reported in laboratory animals can occur in humans at realistic exposure levels. Still, we cannot rule out such effects, given that we studied only a small number of relatively low-risk hypertensive volunteers.

We found important new evidence that pulmonary effects of O_3 potentially increase the hemodynamic load and oxygen demands of the heart, and thereby place patients with cardiovascular disease especially at risk whether or not O_3 directly affects the circulation. Specifically, we found that the alveolar-arterial oxygen gradient increased to a statistically and clinically significant degree after O_3 exposure (relative to filtered air), in conjunction with a small but statistically significant loss in forced expiratory function. This suggests that O_3 affected the airways in a manner causing a decrease in oxygen delivery to the blood. Rate-pressure product also showed a statistically significant increase, suggesting that the heart had to work harder to compensate for this reduced oxygen delivery.

RECOMMENDATIONS

These findings are most significant for people with chronic cardiopulmonary impairments more severe than those of our subjects, i.e., people for whom even a slight further impairment of oxygen delivery to the heart or to the rest of the body threatens serious consequences. Such people (e.g., patients with chronic obstructive pulmonary disease, coronary artery disease, or congestive heart failure) should be studied further to document their short-term responses to ambient air pollution exposures and to carefully controlled laboratory exposures. Relatively noninvasive measures of response (e.g. ambulatory electrocardiogram and blood pressure monitoring, radiologic or echocardiographic imaging to assess heart function) would be preferable in studies of these higher-risk populations.

INTRODUCTION

Ozone (O_3) pollution occurs on warm sunny days within and downwind of most urban areas in North America. Short-term respiratory irritant responses and bronchoalveolar inflammatory changes have been consistently documented in controlled O_3 exposures of human volunteers at concentrations attainable in ambient pollution [Lippmann, 1993; Gong and Linn, 1994; Bascom et al., 1996]. Excess mortality (including cardiovascular and respiratory mortality) has been associated with contemporary O_3 levels in Los Angeles [Kinney and Ozkaynak, 1991; Kinney et al., 1994; Bascom et al., 1996; Ozkaynak et al., 1996] and London [Anderson et al., 1996]. Although specific pulmonary and extrapulmonary causes or mechanisms for these deaths are difficult to identify, cardiovascular dysfunction or diseases are suspected to contribute, given that they are associated with a large proportion of all deaths. If acute cardiovascular illnesses or deaths do result from O_3 exposure, they might reflect either direct effects on the heart or blood vessels, or effects secondary to pulmonary dysfunction, or pulmonary vascular alterations. It is also possible that metabolized cytokine or oxidative products activated by O_3 exposure might result in increased stress for which an already compromised cardiovascular system might be unable to compensate.

Hemodynamic results from controlled O_3 exposure studies of animals and humans are mixed. Studies of rodents suggest acute cardiovascular dysfunction [Uchiyama et al., 1986; Uchiyama and Yokoyama, 1989; Arito et al., 1990], microscopic myocardial pathology [Rahman et al., 1992], and abnormal myocardial protein synthesis [Kelly and Birch, 1993] resulting from O_3 exposures within or modestly above the ambient concentration range. Ozone-induced alterations in core temperatures (producing hypothermia) may also integrally modulate lung uptake of O_3 and subsequent cardiopulmonary toxic effects [Watkinson et al., 1995]. Acute O_3-induced reductions in cardiac output have been reported in anesthetized dogs [Friedman et al., 1983] but not in conscious resting sheep preparations [Schelegle et al., 1990; Gunther et al., 1992], suggesting differences related to species, methodology, and/or study design. Human cardiovascular data are limited to several controlled O_3 studies, all with small number of subjects and whose cardiovascular responses to acute oxidant exposure were assessed by noninvasive techniques [Folinsbee et al, 1978; Superko et al., 1984; Drechsler-Parks, 1995]. Cardiac output and other measured hemodynamic variables did not significantly change after acute oxidant exposure in healthy adults, age 20-85 yrs. [Folinsbee et al, 1978; Drechsler-Parks, 1995], although the exposure combination of 0.60 ppm nitrogen dioxide and 0.45 ppm O_3 was associated with a lower cardiac output than that measured with O_3 and filtered air exposures ($P < 0.05$). No significant O_3 effects on heart rate, blood pressure, rate-pressure product, and electrocardiogram (ECG) were found in 6 male volunteers with coronary artery disease (CAD) after 40 min exercise in 0.2 or 0.3 ppm O_3 [Superko et al., 1984]. Thus, further study of the cardiovascular effects of O_3 is warranted in view of the high prevalence of cardiovascular diseases in the United States, their significant morbidity and mortality rates, and the limited understanding of pollutant-related health effects on patients with cardiovascular disorders. Invasive physiologic monitoring may accurately detect acute cardiovascular effects of O_3 in humans. Accumulating experience and technological improvements has alleviated concerns about cost, discomfort, and risk of complications from invasive monitoring. We hypothesized that O_3 exposure within the ambient range can directly cause clinically significant acute cardiovascular dysfunction, both in stable patients with preexisting cardiovascular disease (essential hypertension) and in otherwise similar healthy volunteers. To test this hypothesis, we performed controlled O_3 exposures of volunteers with right heart and radial artery catheterizations, allowing repeated direct measurements of hemodynamics and blood sampling for assays of enzymes related to myocardial ischemia and catecholamine.

METHODS

Subjects

The study was approved by the institutional review board and hospital administration. Prospective volunteers were recruited by word-of-mouth, printed advertisements, and by referrals from local physicians. Each volunteer was fully apprised of the study's purpose, procedures, benefits, and risks, and signed an institutionally-approved informed consent form, prior to medical screening. Each subject received a participation fee.

We recruited 10 subjects with essential hypertension and 6 healthy controls, all of whom were male, >40 yrs. of age, and nonsmoking >2 yrs. (Table 1). The patient group had physician-documented essential hypertension which was being treated either pharmacologically for >1 year or with nonpharmacologic methods (one subject). Healthy subjects had no cardiopulmonary symptoms or disease according to history and screening evaluation. All subjects had a stable and clinically unremarkable resting ECG and were clinically free of accelerated or uncontrollable hypertension, left ventricular hypertrophy, congestive heart failure (CHF), myocardial infarction, cardiac dysrhythmias, or other acute or active cardiovascular disease, as well as pulmonary diseases including asthma.

Screening tests included a general medical history (including any drugs and allergies); a physical examination; a battery of cardiopulmonary tests, including resting 12-lead ECG; spirometry; venous hemogram, platelet count, prothrombin time, and partial thromboplastin time; urine screening for illicit drugs which might affect hemodynamics; echocardiogram (on a separate day); and cardiac treadmill test, according to a modified Bruce protocol on a CASE 12/15 treadmill stress test system (Marquette Electronics), with ECG monitoring. All subjects underwent exercise stress testing to evaluate possible pre-existing myocardial ischemia and exercise tolerance. The hypertensive subjects remained on their anti-hypertensive medication(s) (Table 1) during the screening evaluation. Otherwise, anti-hypertensive medications, nonsteroidal anti-inflammatory drugs (including aspirin), vitamin supplements, and caffeinated and alcoholic products were stopped at least 7 days prior to and during the exposure sessions. Hypertensive subjects were monitored during their individualized medication withdrawal period. The target blood pressure parameters were a resting systolic blood pressure of 140-180 mm Hg and diastolic blood pressure of 90-110 mm Hg.

All subjects showed normal or unremarkable blood and urine tests, spirometry, 12-lead ECG, and echocardiogram except for a mild obstructive ventilatory defect in one hypertensive ex-smoker (subject 2261). Fourteen subjects were either never smokers or ex-smokers for >3 yr. Hypertensive subjects tended to be older, taller, and heavier than the normal subjects. Three of the hypertensive subjects and one healthy subject demonstrated systolic hypertension (>225 mm Hg) during maximum exercise testing. One hypertensive subject (2263) showed exercise-induced ischemic ECG changes. Exercise results were otherwise normal or unremarkable.

Protocol

Exposures.

Qualified subjects were studied one at a time year-round. Subjects were advised to avoid respiratory irritants including air pollution and environmental tobacco smoke prior to the study. Ambient O_3 concentrations in the laboratory vicinity during the smoggiest summer-autumn days did not exceed 50% of the experimental concentration. Each subject was exposed separately to filtered air (FA) alone and to 0.30 ppm O_3 in FA for 3 hr each with intermittent exercise. This O_3 exposure models a "worst-case" summer afternoon in metropolitan Los Angeles, and is sufficient to elicit reversible lung dysfunction in healthy subjects.

Filtered-air exposure was always performed on the first study day (Day 1), immediately following right-heart and radial-artery catheterizations (described below), and O_3 exposure on the next day (Day 2). Thus, subjects did not undergo both major experimental stresses (catheterizations and O_3 exposure) on the same day. This nonrandomized design was considered ethical and practical for an air pollution study with no direct precedent, involving invasive procedures in human volunteers.

