DOI: 10.1161/CIRCULATIONAHA.112.094359

Controlled Exposure of Healthy Young Volunteers to Ozone Causes Cardiovascular Effects

Running title: *Devlin et al.; Ozone Induces Cardiovascular Changes*

Robert B. Devlin, PhD[1]; Kelly E. Duncan, PhD[2]; Melanie Jardim, PhD[1]; Michael T. Schmitt, MSPH[1]; Ana G. Rappold, PhD[1]; David Diaz-Sanchez, PhD[1]

[1]Environmental Public Health Division, National Health & Environmental Effects Laboratory, US EPA, Research Triangle Park, NC; [2]Center for Environmental Medicine, Asthma, and Lung Biology, University of North Carolina School of Medicine, Chapel Hill, NC

Correspondence:

Robert Devlin

National Health and Environmental Effects

Research Laboratory MD 58D

US EPA

Research Triangle Park, NC 27711

Tel: 919-966-6255

Fax: 919-966-6271

E-mail: devlin.robert@epa.gov

Journal Subject Codes: [91] Oxidant stress; [160] Fibrinolysis; [100] Health policy and outcome research

Downloaded from http://circ.ahajournals.org/ by guest on December 27, 2016

DOI: 10.1161/CIRCULATIONAHA.112.094359

Abstract:

***Background*—**Recent epidemiology studies have reported associations between acute ozone exposure and mortality. Such studies have previously reported associations between airborne particulate matter pollution (PM) and mortality and support for a causal relationship has come from controlled exposure studies which describe pathophysiological mechanisms by which PM could induce acute mortality. In contrast, for ozone, there are almost no controlled human exposure studies which have tested whether ozone exposure can modulate the cardiovascular system.

***Methods and Results*—**Twenty three young healthy individuals were exposed in a randomized crossover fashion to clean air and 0.3 ppm ozone for two hours while undergoing intermittent exercise. Blood was obtained immediately prior to exposure, immediately afterward, and the next morning. Continuous Holter monitoring began immediately prior to exposure and continued for 24 hours. Lung function was performed immediately prior to and immediately after post exposure and bronchoalveolar lavage was performed 24 hours after exposure. Immediately following ozone exposure we observed a 98.9% increase in IL-8, a 21.4% decrease in plasminogen activator inhibitor 1 (PAI-1), a 51.3% decrease in the high frequency component of HRV, and a 1.2 % increase in QT duration. Changes in IL-1B, and PAI-1 were apparent 24 hours after exposure. In agreement with previous studies, we also observed ozone-induced drops in lung function and an increase in pulmonary inflammation.

***Conclusions*—**This controlled human exposure study shows that ozone can cause an increase in vascular markers of inflammation, changes in markers of fibrinolysis, as well markers that affect autonomic control of heart rate and repolarization. We believe these findings provide biologic plausibility for the epidemiology studies that associate ozone exposure with mortality.

***Clinical Trial Registration Information*—**clinicaltrials.gov; Identifier: NCT01492517.

Key words: fibrinolysis; inflammation; nervous system, autonomic

Downloaded from http://circ.ahajournals.org/ by guest on December 27, 2016

DOI: 10.1161/CIRCULATIONAHA.112.094359

Introduction

Ozone, a ubiquitous air pollutant, has been studied more extensively than perhaps any other environmental toxicant. Numerous papers have described health effects associated with exposure to ozone. However, until recently nearly all of them have focused on characterizing respiratory effects.[1] Although epidemiology studies have associated exposure to particulate matter (PM) with acute mortality and morbidity,[2] only recently have they found associations between ozone and mortality. It has been challenging to disentangle the effects of ozone from those of PM in these studies because the two pollutants are often closely correlated temporally and geographically. Despite this, there is an emerging body of studies that report robust associations between ozone and cardiovascular mortality[3-7] and morbidity.[8-10]

Unlike for PM, there has been relatively little work establishing biological plausibility for the observed association between ozone exposure and mortality. Epidemiological studies have shown that PM levels are associated with increased hospitalizations and alterations in markers of cardiovascular risk such as arrhythmia, changes in heart rate variability, vascular inflammation, and endothelial cell dysfunction.[2] Evidence for a causal link between PM and mortality has been bolstered by controlled exposure studies to PM that have described pathophysiological processes by which acute PM exposure could cause death as well as molecular pathways which underlie these processes.[2] However, this type of coherence between epidemiological, clinical and molecular studies does not yet exist for ozone.