Ozone was generated from medical oxygen by high-voltage discharge (Sander IV ozonator). Chamber levels of O_3 were monitored continuously during both exposure sessions by calibrated ultraviolet photometers (Dasibi). Nitrogen oxides were monitored by chemiluminescent analyzers (Thermo-Electron and Monitor Labs) which were calibrated daily.

Each subject was admitted to the hospital on a Wednesday morning (Day 1). Symptom ratings, vital signs, cardiopulmonary examination, 12-lead ECG, and spirometry were initially obtained during rest in a special procedures room adjacent to the exposure laboratory. Continuous ECG monitoring was initiated, and blood was collected from an arm vein for determination of pre-catheterization plasma catecholamine levels. Once the vascular catheters were passed and secured, baseline supine measurements were recorded after a 30- to 60-min stabilization period. The subject then underwent a 3-hr single-blinded exposure to FA inside an environmentally controlled chamber [Weymer et al., 1994] at 70°F and 50% relative humidity. Approximately 0.05 ppm O_3 was transiently generated in the chamber prior to the subject's entry to produce the characteristic odor and maintain the single-blinded exposure conditions. A study physician (HG) was continuously inside the chamber to monitor the subject's clinical status, catheter integrity, cardiac rhythm, and hemodynamic readings. During exposure, the subject alternately exercised moderately on an electric cycle ergometer (Corival, Quinton) for 15 min (target ventilation rate 30-40 L/min) and rested for 15 min, with continuous ECG and hemodynamic monitoring, visual observation, and symptom assessment. Symptoms and spirometry were also determined hourly during exposure. Breathing was unencumbered except for 5-min intervals during the last rest and exercise periods of each hour, at which time minute ventilation was measured using a mouthpiece, non-rebreathing valve, and dry gas meter. For 7 hypertensive and 4 healthy subjects, catheter-derived hemodynamic measurements (heart rate, blood pressure, and pulmonary artery pressure) were recorded in the upright position during the final minutes of each rest and exercise period. Heart rate and blood pressure were recorded by cuff measurement during the final minutes of each rest and exercise period for the other subjects. The subject consumed fluids *ad libitum* and lunch during the exposure. Within 30-60 min following completion of exposure, the subject underwent resting cardiovascular measurements, 12-lead ECG, blood collection, and symptom recording, as well as cardiopulmonary auscultation by the study physician. Follow-up symptom and hemodynamic measurements were also obtained 2 hr later.

With catheters still in place and the pulmonary artery catheter tip retracted to the right atrium, the subject stayed overnight in the hospital's observation unit under continuous ECG monitoring and nursing supervision. The subject received meals with noncaffeinated and nonalcoholic beverages and symptomatic management of catheter discomfort (as-needed oral acetaminophen and temporary ice packs). No sedative or anti-hypertensive drugs were administered between or during exposures (one exception: see Complications).

During the next morning (Thursday, Day 2), the pulmonary artery catheter tip was refloated to a pulmonary wedge position. The subject then underwent all procedures (except catheterization) at approximately the same times as on Day 1, including an exposure to 0.3 ppm O_3 in FA. Catheters were removed after completion of all measurements, and the catheter sites were cleaned and bandaged with adequate hemostasis. The study physician evaluated each subject, and then discharged the subject from the hospital. The subject then resumed his anti-hypertensive medication(s), if any.

Catheterization and Hemodynamic Measurements.

Pressure transducers (Telos Medical) were zero-referenced to the atmosphere with phlebostatic access at the level of the left atrium before and after exposures (and during exposure when measurements were conducted). Pulmonary artery and radial artery catheters were placed percutaneously after local infiltration with 1% lidocaine, under sterile conditions [Swan et al., 1970; Hathaway, 1978]. An 8.5-French sheath introducer (Arrow International) was placed in the right internal jugular vein, followed by a 7.5-French flow-directed thermodilution pulmonary artery catheter (Swan-Ganz; VIP with Thromboshield coating, American Edwards Laboratories) with a catheter contamination shield (Cath-Gard, Arrow) connecting the sheath adapter and proximal catheter. The catheter tip was then passed into the central circulation, with reference to characteristic right-heart pressures and waveforms, until the pulmonary wedge pressure was determined. Cardiac output was measured at least in triplicate by the thermodilution method, via infusion of iced saline and a computer for cardiac output (Hewlett-Packard). Core temperature was measured continuously with a thermistor in the pulmonary artery catheter. A portable chest radiograph was always performed following catheterization to confirm the vascular location of the catheter tip (always in the right pulmonary artery) and to rule out pneumothorax and other acute lung problems. A 20-gauge radial artery catheter (Arrow) was usually placed in the left radial artery under sterile conditions (following an Allen test for collateral ulnar arterial flow and local anesthesia) for continuous arterial pressure monitoring and scheduled blood sampling for blood gases and chemistries. The indwelling catheters were connected to an integrated physiologic monitoring and recording system (Eagle monitor using 7025 software, Marquette), which displayed real-time waveforms and digital values for configured ECG, heart rate, hemodynamic pressures, cardiac output, arterial-catheter (a-line) blood pressure, noninvasive (cuff) blood pressure, and (oximetric) SaO_2, and core temperature. Catheter patency was maintained with continuous infusion of saline containing heparin solution (6 units per hour). An automated oscillometric cuff sphygmomanometer (placed on the noncatheterized arm) was interfaced with the Eagle monitor and measured blood pressure every 5-10 min. ECG, pressures, and other physiologic parameters were recorded on thermal paper tracings (Archivist, Marquette).

A complete set of hemodynamic variables was always measured before and after each exposure during quiet supine rest. Measured hemodynamic variables included the heart rate, central venous pressure, pulmonary wedge pressure, cardiac output, and systolic, diastolic, and mean arterial and pulmonary artery pressures. The following variables were calculated (via the programmed Eagle computer) according to standard formulas: cardiac index = cardiac output/body-surface area; stroke volume = cardiac output/heart rate X 1000; systemic vascular-resistance index = 79.92 X (mean arterial pressure - central venous pressure)/cardiac index; pulmonary vascular resistance index = 79.92 X (mean pulmonary-artery pressure - pulmonary wedge pressure)/cardiac index; left ventricular stroke-work index = stroke-volume X (mean arterial pressure - pulmonary wedge pressure) X 0.0136; right ventricular stroke-work index = stroke-volume X (mean pulmonary-artery pressure - central venous pressure) X 0.0136; and rate-pressure product = heart rate X systolic blood pressure. Table 2 shows the units of measurement and abbreviations for these variables. Automated sphygmomanometer (cuff) blood-pressure data were more complete than a-line data, which were unobtainable or incomplete in 7 subjects. (Locally anesthetized brachial artery punctures supplied the necessary blood samples in those instances.)

Blood Analyses. Blood samples were collected from the pulmonary artery and arterial catheters (after discarding heparinized catheter volume) immediately before and after each exposure. Blood for lactate dehydrogenase (LDH) and creatine kinase (CK) was collected from both catheters in coded vacuum tubes pretreated with preservatives, and absolute serum levels and percentages of isoenzymes were measured during each study day in the hospital's Chemistry Laboratory, according to standard methods. Aliquots were also processed, coded, and frozen at -70°C in coded tubes with preservatives for subsequent assays of serum troponin T (from both catheters), epinephrine, and norepinephrine (from pulmonary artery

catheter only), according to established radioenzymatic methods [Peuler et al., 1977]. Troponin T, a sensitive biochemical marker of myocardial-cell injury [Katus et al., 1991; Hamm et al., 1992; Adams et al., 1993], was quantitatively determined with an enzyme immunologic assay (Boehringer). Routine venous blood biochemistries (Chemzyme Plus, SmithKline Beecham Clinical Laboratories) were also measured before and after exposures and on Day 3. All laboratory technicians were blinded to the study conditions.

Respiratory Testing.

Immediately before and after each exposure, symptoms were recorded and forced vital capacity (FVC) and forced expired volume in one second (FEV_1) were measured with a Spirotech rolling seal spirometer, calibrated daily with a 3-L volumetric syringe. Respiratory rate was counted visually and ventilation rate was determined from a dry gas meter. Arterial blood samples were anaerobically collected, kept on ice, and analyzed within an hour after each collection for blood gas parameters in the hospital's pulmonary function laboratory (ABL520 analyzer, Radiometer) by a technician blinded to exposure conditions. The abbreviated alveolar air equation was used to calculate the alveolar-arterial oxygen tension gradient [(A-a)PaO_2]. Values for SaO_2 were also derived from arterial blood gas analyses. Noninvasive arterial oxygen saturation (SaO_2) was continuously monitored before, during, and after exposure with a pulse oximeter (Nellcor) interfaced with the Eagle monitor. Statistical results from pulse oximetry and derived SaO_2 data were generally similar. The derived SaO_2 data are reported here, since they were complete, whereas some pulse oximeter data were missing.

Symptom Assessment.

Immediately before each day's exposure and at the end of each rest/exercise cycle, the subject filled out a standardized symptom questionnaire. The questionnaire recorded 11 respiratory and systemic symptoms commonly reported in clinical studies of respiratory irritants [Linn et al., 1983a; Hackney and Linn, 1985], plus 4 additional symptoms related to acute cardiovascular dysfunction. The questionnaire also included an item for other (miscellaneous) symptoms. The subject was instructed to score each symptom in a range from 0 (not present) to 40 (incapacitating). Scores for particular symptoms were summed to determine a total score, a subtotal for respiratory symptoms, and a subtotal for cardiovascular symptoms. (For details of scoring, see footnote to Table 8 in Results section.)

Followup.

Approximately 24 hours after the last exposure (Friday: Day 3), each subject returned to the laboratory for medical assessment by the study physician. At that time the physician evaluated the catheter sites, symptom ratings, vital signs (including cuff blood pressure via aneroid sphygmomanometer), and performed a cardiopulmonary examination, a 12-lead ECG, spirometry, and venous sampling for measurement of routine blood biochemistry's as well as cardiac enzymes.

Data Analysis

BMDP-Dynamic statistical software (SPSS, Inc., Chicago) was used in all analyses. Our experimental hypothesis would be supported if cardiovascular function changed during or following exposure on Day 2 (O_3) more unfavorably than on Day 1 (FA), to a statistically significant ($P < 0.05$) degree. To test the hypothesis, pre-exposure and immediate post-exposure data for each experimental variable were subjected to analysis of variance (ANOVA) with repeated measures. The analytical model included two repeated-measures factors, day (1 vs. 2) and time (pre vs. post), and a grouping factor, clinical status (normal vs. hypertensive). A significant day-time interaction, with the most unfavorable mean measurement post-O_3, would support the hypothesis. For variables showing significant O_3 effects, followup ANOVA assessed the rate of recovery by comparing later (2-hr)

measurements with immediate post-exposure measurements. A significant grouping effect would indicate overall differences between normal and hypertensive subjects, while a significant interaction between grouping and day or time would indicate a different response to the exposure protocol between normals and hypertensives. Additional ANOVA were performed for normal and hypertensive subjects separately, as well as for all subjects pooled (ignoring clinical status). Also, pairwise statistical comparisons were performed between pre-FA and pre-O_3 measurements. A significant difference between the two pre-exposure measurements (a uniform baseline condition) would suggest that the stress of catheterization influenced the pre-FA measurement. In such as case, the pairwise comparison of post-exposure measurements might be a more reliable test for an O_3 effect than the day-time interaction described above.

Data were incomplete for certain variables, due to occasional technical problems. These variables were analyzed as described above, excluding subjects with incomplete data, and were reanalyzed with maximum likelihood estimation (MLE) of missing data using the Newton-Raphson algorithm [Dixon, 1992]. The conventional analysis and the reanalysis with MLE usually gave similar statistical conclusions (see Results).

Additional ANOVA were performed on heart rate, ventilation rate, blood pressure, and mean arterial pressure data collected during exposures, in the first and last periods of exercise/rest. These analyses were similar to the analyses previously described, except that they included an additional repeated-measures factor, i.e., exercise vs. rest.

For systolic and diastolic blood pressures, data were available from automated sphygmomanometry (cuff) and the arterial catheter (a-line). The a-line data were expected to be more accurate, but were more often missing than the cuff data. Accordingly, three separate analyses were performed, i.e., with cuff data only, with a-line data only, and with a "complete" data set including all a-line measurements plus cuff measurements to replace missing a-line data. Statistical results from all three were generally similar, so only the "complete" data analysis is presented here. (A more accurate prediction of missing a-line data from cuff data was attempted using regression analysis. It was unsuccessful because the apparent regression relationships differed between subjects, and were often non-significant, probably because of few usable data points and narrow pressure ranges for a given subject.)

RESULTS

Exposure Conditions

The 3-hour-average O_3 concentrations during all O_3 chamber exposures (recorded at 5-min intervals) ranged from 0.294 to 0.312 ppm. Chamber O_3 levels on FA exposure days did not exceed 0.01 ppm. This represents the background O_3 concentration resulting from ambient reactions within the exposure chamber. The maximum standard deviations of O_3 levels did not exceed 0.027 ppm. It should be noted that the background O_3 concentration is less than one-half of the standard deviation for the O_3 exposures, and thus represents a concentration of 0.0 ppm within the context of the measurements. The chamber concentration of NOx did not exceed 0.02 ppm.

Climate control in the chamber during the 3-hr exposures was less precise; temperature ranged from 22 to 25 C and relative humidity ranged from 46% to 64%. These ranges are typical of the exposure facility.

Cardiovascular Comparison of Controls and Hypertensives

Screening cuff BP averaged 122/82 and 147/88 mm Hg in the control and hypertensive subjects (on medication), respectively. Pre-catheterization BP on Day 1 averaged 119/74 and 147/91 mm Hg in the controls and hypertensives (off medication), respectively. Three of 10 hypertensive subjects had elevated screening BPd (90 mm Hg), whereas 6 of 10 had diastolic hypertension prior to catheterization on Day 1. Thus, the withdrawal of anti-hypertensive therapy modestly increased BPd without changing BPs. Differences between screening and pre-catheterization BP were non-significant ($P > 0.3$, ANOVA) for the hypertensives and for all subjects. Overall differences in BPs and BPd were significant ($P = 0.005$ and $P = 0.04$, respectively) between controls and hypertensives.

In general, expected differences in resting hemodynamics between the healthy and hypertensive subjects were observed (26). Overall mean BPs from pre- and post-exposure measurements on both exposures was 154 mm Hg in hypertensives, compared to 128 mm Hg in normals ($P = 0.01$). Mean BPd tended to be higher in hypertensives than in controls (83 vs. 70 mm Hg, respectively; $P = 0.07$). Mean arterial pressure was higher in hypertensives (107 mm Hg) than in the control subjects (92 mm Hg; $P = 0.05$). Corresponding MPAP was 17 mm Hg for hypertensives and 12 mm Hg for controls ($P = 0.03$). The initial (pre-FA) CI was higher in hypertensives than in the control subjects (mean 3.67 vs. 2.68 L/min/m^2; $P = 0.003$), although the overall means were less divergent (3.69 for hypertensives vs. 3.21 L/min/m^2 for controls; $P = 0.07$). Other cardiovascular end points did not show statistically significant differences between the two groups.

Cardiovascular Responses

Table 3 summarizes the primary physiologic results for hypertensives, controls, and both groups combined. Within the limitations of this small data set, the hypertensive and control subjects appeared to respond similarly to exposures. That is, the day-status interaction was non-significant for all primary endpoints. Changes pre- to post-exposure in BPs, BPd, MAP, MPAP, PAWP, and CI were not significantly different between FA and O_3. Central venous pressure was the only pulmonary-artery catheter measurement to show a statistically (but not clinically) significant O_3 effect both by the day-time interaction and by the comparison of post-O_3 vs. post-FA data for all subjects.

Mean CVP decreased by 1.4 mm Hg during O_3 exposure, compared to an increase of 0.5 mm Hg with FA (interaction $p < 0.049$ in 2-factor ANOVA including all subjects). Mean CVP was also lower overall on Day 2, relative to Day 1 (day $P < 0.02$). Pairwise comparison showed that the post-O_3 mean of 3.7 mm Hg was significantly lower than the post-FA mean of 5.9 mm

Hg (P = 0.007). The apparent O_3 effect did not reverse within 2 hr, according to a followup ANOVA which showed only a minimal, non-significant difference between immediate-post-exposure and 2-hr-post-exposure mean CVP.

Resting heart rate increased after O_3, relative to its pre-exposure value, significantly more than after FA. The increase in HR, with little change in BPs, gave rise to a significant increase in RPP. If RPP was calculated using pulse pressure (thus allowing influence by BPd as well as BPs), the statistical conclusions did not change. There were no clinically significant changes in 12-lead ECGs or on ECG telemetry throughout the exposures (see Complications). Stroke volume, pulmonary vascular resistance index, and left- and right-ventricular stroke work indices showed no significant exposure-related variation for pooled data (Table 3). However, stroke volume showed a difference in O_3 response between hypertensive and normal subjects (Table 3), which was statistically significant in the analysis with MLE ($P < 0.01$) and marginally significant in the conventional analysis which excluded one subject with incomplete data ($P < 0.05$). Normals' mean stroke volume rose in FA but fell (from a higher pre-exposure value) in O_3. Hypertensives' mean stroke volume changed little in FA but rose (from a lower pre-exposure value) in O_3. This difference was not considered clinically significant. Cardiac index showed a statistically significant increase from pre- to post-exposure on both days ($P<0.05$); the increase was larger with O_3, but not significantly different from that with FA.