Indeed, there are only a handful of controlled exposure studies that have examined cardiovascular effects due to ozone exposure. Foster et al.[11] found a reduction in serum levels of the free radical scavenger α-tocopherol after ozone exposure and Gong et al.[12] observed an increase in alveolar-to-arterial PO_2 gradient which could be due to impaired alveolar-arterial

Downloaded from http://circ.ahajournals.org/ by guest on December 27, 2016

DOI: 10.1161/CIRCULATIONAHA.112.094359

oxygen transfer. Some animal toxicology studies have reported increased systolic blood pressure and heart rate[13] which are thought to be related to a drop in core temperature in rats exposed to ozone.[14] Chuang et al. also reported ozone-induced increase in vascular oxidative stress and mitochondrial DNA damage, and Perepu et al.[15] observed enhanced sensitivity to myocardial ischemia reperfusion injury in rats exposed to ozone.

Taken as a whole, there is weak coherence between the recent epidemiological associations of cardiovascular mortality and morbidity and ozone exposure, and the few controlled exposure studies that have attempted to provide biological plausibility and possible biological mechanisms that explain the association between ozone and acute mortality. We hypothesized that if such an association is real then the effects of ozone exposure should be apparent outside of the airways and should be manifested in systemic changes in the cardiac or vascular systems. To test this we characterized cardiovascular changes after exposure of healthy young volunteers to ozone. Here we report changes in vascular markers of fibrinolysis, markers of vascular inflammation, changes in heart rate variability, and repolarization.

Downloaded from http://circ.ahajournals.org/ by guest on December 27, 2016

Methods

Study Population

Participants were recruited under a contract to the Westat Corporation, which identified potential subjects through their web site, newspaper advertising, and targeted calls to qualified subjects who had completed other EPA studies. A total of 23 volunteers completed the study. The median age was 28.8 years (youngest participant was 19, oldest was 33); there were 20 males and 3 females (21 White, 1 Hispanic, 1 Black). The use of young healthy subjects could be seen as a limitation of the study since it is not the population that might be expected to respond in a

DOI: 10.1161/CIRCULATIONAHA.112.094359

clinically significant manner to ozone. However, for safety reasons we chose not to study a more at risk population. The ozone-induced effects reported here could conceivably be greater in at risk individuals, or even if the same, could potentially place them at greater risk than healthy young individuals.

Mean resting heart rate of the study population was 68.3 beats per minute (51 – 88). Mean diastolic blood pressure was 74.9 mm Hg (64 -86) and mean systolic blood pressure was 123.3 mmHg (103 – 136). They were free of cardiopulmonary diseases and allergies as determined by a detailed medical history and physical examination. All subjects had FEV_1 and FVC baseline values of at least 80% predicted for height and age and were lifetime non-smokers, except for one subject who had smoked ½ pack per year until 1998) s. Subjects were informed of the procedures and potential risks and signed an informed consent. The protocol and consent forms were approved by the University of North Carolina School of Medicine Committee on the Protection of the Rights of Human Subjects and the US Environmental Protection Agency.

Study Design

This was a randomized single-blind crossover study, where each subject was exposed twice for two hours: once to clean air and once to 0.3 ppm ozone. This concentration is comparable to that used in many previous controlled human exposure studies. Although higher than the EPA NAAQS standard of 0.076 ppm and not seen in American cities, it is only slightly higher than peak hourly concentrations observed in heavily polluted cities such as Beijing or Mexico City. [16] [17] In addition, the subjects in this study were only exposed to elevated levels of ozone for two hours, in contrast to "real life" situations which would involve much lengthier ozone exposures. Each exposure was separated by at least two weeks. During the two hour exposure, each subject alternated 15 minutes of rest with 15 minutes of exercise on a cycle ergometer. Minute