Mean arterial pressure, pulmonary artery wedge pressure, and systemic vascular resistance index showed significant overall differences between Days 1 and 2 (being lower overall on Day 2), and significant decreases pre- to post-exposure on both days, but no significant O_3 effect in two-factor ANOVA. Post-exposure PAW was significantly lower post-O_3 than post-FA ($P = 0.02$) by pairwise comparison. Mean pulmonary artery pressure was significantly lower overall on Day 2, but showed no significant change pre- to post-exposure. There were no clinically significant changes in 12-lead ECGs before and after exposures. No ischemic changes or significant arrhythmias were noted on ECG monitoring during and after the exposures.

Mean core body temperature significantly increased during both exposures, by 0.2-0.3° C from a baseline of 36.4-36.5° C, similarly in hypertensives and controls. .

Table 4 summarizes physiologic results obtained during the first and last exercise/rest periods in both groups. Exercise significantly increased BPs (but not BPd), HR, MPAP, and V_E, as expected. Overall BP during exercise averaged 145/80 mm Hg in controls and 169/88 mm Hg in the hypertensives. Neither exercise BP nor RPP showed statistically significant day, time, or interaction effects in 2-factor ANOVA.. Heart rate tended to rise during the final measurements, to a greater degree in O_3 than in FA ($P < 0.06$). This response pattern was consistent with that for pre- and post-exposure resting HR, which showed a statistically significant O_3 effect, as described previously.

Cardiac Enzymes

Enzyme values were not statistically different between the PA and arterial sources. Values from the PA catheter (representing mixed venous blood) were used in the statistical analyses since clinical reference values are based on venous samples. The concentrations of CK, LDH, the percentages of their respective isoenzymes, and troponin T were within the normal clinical range in all samples, including those on the followup day (Day 3). Total CK was significantly higher overall in hypertensives than in controls. As expected, the isoenzyme CK-BB was never detected (normal range 0%) and, in most samples, CK-MM (normal range 95-100%) constituted 100% of CK. LDH-3 was the only LDH isoenzyme to show a statistically significant change related to O_3, rising from 23.4 to 24.6 during FA exposure, and falling from 26.3 to 25.6 during O_3 exposure, in terms of mean percentage concentrations for all subjects. The heart-associated fraction of LDH (LDH-1) decreased during both exposures. The heart-associated fraction of CK (CK-MB) was detected in only 6 of 16 subjects, and in them, only at low levels. Most values for troponin T (normal range: 0.0 - 0.1 ng/ml) were below the limit of

detection (0.04 ng/ml), and preliminary inspection suggested that the few samples with detectable (but low) concentrations occurred more or less at random, so no formal analysis was attempted.

Hormonal Responses

Both catecholamines were substantially above the normal basal range throughout the study (Table 6). Pre- and post-exposure EPI levels showed a statistically significant O_3 effect, while NOREPI showed a similar response approaching statistical significance for hypertensives and for all subjects combined. The catecholamine response pattern was not significantly different between the two groups. However, the direction of change was unexpected, i.e., the overall catecholamine changes were modest during Day 1 (FA exposure), increased at the pre-O_3 measurement on Day 2, and then decreased after O_3 exposure to a level appreciably below the Day 1 levels. The catheterization procedure (Day 1) had no statistically significant effect on circulating catecholamine levels for hypertensives, controls, and all subjects combined, according to comparisons of peripheral venous blood obtained before catheterization versus PA samples obtained just after the catheterization. However, mean EPI rose from 45 to 82 ng/L in the control subjects. Atrial natriuretic factor and its components did not show significant O_3-induced changes in either group or for all subjects combined, except for a small decrease in pro-ANF 1-30 in the control subjects ($P = 0.04$).

Respiratory Responses

Respiratory responses are summarized in Table 7. Mean resting V_E in FA and O_3 was similar overall (14 L/min) and in the hypertensive (15 L/min) and control (11 L/min) groups. Hypertensives had higher overall exercise V_E (mean 36 L/min) than controls (mean 30 L/min) during exposures. Exercise V_E averaged 35 L/min in FA and 33 L/min in O_3 for all subjects combined. The diminution in V_E with O_3 was significant ($P < 0.007$, 2-factor ANOVA with all subjects combined) and contrasted with the significant rise in HR during O_3 exposures compared to FA (see above). Ventilation increased from early to later exercise sessions during both exposures (time effect $P < 0.012$).

Lung function differences between hypertensives and normals were non-significant. Both control and hypertensive subjects maintained essentially normal baseline lung function throughout both exposures. Mean FEV_1 decreased significantly in hypertensive and control subjects considered separately and in all subjects considered together, with O_3 exposures as compared to FA response (Table 7). Mean FVC also decreased significantly in the entire group, and in the controls considered separately. For all subjects combined, both mean FVC and FEV_1 decreased by approximately 6% during O_3 exposure, but changed little during FA exposure.

All subjects maintained arterial oxygenation throughout both exposures. All subjects' mean SaO_2 decreased significantly from pre- to post-exposure on both days, and was significantly lower overall on Day 2. The excess loss during O_3 exposure relative to FA (SaO_2, -0.3%) was not significant. However, in pairwise comparison, the post-O_3 mean of 94.7% was significantly lower than the post-FA mean of 95.7% ($P < 0.04$). Thirteen of the 16 subjects showed increased pre- to post-exposure (A-a)PO_2 during O_3 exposure, compared to FA (Figure). Mean (A-a)PO_2 increased <1 mm Hg in FA , but more than 10 mm Hg in O_3 from similar pre-exposure values in all subjects ($P < 0.005$). The difference between hypertensives and controls was non-significant.

Symptoms

The mean total symptom score (Table 8) changed little in FA, and increased during O_3 exposure to an extent that suggested a minimal (barely perceptible) worsening of one symptom in a typical subject. This increase was not statistically significant. However, the subtotal score for respiratory symptoms significantly increased during O_3 exposure ($P< 0.04$) for all subjects. When the hypertensive and control groups were compared, the difference in respiratory symptoms was not significant. The increase attributable to O_3 averaged 9 points, representing mild worsening of one symptom. One subject reported a severe sore throat during O_3 exposure. Otherwise, nearly all reported symptoms were rated minimal or mild. The subtotal score for cardiovascular symptoms showed no significant variation during exposures, and any minimal or mild symptoms were reported only rarely.

Complications

There were no short- or long-term medical complications related to the temporary withdrawal of anti-hypertensive medications or during placement, maintenance, or removal of the catheters in the subjects who completed the protocol. Catheter-related discomfort was well tolerated with symptomatic management. Chest radiographs following catheterizations were unremarkable. Hypertensive subject #2263 performed only the first two exercise sessions on both study days, because, on Day 1, he exhibited frequent ventricular ectopic beats during exercise that resolved at rest. No clinically significant arrhythmias were observed subsequently. This subject also required a single oral dose of short-acting nifedipine (10 mg) for increasing diastolic hypertension over 2 hr (Day 1) and 6.5 hr (Day 2) prior to the pre-exposure measurements.

Five additional hypertensive subjects were removed from the study early on Day 1 and their data were not used in the analysis. Two subjects declined to proceed shortly after beginning the initial catheterization. The protocol was terminated by the investigators in 3 subjects because the balloon-inflated PA catheter could not be successfully passed and/or transient but recurrent cardiac arrhythmias occurred (2 subjects), and transient hypotension occurred during the initial exercise period (one subject). None of the subjects developed further complications.

DISCUSSION

This first-ever study with detailed direct measurement of cardiovascular responses to O_3 in both healthy and hypertensive volunteer human subjects has shown little direct unfavorable effect of O_3 on the heart and vasculature. The effective dose used in this study was comparable to a worst-case Southern California ambient exposure. Thus, our results do not strongly support the hypothesis that direct O_3 effects previously reported in laboratory animals occur in humans at realistic exposure levels. Of course, the study design cannot wholly rule out such effects either, given that we studied only a small number of relatively low-risk hypertensive volunteers. As discussed later, the results provide important new evidence that pulmonary effects of O_3 potentially increase the hemodynamic load and oxygen demands of the heart, and thereby place patients with cardiovascular disease especially at risk whether or not O_3 directly affects the circulation.

We initially considered evaluating patients with inactive congestive heart failure (CHF) and stable coronary artery disease (CAD). The population with these diseases in the United States is large. The American Heart Association [1995] estimated that there are almost 5 million patients with CHF and over 6 million people with CAD, including 3 million with angina pectoris. However, CHF patients were considered to be at increased risk from the procedures, could not ethically undergo drug withdrawal, and have complex (confounding) neurohormonal interactions. Although CAD patients were actively recruited, few candidates ultimately agreed to participate or fulfilled the entry criteria. Future clinical studies may specifically target volunteers with stable CAD or CHF. Patients with stable essential hypertension were considered a practical and optimal group for this clinical investigation. An estimated 60 million adults have elevated blood pressure in the United States. Hypertension is both a dependent and an independent risk factor for serious cardiovascular morbidity and mortality [Frohlich et al., 1992].