Downloaded from http://circ.ahajournals.org/ by guest on December 27, 2016

ventilation was measured during each exposure and exercise levels adjusted to obtain a minute ventilation of 25 L/min/m^2 body surface area. This exercise regiment is comparable to that done in most previous studies in which human volunteers were exposed to ozone under controlled conditions. It is meant to mimic people performing physical labor or exercising outdoors. The exposures were conducted at the EPA Human Studies Facility on the campus of the University of North Carolina and the exposure chamber and generation of ozone have been described previously.[18] Briefly, ozone was generated by a silent electric discharge method (Model 502; Meckenheim, Bonn, Germany) and introduced into a chamber that was maintained at 22.6 ± 1.0^{o}C and 40 ± 6.5% relative humidity.. All exposures were conducted at the same time of day to avoid confounding by circadian variations.

Measurements

Spirometry (FVC, FEV_1) was performed immediately prior to exposure, immediately after exposure and again the next morning as described earlier.[18] Spirometry was done using a Sensormedics Vmax 220 instrument and software (Sensormedics Corp., Yorba Linda CA) according to ATS guidelines.

Each subject underwent bronchoscopy with bronchoalveolar lavage (BAL) 18 hours after the exposure as described previously.[19] Cells were counted using a hemocytometer and cell differentials performed on cytocentrifuged slides stained with a modified Wright Stain (Leukostat Solution, Fisher Scientific, Pittsburgh, PA). At least 200 cells per slide were counted.

Venous blood was sampled immediately before, 1 hour after, and 18 hours after each exposure. A differential blood count, and blood lipid panel was performed by LabCorp (Burlington, NC). Commercially available ELISA kits were used to quantify levels of C-reactive protein (Alpco Diagnostics, Windham, NH); d-dimer and von Willebrand's factor (vWF)

Downloaded from http://circ.ahajournals.org/ by guest on December 27, 2016

DOI: 10.1161/CIRCULATIONAHA.112.094359

(Diagnostica Stago, Parsippany, NY); tissue plasminogen activator (tPA), plasminogen, (Enzyme Research Laboratories, South Bend, IN); and plasminogen activator inhibitor 1 (PAI-1) (DakoCytomation, Carpenteria, CA).

Continuous ambulatory electrocardiograms (Holter ECGs) were collected for approximately 24 hours using a Mortara H12+ 12-Lead ECG Recorder (Mortara Instrument Co., Milwaukee, WI). The digitally-recorded ECGs were sampled at 1000 Hz and a trained research nurse blinded to the exposure randomization manually edited the sequence of ECG complexes to ensure proper labeling of each QRS complex. Time-domain parameters (SDNN, PNN_{50}) were calculated over a 24-hour period starting at the beginning just prior to each exposure. Frequency-domain parameters were measured during three 30-minute periods (immediately prior to exposure and approximately 1 hour and 20 hours after the completion of exposure) while the subjects rested quietly in a darkened room. The final five minutes of recording during these resting periods was used for calculation of frequency domain and repolarization variables. High frequency (HF, 0.15 - 0.4 Hz) and low frequency (LF, 0.04 - 0.15 Hz) were calculated in msec. Premature atrial contractions (PAC) and premature ventricular contractions (PVC) were calculated over the entire 24 hour monitoring period.

The effects on cardiac repolarization were assessed by measuring the QT interval corrected for heart rate (QTc). QTc was calculated from the raw Holter ECG data using proprietary analysis software from Mortara, Inc., which corrects for heart rate by using a subject-specific QT/RR slope. The complexity of the QRS complex was also calculated using Mortara software and is defined as the ratio of the second eigenvalue to the first.

Statistical Analysis

All endpoints measured one hour (post) and 24 hours (followup) following exposure were

Downloaded from http://circ.ahajournals.org/ by guest on December 27, 2016

divided by pre exposure values and expressed as percent of the baseline (pre-exposure), with the exception of bronchoalveolar lavage, the 24 hr Holter time domain data, and ectopic beats that were only collected 24 hours after exposure. Normalization against pre-exposure values is regularly performed in controlled-exposure studies to account for day to day variability of baseline levels in subjects. By normalizing against pre-exposure values and comparing each person's response to air exposure with the same person's response to ozone exposure, a number of other confounding parameters are also controlled; e,g. exercise, age, gender, medication use, diurnal variation etc. The post/pre and followup/pre values following both air and ozone exposure were used to calculate statistical significance.