Hemodynamics.

In general, myocardial function and systemic hemodynamics were maintained in the expected or normal range during and after acute O_3 exposure. The statistically most convincing cardiovascular change attributable to O_3 was the larger increase in resting heart rate after O_3 than after FA, relative to pre-exposure levels which were similar on both days. There was also a suggestion that O_3 tended to increase heart rate during exposure, in that exercise heart rates rose slightly on Day 2, while ventilation rates declined slightly. The accompanying increase in rate-pressure product was more difficult to interpret, in that it reflected pre-exposure differences between O_3 and FA, more than post-exposure differences. A slight decline in central venous pressure after O_3 was the only other cardiovascular change which was significant both by 2-factor (pre-post) and by pairwise (post-exposure only) analyses. Pulmonary artery wedge pressure also showed a slight decline relatable to O_3 but was significant only in the analysis restricted to post-exposure data. Accordingly, there was no evidence of pulmonary congestion or left ventricular dysfunction. Although other changes were non-significant, the overall pattern suggested that cardiac index tended to increase modestly and SVRI tended to decrease modestly following O_3 exposure. The clinical significance of these cardiovascular changes is uncertain. They could represent a homeostatic (hormonal?) response to maintain tissue oxygenation in the face of lung dysfunction and the resulting decreased oxygenation of the pulmonary circulation.

The previously reported hemodynamic findings related to acute O_3 exposure appear to be influenced by species, exposure conditions, and measurement techniques. Friedman et al [1983] reported reductions in arterial blood pressure (as well as PaO_2) in anesthetized, intubated dogs after 4-hr exposure to 0.3 and 1.0 ppm O_3. The relative hypotensive effect of acute O_3 exposure was subsequently supported by the finding of reduced mean blood pressure and heart rate in conscious rats [Uchiyama et al., 1986; Uchiyama et al., 1989; Arito

et al., 1990]. However, Tepper et al [1990] observed that 135-min exposures to 0.12 to 1.0 ppm O_3 (with intermittent carbon dioxide inhalation to increase ventilation) did not significantly affect mean blood pressure or arterial blood gases in conscious rats. However, there was a slight increase in $PaCO_2$ despite dose-dependent increases in respiratory frequency and reductions in tidal volume. In addition, other investigators [Schelegle et al., 1990; Gunther et al., 1992] found no changes in cardiac output, mean aortic pressure, pulmonary artery pressure, and arterial blood gases in instrumented, awake sheep exposed to O_3 levels as high as 4 ppm for 3 hr. Similarly, noninvasive data derived from several controlled human studies have indicated nonsignificant cardiovascular findings. Cardiac output and other measured hemodynamic variables did not significantly change after acute oxidant exposure in 13 healthy adults, age 20-85 yr. [Folinsbee et al, 1978; Drechsler-Parks, 1995]. However, exposure to a combination of 0.60 ppm nitrogen dioxide and 0.45 ppm O_3 was associated with a lower cardiac output than that measured with O_3 and filtered air exposures ($P < 0.05$). No significant O_3 effects on heart rate, blood pressure, rate-pressure product, and electrocardiogram were found in 6 male volunteers with CAD after 40 min exercise in 0.2 or 0.3 ppm O_3 [Superko et al., 1984]. The above human studies [Folinsbee et al, 1978; Superko et al., 1984; Drechsler-Parks, 1995] involved small numbers of healthy subjects, noninvasive cardiovascular techniques, and different study design and types of subjects. Nonetheless, our results generally support previous results in man with, for the first time, directly measured hemodynamics via indwelling vascular catheters.

Temperature.

Core temperatures increased slightly during both exposures, unlike the finding of hypothermia in unanesthetized, unrestrained O_3-exposed rats with radiotelemetric monitoring [Watkinson et al., 1995, 1996]. Core temperatures were also unchanged in unanesthetized sheep despite exposure to O_3 levels up to 4 ppm for 3 hr [Schelegle et al, 1990]. Thus, differences in species (and strains) are likely factors accounting for the different thermal responses.

Cardiac Enzymes.

Three types of biochemical markers for myocardial ischemia or injury were measured; all values were either within normal range or non-detectable in all subjects. Although the blood collection schedule for CK and LDH over the 3-day protocol may have missed early, transient changes (especially for CK-MB), troponin T was included in this assay battery since it is reportedly a highly sensitive and specific marker of myocardial-cell injury [Katus et al., 1991; Hamm et al., 1992; Adams et al., 1993]. Notably, there was no evidence of biochemical ischemia after either exposure or on the third (follow-up) day. Unchanged 12-lead ECGs and ECG monitoring corroborated the biochemical findings.

Hormones

Plasma catecholamine concentrations were measured to determine the overall stress elicited by the catheterizations and exposures. In particular, plasma norepinephrine is an established marker for systemic sympathetic activity. The expected adrenergic stimulation and elevated catecholamines were considered likely to exert active effects on blood pressure, heart rate, coronary artery tone, and myocardial function (increasing oxygen consumption and demand) which might, in turn, precipitate cardiovascular events in hypertensive patients [Superko et al., 1984]. The results were generally inconsistent with these expectations. Even at the time of venous blood sampling before the experimental procedures, catecholamine levels (particularly for norepinephrine) indicated an appreciable stress level. The catheterization procedure did not further elevate these levels appreciably, except possibly for epinephrine in normals. The subsequent FA exposure did not induce meaningful change. However, the catecholamine levels rose further after a night in the hospital with catheters in place and then fell during the O_3 exposure. Other indicators of stress, such as heart rate and blood pressure, did not follow this pattern. Thus, the relationship of circulating catecholamines to O_3 effects is not clear.

Respiratory Responses

The observed modest FVC and FEV_1 losses with O_3 exposure were typical for middle-aged and older adults, while the symptom increases appeared, if anything, disproportionately mild. Subjects' perception of O_3-related respiratory symptoms might well have diminished (relative to noncatheterized healthy, younger subjects in previous studies) because of competing symptoms associated with catheterization. However, few symptoms were reported in the "other" category on the questionnaire that could relate directly to catheters.

We conclude that the post-O_3 increase in (A-a)PO_2 represents a true effect of O_3, in light of the stability of (A-a)PO_2 at all three measurement times prior to O_3 exposure, and prior evidence (admittedly inconsistent) that short-term O_3 exposure may impair blood oxygenation. In the earliest (to our knowledge) study which addressed that issue, O_3 reportedly increased (A-a)PO_2 (estimated from "arterialized" earlobe capillary blood) even in healthy subjects exposed to 0.1 ppm who experienced no meaningful spirometric changes [Von Nieding et al., 1977]. Two subsequent studies failed to confirm that finding [Islam et al., 1979; Linn et al., 1979]. Multiple investigations of O_3-exposed volunteers with chronic obstructive pulmonary disease, measuring SaO_2 changes by noninvasive oximetry, also have yielded mixed positive and negative results [Solic et al., 1982; Linn et al., 1982, 1983b; Kehrl et al., 1985]. The present observation of significantly increased (A-a)PO_2 after a 3-hr 0.3-ppm O_3 exposure, accompanied by a small and equivocally significant loss in SaO_2, does not resolve the uncertainty about previous findings, all of which related to lower effective O_3 doses. Nevertheless, the present finding considerably strengthens the case that O_3 exposure levels sufficient to cause modest FVC and FEV_1 losses and only minimal increases in respiratory symptoms can also impair alveolar-arterial gas exchange. Medically, the observed (A-a)PO_2 effect implies an important risk of acute arterial desaturation for anyone with pre-existing marginal oxygenating capacity exposed at similar O_3 levels. Finding only a very slight SaO_2 loss in these subjects is not surprising, since all had normal baseline SaO_2 and thus would fall within the relatively flat portion of the oxyhemoglobin dissociation curve. Mechanistic explanation of the increased (A-a)PO_2 requires further investigation. Plausible mechanisms would involve general airways narrowing and/or abnormal alveolar-capillary interface as a result of pulmonary inflammation and enhanced epithelial permeability (leading to interstitial edema). However, the hemodynamic data argue against pulmonary edema secondary to left ventricular failure.