Paired tests were used to assess differences between air and ozone, with each subject serving as their own control, minimizing variation in response among subjects. Since data were not distributed normally for some end points, to be consistent all end points were log transformed prior to analysis. Changes following ozone exposure are expressed relative to changes following air exposure (percent of air exposure). A p value of 0.05 or less was considered significant, although it might also be appropriate to use a value of 0.025 for significance since data from two time points (post and follow-up) are being analyzed. It is also recognized that due to the number of variables being analyzed in this study, some of the variables may appear significant due to chance alone. In addition to paired t tests, the data were also analyzed by mixed effects models incorporating post and follow-up findings independently or in the same model, and by a bootstrap model. Since the findings from all four tests were consistent, only paired t test findings are shown in the manuscript.

Downloaded from http://circ.ahajournals.org/ by guest on December 27, 2016

DOI: 10.1161/CIRCULATIONAHA.112.094359

Results

Effect of Ozone on Vascular Markers of Inflammation

None of the participants reported any complaints or symptoms following exposure to air or ozone. Baseline values and values post and followup exposure are shown in **Table 1**, as well as the post/pre and followup/pre changes. Statistically significant ozone-induced changes are shown in **Figure 1**. Immediately following ozone exposure we observed statistically significant post/pre increases in blood levels of IL-1β and near significant increases in IL-8 and TNFα compared with post/ pre changes following air exposure. There was an 85.3% increase in IL-8 (CI 44.1, 138.5), a 55.7% increase in IL-1β (confidence intervals of -5.1, 152.9) and a 10.1% increase in TNFα (CI -0.75, 22.1). Some markers of inflammation were present as long as 24 hours after exposure. There was a 103.8% increase in followup/pre blood levels of IL-1β (CI 32.5, 213.9) and a 65.4% increase in followup/pre CRP levels 24 hours after ozone exposure (CI 8.1, 152.9), relative to followup/pre values following air exposure.

Effect of Ozone on Vascular Markers of Thrombosis

In addition to inducing vascular inflammation, we also determined if exposure to ozone could cause a more pro-thrombogenic environment. We measured blood concentrations of several proteins involved in the formation or dissolution of blood clots. Baseline values of vascular thrombosis and values post and followup exposure are shown in **Table 2**, as well as the post/pre and followup/pre changes. Statistically significant ozone-induced changes are shown in **Figure 2**. There was a 32.8% decrease in post/pre PAI-1 concentration (CI -53.8, -2.4) which persisted for 24 hours at which point there was a 42.7% decrease in followup - pre (CI -65.5, -5.1)]. There was also a 41.5% decrease in followup/pre plasminogen levels (CO -67.1 – -16.0). Finally, there was a trend for 44.2% increase in tPA post/pre levels (CI -1.7 – 63.1) which was near significant

Downloaded from http://circ.ahajournals.org/ by guest on December 27, 2016

(p = 0.065).

Effect of Ozone on Heart Rate Variability and Repolarization

Particulate air pollution (PM) has been associated with altered autonomic nervous system control of the heart, as evidenced by both changes in heart rate variability (HRV) and repolarization. To determine if ozone can affect the autonomic nervous system as well we measured changes in both time and frequency domain for HRV as well as markers of repolarization. Baseline values of HRV and repolarization and values post and followup exposure are shown in **Table 3**, as well as the post/pre and followup/pre changes. Statistically significant ozone-induced changes are shown in **Figure 3**. There was a 51.2% percent reduction in the post/pre high frequency (HF) component of HRV immediately after ozone exposure (CI -69.2. -23.7) compared with post/pre values following air exposure. There was a trend for decreased HF twenty four hours following exposure at which time there was a 32.8% decrease in HF (CI -57.9, 7.1). There was a small, but significant, ozone-induced 1.2% increase in the duration of the QT interval immediately after exposure, when corrected for heart rate by using a subject-specific QT/RR slope (CI 0.40, 2.0). We also observed an ozone-induced 5.8% decrease in the complexity of the QRS wave immediately following exposure (CI -10.5, -1.0). The QRS complexity is defined as the ratio of the second eigenvalue to the first and represents the spread of depolarization through the ventricular muscle. Exposure to ozone did not cause changes in other measures of HRV or repolarization.