This study parallels previous investigations of patients with documented CAD who were exposed to controlled concentrations of inhaled carbon monoxide (CO) [Adams et al., 1988; Allred et al., 1989; Kleinman et al., 1989; Sheps et al., 1990]. The CO studies used noninvasive procedures to detect the early onset of exercise-related ischemic ST-segment changes, angina, and ventricular arrhythmias and dysfunction. The mechanism for the myocardial ischemia and reduced cardiac performance is CO-induced tissue hypoxia, which impairs effective oxygen delivery to the working myocardium, in conjunction with the limited compensatory capability in CAD to increase coronary blood flow in response to increased myocardial oxygen demands during exercise. Our study differed from the CO studies in that the subjects were predominantly hypertensive without clinical ischemic heart disease and exercise stress testing was not used as a provocative end point. Active smokers were excluded (as in the CO studies) to avoid the confounding effects of exposure to inhaled carbon monoxide and nicotine, which acutely exerts a dose-related sympathomimetic stimulation and resulting hemodynamic changes [Le Houezec and Bencowitz, 1991]. Although inhaled O_3 decreased arterial oxygenation, significant myocardial hypoxia, ECG changes, or ischemia (with enzyme elevations) did not occur since the subjects had normal baseline oxygenation, the decline was relatively modest, and presumably normal or sufficient myocardial function and reserve were present.

Limitations of the Protocol.

The study design and the small number of subjects limit interpretation of the hemodynamic results. These limitations were largely related to ethical and logistical aspects of the protocol and its procedures, as well as the lack of direct precedent for this type of air pollution research. We did not randomize the order of exposures since that would have required a greater number of volunteers, and/or catheterizations of subjects on two separate days. Therefore, we cannot exclude the possibility that time-dependent confounding factors either caused cardiopulmonary changes that were mistakenly attributed to O_3, or masked the real effects of O_3. Confounding seems unlikely with respect to the declines in FVC and FEV_1 after O_3: preexposure baselines were similar on both days, and responses both to filtered air and to O_3 were within the range found in multiple previous studies [Lippmann, 1993; Gong and Linn, 1994; Bascom et al., 1996]. Accordingly, we conclude that these lung function changes represent true responses to O_3 typical of middle-aged and older men without chronic respiratory disease.

Conclusions

Conclusions concerning cardiovascular physiologic effects must be more tentative, in that the cardiovascular variables were generally less stable than respiratory variables, and have fewer precedents to aid interpretation. Occasional missing data required maximum likelihood estimation or substitution of alternative measurements, and the 30-60-min delay between the end of exposure and the initial post-exposure hemodynamic measurements precluded detection of possible transient hemodynamic responses. In addition, women were excluded from this pilot study in order to avoid confounding hormonal effects [Fox et al., 1993] and to ensure a more homogeneous study population, as well as for practical reasons.

The mean pre-catheterization blood pressure values on Day 1 were not elevated in all hypertensive subjects. In general, the blood pressures tended to rise during their drug withdrawal process: 6 hypertensive patients had higher pre-catheterization systolic and diastolic blood pressures on Day 1 than during screening, and 6 of the 10 patients presented with mildly or moderately elevated diastolic blood pressure. The individualized withdrawal of different anti-hypertensive medications prior to the initial exposure session may have been inadequate in terms of duration (at least one week prior), and drug levels may have persisted to some extent, thereby protecting against possible hypertensive effects of O_3 in some patients. The investigators did not believe that prolonged medication withdrawal was either ethically or medically prudent for the purposes of this research study. As a compromise, either an abrupt drug stoppage or gradual withdrawal (beta-blocking agents only), with subsequent monitoring of clinical status, was performed 1-3 wks. prior to the initial exposure session. Blood pressure may be underestimated from single cuff measurements shortly following withdrawal of anti-hypertensive drugs [Beltman et al., 1996], providing another possible explanation for the absence of significant hypertension in some subjects.

The general safety of the invasive hemodynamic procedures used in this research study warrants discussion. Overall, the subjects tolerated the insertion and maintenance of the two catheters well. Although the catheters produced temporary discomfort after the effects of the local anesthesia (lidocaine) dissipated, the subjects generally tolerated the catheters with symptomatic management. There were no clinically significant acute or subsequent complications (i.e., persistent arrhythmias, pneumothorax, hemoptysis, local bleeding, infection, or vascular insufficiency) related to the catheters. None of the subjects voluntarily withdrew from the study once the catheters were successfully inserted and functioning. However, carefully performed procedures with sterile technique, continuous clinical monitoring of the subject, catheters, and hemodynamics, experienced medical and technical staff, and coordination of accessory physical and instrumentational resources are necessary for successful and safe implementation of this type of air pollution-health effects research.

Our study has demonstrated that this type of hemodynamic research can be effectively and safely conducted during both rest and exercise and may have implications for application in

future studies, e.g., in controlled human exposures to inhaled particulates. Epidemiological studies [Schwartz and Dockery, 1992; Pope et al., 1994; Schwartz, 1996] indicate a consistent association between particulate air pollution and deaths from cardiovascular causes. Some epidemiological data suggest that particulate air pollution may indirectly promote cardiac events via a respiratory mechanism (e.g., hypoxia). Preliminary results from an animal model [Nearing et al., 1996] suggest that acute cardiac electrophysiologic abnormalities are induced by exposure to inhaled particles (fly ash).

In summary, the experimental results did not support our hypothesis of acute cardiovascular dysfunction in subjects with chronic cardiovascular disease (hypertension) exposed to O_3. We did not find convincing evidence of significant direct cardiovascular effects from O_3 exposure, analogous to effects reported in laboratory animals. Either no such direct effects occurred, or they did occur but were not detected due to limitations of experimental design and sample size. More importantly, the results did support our hypothesis in a more general sense, in showing that O_3 can exert cardiovascular effects indirectly by impairing alveolar-arterial oxygen transfer, and potentially reducing oxygen supply to the myocardium, thus increasing the need for compensatory cardiovascular adjustments. The increased (A-a)PO_2 and cardiovascular responses were not obviously different between the exposed healthy and hypertensive subjects. Presumably, these cardiopulmonary responses to O_3 could occur also in persons with more severe cardiopulmonary disease, with potentially more adverse or severe cardiovascular consequences. Thus, even without demonstrating direct cardiovascular effects, this study strengthens the case that persons with preexisting cardiovascular disease (e.g., CAD) are at risk from ambient O_3 pollution.

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TABLE 1: CHARACTERISTICS OF INDIVIDUAL SUBJECTS

ID #	Age yr	Weight lbs	Height ins	FEV1 % predicted	Medications†
Hypertensive Group:					
2261	47	195	69	72	a,b,c
2263*	78	170	65	120	d
2271	47	189	75	85	b
2272	61	159	67	129	None
2275	51	284	73	106	a
2284	42	179	66	102	a
2313	52	262	71	86	a,c
2314	48	184	74	92	d
2315	53	209	66	92	a
2316	51	250	70	95	a,b,c
[Mean]	53	208	70	98	
[SD]	10	42	4	17	
Control Group:					
683	44	176	66	84	None
1082	40	171	66	97	None
2270	46	206	68	97	None
2276	41	168	72	92	None
2277	42	195	70	111	None
2278	49	170	68	107	None
[Mean]	44	181	68	98	
[SD]	3	16	2	10	
All Subjects:					
Mean	50	198	69	98	
SD	9	37	3	14	

* This subject also has ischemic heart disease and prior angioplasty.

† Medications: a = angiotensin-converting enzyme inhibitor; b = calcium-channel blocker; c = diuretic; d = beta blocker.

TABLE 2: PRIMARY HEMODYNAMIC END POINTS

END POINT	ABBREVIATION	UNITS
Cardiac output	CO	L/min
Cardiac index	CI	L/min/m2
Stroke volume	SV	ml/beat
Left ventricular stroke work index	LVSWI	g m/m2
Right ventricular stroke work index	RVSWI	g m/m2
Central venous pressure	CVP	mm Hg
Mean pulmonary artery pressure	MPAP	mm Hg
Pulmonary artery wedge pressure	PAWP	mm Hg
Pulmonary vascular resistance index	PVRI	dyn sec cm-5 m2
Systemic vascular resistance index	SVRI	dyn sec cm-5 m2
Heart rate	HR	beats/min
Systolic blood pressure	BPs	mm Hg
Diastolic blood pressure	BPd	mm Hg
Mean arterial pressure	MAP	mm Hg
Rate-pressure product	RPP	beats/min mm Hg*

* Systolic blood pressure.

TABLE 3: MEAN (±S.D.) HEMODYNAMIC RESPONSES TO FILTERED AIR (FA) AND OZONE EXPOSURES

	HYPERTENSIVE SUBJECTS (n=10)				CONTROL SUBJECTS (n=6)				ALL SUBJECTS (n=16)			
End Point	Pre-FA	Post-FA	Pre-O3	Post-O3	Pre-FA	Post-FA	Pre-O3	Post-O3	Pre-FA	Post-FA	Pre-O3	Post-O3
CI	3.67 +/-0.68	3.82 +/-0.56	3.31 +/-0.45	3.96 +/-0.82	2.68 +/-0.28	3.33 +/-0.65	3.08 +/-0.26	3.75 +/-0.54	3.29 +/-0.6	3.62 +/-0.6	3.23 +/-0.61	3.87 +/-0.61
SV	110 +/-18	111 +/-20	104 +/-15	109 +/-21	88 +/-14	96 +/-10	100 +/-13	93 * +/-16	103 +/-17	106 +/-17	104 +/-18	103 +/-18
LVSWI	78.9 +/-16.3	65.6 +/-16.3	64.4 +/-17.1	66.4 +/-17.1	56.2 +/-7.9	57.0 +/-7.9	55.1 +/-7.9	54.1 +/-7.9	70.4 +/-15.6	62.4 +/-15.6	61 +/-15.9	61.8 +/-15.9
RVSWI	9.4 +/-3.0	8.6 +/-3.0	7.0 +/-3.1	8.8 +/-3.1	5.6 +/-1.6	5.9 +/-1.6	6.3 +/-1.6	6.7 +/-1.6	7.9 +/-2.9	7.6 +/-2.9	6.7 +/-2.9	8 +/-2.9
CVP	5.3 +/-3.0	5.7 +/-4.1	5.1 +/-2.6	4.1 +/-3.0	5.5 +/-3.5	6.3 +/-3.6	5 +/-2.1	3 +/-1.5	5.4 +/-3.1	5.9 +/-3.8	5.1 +/-2.4	3.7 * +/-2.5
MPAP	19.1 +/-5.9	18.6 +/-5.2	14.9 +/-3.1	15.6 +/-4.6	14.7 +/-5.7	11.5 +/-5.5	12.2 +/-3.8	10.8 +/-4.1	17.4 +/-6.1	15.9 +/-6.3	13.9 +/-3.5	13.9 +/-4.9
PAWP	12.0 +/-6.6	10.8 +/-5.5	9.6 +/-4.7	8.9 +/-6.6	9.2 +/-4.3	7.7 +/-4.2	9.3 +/-3.6	5.7 +/-5.2	10.9 +/-5.8	9.6 +/-5.1	9.5 +/-4.2	7.7 +/-6.1
PVRI	154 +/-83	155 +/-80	133 +/-83	145 +/-83	170 +/-74	192 +/-74	128 +/-74	154 +/-74	160 +/-83	169 +/-81	131 +/-83	148 +/-83
SVRI	2575 +/-899	2080 +/-500	2365 +/-458	2034 +/-477	2848 +/-386	2146 +/-516	2184 +/-355	1782 +/-300	2723 +/-510	2096 +/-510	2298 +/-524	1939 +/-524

HR resting	69.0 +/-7.1	72.7 +/-7.5	66.3 +/-6.6	77.7 +/-9.5	59.7 +/-6.1	67.8 +/-12.8	61 +/-8.0	76.7 +/-14.9	65.5 +/-8.0	70.9 +/-9.7	64.3 +/-7.4	77.3* +/-11.14
BPs	159 +/-28	156 +/-24	153 +/-19	149 +/-18	132 +/-17	131 +/-16	125 +/-18	125 +/-6	149 +/-27	146 +/-24	142 +/-23	140 +/-19
BPd	92 +/-19	79 +/-13	82 +/-18	78 † +/-13	75 +/-15	69 +/-17	66 +/-7	68 +/-12	86 +/-19	75 +/-15	76 +/-16	74 † +/-13
MAP	118.6 +/-16.2	103.6 +/-13.3	105.8 +/-14	101.4 +/-13.2	100.2 +/-14.1	92.8 +/-11	88.8 +/-9.7	85.8 +/-18.0	113.8 +/-18	98.9 +/-18.4	99.8 +/-18.4	95.6 +/-18.4
RPP	11036 +/-2464	11357 +/-2338	10125 +/-1494	11701* +/-2563	7864 +/-947	8991 +/-2563	7667 +/-1724	9647 +/-2096	9846 +/-2541	10469 +/-2621	9203 +/-1959	10931* +/-2542
Core Temp	36.5 +/-0.3	36.7 +/-0.3	36.4 +/-0.3	36.7 +/-0.3	36.5 +/-0.2	36.7 +/-0.2	36.5 +/-0.2	36.8 +/-0.2	36.5 +/-0.3	36.7 +/-0.3	36.4 +/-0.3	36.7 +/-0.3

* P <0.05; † P <0.01 for O3 effect (ANOVA). Differences between hypertensives and controls were non-significant.

TABLE 4: MEAN (±S.D.) PHYSIOLOGIC RESPONSES DURING EXPOSURE IN HYPERTENSIVE AND CONTROL SUBJECTS

HYPERTENSIVE SUBJECTS (n=7)

End Point	FA - Early exer.	FA - Early rest	FA - Late exer.	FA - Late rest	O3 - Early exer.	O3 - Early rest	O3 - Late exer.	O3 - Late rest
BPs *,†	178 +/-24	148 +/-25	167 +/-26	153 +/-25	174 +/-23	143 +/-23	163 +/-24	153 +/-23
BPd *	93 +/-17	93 +/-18	92 +/-19	95 +/-18	89 +/-17	88 +/-17	94 +/-17	90 +/-17
MPAP ‡	20.3 +/-5.0	12.2 +/-5.4	19.2 +/-5.4	11.5 +/-5.4	18.3 +/-5.1	11.4 +/-5.1	18.9 +/-5.1	11.7 +/-5.1
HR †,§	111 +/-10	93 +/-11	115 +/-11	89 +/-10	111 +/-10	89 +/-10	118 +/-11	92 +/-10
VE *,†,‡	36.1 +/-5.7	14.8 +/-5.9	39.2 +/-5.9	16.9 +/-5.7	35.1 +/-5.7	14.8 +/-5.7	37.8 +/-5.9	16.8 +/-5.7

CONTROL SUBJECTS (n=4)

End Point	FA - Early exer.	FA - Early rest	FA - Late exer.	FA - Late rest	O3 - Early exer.	O3 - Early rest	O3 - Late exer.	O3 - Late rest
BPs *,†	147 +/-18	135 +/-19	155 +/-18	128 +/-18	144 +/-17	130 +/-19	134 +/-19	134 +/-19
BPd *	92 +/-14	81 +/-16	69 +/-14	79 +/-14	80 +/-13	77 +/-16	81 +/-16	75 +/-16
MPAP †	17.0 +/-5.8	11.0 +/-5.8	17.0 +/-5.8	12.0 +/-5.8	16.7 +/-5.8	9.0 +/-5.8	16.0 +/-5.8	10.7 +/-5.8
HR †,‡,§	111 +/-13	81 +/-12	121 +/-12	91 +/-12	113 +/-12	87 +/-12	126 +/-12	102 +/-12
VE *,†	30.1 +/-3.8	10.9 +/-3.8	31.1 +/-3.8	10.9 +/-3.8	27.6 +/-3.8	9.9 +/-3.8	30.7 +/-3.8	11.9 +/-3.8

* Significant ($P < 0.05$) difference between hypertensives and controls.
† Significant ($P < 0.05$) difference between exercise and rest.
‡ Significant ($P < 0.05$) difference between early and late.
§ Day-time interaction (O3 effect) $P = 0.06$, with all subjects combined.

TABLE 5: MEAN (±S.D.) BIOCHEMICAL RESPONSES TO FILTERED AIR (FA) AND OZONE (O3) EXPOSURES

	HYPERTENSIVE SUBJECTS (n=10)				CONTROL SUBJECTS (N=6)				ALL SUBJECTS (N=16)			
End Point	Pre- FA	Post- FA	Pre-O3	Post-O3	Pre-FA	Post-FA	Pre-O3	Post-O3	Pre-FA	Post-FA	Pre-O3	Post-O3
CK, IU/L	110 +/-64	122 +/-64	100 +/-64	126 +/-64	57 +/-28	61 +/-28	53 +/-28	65 +/-28	90 +/-60	99 +/-60	83 +/-60	103 +/-60
CK-MB, %	0.7 +/-1.0	0.2 +/-1.0	0.1 +/-0.9	0.3 +/-0.9	0.0 0	0.0 0	0.0 0	0.8 +/-1.9	0.5 +/-0.3	0.2 +/-0.3	0.1 +/-0.3	0.6 +/-0.3
LDH, IU/L	482 +/-119	502 +/-121	495 +/-119	556 +/-119	444 +/-88	477 +/-88	474 +/-88	564 +/-88	467 +/-110	491 +/-112	489 +/-109	557 +/-110
LDH-1, %	18.8 +/-3.3	16.8 +/-3.4	18.5 +/-3.3	17.7 +/-3.3	20.8 +/-2.6	18.9 +/-2.5	18.1 +/-2.5	17.8 +/-2.6	19.5 +/-3.1	17.6 +/-3.2	18.4 +/-3.1	17.8 +/-3.1
LDH-2, %	32.4 +/-2.9	30.6 +/-3.0	33.6 +/-2.9	32.5 +/-2.9	32.8 +/-2.8	31.9 +/-2.8	33.4 +/-2.8	32.0 +/-2.8	32.6 +/-2.9	31.1 +/-3.0	33.6 +/-2.9	32.4 +/-2.9
LDH-3, %	23.8 +/-2.9	25.2 -2.9	27.3 +/-2.9	26.1 ‡ +/-2.9	22.7 +/-1.5	23.5 +/-1.6	24.5 +/-1.5	24.8 +/-1.6	23.4 +/-2.6	24.6 +/-2.7	26.3 +/-2.6	25.6* +/-2.7
LDH-4, %	10.9 +/-2.3	12.6 +/-2.4	10.7 +/-2.3	11.2 +/-2.3	11.5 +/-2.2	11.9 +/-2.2	11.0 +/-2.2	13.0 +/-2.2	11.1 +/-2.3	12.4 +/-2.4	10.8 +/-2.3	11.8 +/-2.3
LDH-5, %	14.1 +/-2.9	14.7 +/-3.0	9.9 +/-2.9	12.5 +/-2.9	12.3 +/-2.2	13.8 +/-2.2	12.6 +/-2.2	12.1 +/-2.2	13.5 +/-2.8	14.4 +/-2.9	10.8 +/-2.8	12.4 +/-2.8
NOREPI, ng/L	560.0 +/-243	590.0 +/-243	668.0 +/-243	502.0 +/-250	631.0 +/-203	580.0 +/-189	683.0 +/-135	574.0 +/-112	587.0 +/-214	586.0 +/-214	674.0 +/-214	530.0 +/-219

EPI, ng/L	68 +/-37	75 +/-37	106 +/-37	58.0 * +/-39	82 +/-58	77 +/-66	102 +/-59	78 +/-31	74 +/-43	75 +/-43	104 +/-43	62 † +/-44
ANF fmol/ml	14.7 +/-6.9	12.9 +/-4.0	12.8 +/-4.4	12.0 +/-4.2	14.0 +/-3.3	10.1 +/-2.3	11.4 +/-3.9	10.9 +/-2.6	14.3 +/-5.7	12.0 +/-3.7	12.3 +/-4.0	11.6 +/-3.7
Pro-ANF 1-30	291 +/-206	231 +/-155	273 +/-123	212 +/-136	331 +/-120	199 +/-47	207 +/-82	177* +/-79	306 +/-175	219 +/-125	224 +/--107	199 +/-117
Pro-ANF 31-67 fmol/ml	358 +/-181	294 +/-206	309 +/-147	274 +/-137	356 +/-60	230 +/-62	284 +/-74	211 +/-56	357 +/-145	270 +/-134	300 +/-122	250 +/-115

Normal values: Total CK = 57-374 IU/L; % CK-MB = 0 - 5%; Total LDH = 313-618 IU/L;
% LDH-1 = 17 - 27%; % LDH-2 = 28 - 38%; % LDH-3 = 19 - 27%; % LDH-4 = 5 - 16%; % LDH-5 = 5 - 16%;
NOREPI (norepinephrine) = 148 +/- 45 (SD) ng/L (basal); EPI (epinephrine) = 42 +/- 35 ng/L (basal); ANF = 22 +/- 1 (SEM) fmol/ml;
pro-ANF 1-30 = 531 +/- 25 fmol/ml; pro-ANF 31-67 = 371 +/- 18 fmol/ml.

* $P < 0.05$; † $P = 0.01$; ‡ $P = 0.003$ for O3 effect (ANOVA).

TABLE 6: MEAN (±SD)* PLASMA CATECHOLAMINE LEVELS BEFORE AND AFTER CATHETERIZATION (PRIOR TO EXPOSURE) ON DAY 1

CATECHOLAMINE	GROUP	BEFORE CATH ng/L †	AFTER CATH. ng/L ‡
Norepinephrine	Hypertensive	707 +/- 243	560 +/- 268
	Control	620 +/- 95	631 +/- 203
	All	674 +/- 201	587 +/- 241
Epinephrine	Hypertensive	64 +/- 16	68 +/- 37
	Control	45 +/- 30	82 +/- 64
	All	57 +/- 23	74 +/- 44

Normal values: Norepinephrine = 148 +/- 45 (SD) ng/L (basal).
Epinephrine = 42 +/- 35 ng/L (basal).

* Before-after differences are non-significant ($P > 0.05$).
† From venous samples.
‡ From PA samples.

TABLE 7: MEAN (±S.D.) RESPIRATORY RESPONSES TO FILTERED AIR (FA) AND OZONE (O3) EXPOSURES

End Point	HYPERTENSIVE SUBJECTS (n=10)				CONTROL SUBJECTS (n=6)				ALL SUBJECTS (n=16)			
	Pre-FA	Post-FA	Pre-O3	Post-O3	Pre-FA	Post-FA	Pre-O3	Post-O3	Pre-FA	Post-FA	Pre-O3	Post-O3
FVC (L)	4.41 +/-0.78	4.39 +/-0.66	4.35 +/-0.67	4.08 +/-0.82	4.52 +/-0.58	4.56 +/-0.63	4.41 +/-0.58	4.21* +/-0.61	4.46 +/-0.69	4.46 +/-0.63	4.37 +/-0.62	4.13* +/-0.73
FEV1 (L)	3.45 +/-0.76	3.5 +/-0.7	3.41 +/-0.66	3.20* +/-0.82	3.68 +/-0.55	3.7 +/-0.52	3.58 +/-0.52	3.36 † +/-0.52	3.54 +/-0.68	3.58 +/-0.63	3.47 +/-0.6	3.26 † +/-0.71
SaO2 %	96.6 +/-2.1	95 +/-2.6	95.9 +/-2.0	94.4 +/-2.3	97.2 +/-0.4	96.8 +/-0.4	96.8 +/-0.8	95.2 +/-1.7	96.9 +/-1.7	95.7 +/-2.2	96.2 +/-1.7	94.7 +/-2.1
(A-a)PO2 mmHg	17.0 +/-10.8	18.3 +/-13.3	16.6 +/-11.2	26.8* +/-8.7	9.8 +/-10.8	8.8 +/-9.4	11.2 +/-10.7	22.9* +/-12.5	14.3 +/-11.1	14.7 +/-12.6	14.6 +/-11.0	25.4 † +/-10.1

* $P < 0.05$; † $P < 0.01$ for O3 effect (ANOVA).

TABLE 8: PRE- AND END-EXPOSURE SYMPTOM SCORES: TOTAL, RESPIRATORY, AND CARDIOVASCULAR SYMPTOMS

GROUP	Mean (+SD)				P Values, ANOVA		
	Pre-FA	Post-FA	Pre-O3	Post-O3	Day	Time	Int.
ALL SUBJECTS:							
Total	12.5 +/-14.9	13.1 +/-15.4	15.9 +/-14.3	21.9 +/-18.2	0.13	0.40	0.36
Resp.	7.2 +/-10.1	4.4 +/-7.5	5.0 +/-7.3	11.3 +/-12.6	0.28	0.44	0.04
Card.	0.9 +/-2.7	0.9 +/-2.7	0.9 +/-2.0	2.2 +/-4.5	0.51	0.30	0.22
HYPERTENSIVE SUBJECTS:							
Total	12.5 +/-16.4	10.0 +/-15.5	13.0 +/-14.8	19.0 +/-20.9	0.36	0.75	0.35
Resp.	7.5 +/-12.1	2.0 +/-4.8	4.0 +/-8.1	10.5 +/-14.4	0.38	0.96	0.05
Card.	1.5 +/-3.4	1.0 +/-3.2	0.5 +/-1.6	2.0 +/-4.8	0.99	0.56	0.22
CONTROL SUBJECTS:							
Total	12.5 +/-13.7	18.3 +/-15.1	20.8 +/-13.2	26.7 +/-12.9	0.23	0.31	0.99
Resp.	6.7 +/-6.8	8.3 +/-9.8	6.7 +/-6.1	12.5 +/-9.9	0.59	0.36	0.52
Card.	0.0 +/-0.0	0.8 +/-2.0	1.7 +/-2.6	2.5 +/-4.2	0.17	0.36	0.99

Total symptoms includes respiratory (Resp.) and cardiovascular (Card.) symptoms plus fatigue, headache, eye irritation, and "other." Respiratory symptoms are cough, sputum, substernal irritation, wheeze, chest tightness, sore throat, and nasal discharge. Cardiovascular symptoms are palpitations, irregular heartbeat, weakness, and chest pain. Each symptom was scored for intensity as: 0 = not present, 5 = minimal (barely noticeable), 10 = mild, 20 = moderate, 30 = severe, 40 = incapacitating. (No scores of 40 were reported.) Relevant symptom scores were summed to determine the composite scores for which summary statistics are reported.