Effect of Ozone on Lung Function and Pulmonary Inflammation

Although the primary emphasis in this study was to assess cardiovascular changes caused by exposure to ozone, we also measured ozone-induced changes in FEV_1 and BAL inflammatory cells to ascertain if the participants in this study responded to ozone in a manner consistent to

Downloaded from http://circ.ahajournals.org/ by guest on December 27, 2016

DOI: 10.1161/CIRCULATIONAHA.112.094359

that reported in previous studies. Ozone-induced changes in FEV_1 and BAL neutrophils are shown in **Figure 4**. Immediately after exposure to ozone, post/pre FEV_1 valued following ozone exposure were decreased relative to post /pre air exposure values by 10.9% (confidence intervals of -6.5, -15.2), which is consistent with changes reported in other studies in which participants were exposed to 0.2 – 0.4 ppm ozone.[20] The difference between neutrophils found in BAL fluid 24 hours after ozone exposure relative to air exposure was 7.5% (CI 3.7, 15.3) which is consistent with what we and others have reported previously in participants exposed to 0.2 - 0.4 ppm ozone.[21-23]

Downloaded from http://circ.ahajournals.org/ by guest on December 27, 2016

Discussion

We believe the changes in ozone-induced vascular inflammation, fibrinolysis markers and heart rate variability (HRV) detailed in this study provides the most compelling data to date of the potential for ozone to modulate the cardiovascular system. Previous attempts to study these outcomes in epidemiology studies have resulted in inconsistent results, with some showing positive associations between ozone and cardiovascular changes and others not. These inconsistencies are likely due to differences in exposure metrics and windows of exposure, different methodologies used to assess CV changes, and the difficulty of attributing associations to ozone itself given its co-location across time and space with other pollutants (notably PM). [4]

In this study we observed robust changes in several pro-inflammatory cytokines in the blood within an hour after exposure to ozone. One of them (IL-1β) persisted for at least 24 hours with no diminution. These increases in markers of inflammation reported in this study were both substantial and highly significant, and thus not likely observed just due to chance. We also observed changes in CRP as well as a trend for increased concentration of neutrophils in the

blood immediately following ozone exposure. In contrast, previous epidemiology studies reporting associations between ozone and inflammatory markers have been equivocal. A retrospective repeated measures analysis of 45 Canadian adults reported a positive association between ozone and IL-6 with the strongest effects observed for a 4-day moving average of ozone.[24] Similarly, a study of 76 healthy individuals in Taiwan found associations between ozone and increased levels of blood CRP;[25] the strongest associations were with a 2 day averaging time. However, no association between ozone and CRP was observed in a repeated measures study of healthy individuals living in the Netherlands[26] or in more than 3000 healthy individuals living in Israel.[27] The causal nature of the findings from our controlled exposure study support the epidemiology studies that have reported positive associations between ozone and markers of inflammation.

We observed ozone-induced changes in several markers associated with fibrinolysis. A small increase in tissue plasminogen activator (tPA) was seen immediately following a two hour exposure to ozone. This serine protease catalyzes the conversion of plasminogen to plasmin, the major enzyme responsible for the breakdown of blood clots. There were more substantial and prolonged decreases in plasminogen activator inhibitor (PAI-1). PAI-1 levels were decreased both immediately and 24 hours after ozone exposure. PAI-1 is a serine protease inhibitor encoded by the Serpine1 gene and is the principal inhibitor of tPA. The combination of an increase in tPA and a decrease in PAI-1 suggests the possibility that ozone might activate the fibrinolysis system, perhaps in response to fibrin deposition caused by ozone exposure, though other explanations are also possible. We have previously reported that activation of fibrinolysis pathways, as well as increased levels of d-dimer (a fibrin breakdown product), are associated with exposure to particulate matter air pollution in humans.[28]

Downloaded from http://circ.ahajournals.org/ by guest on December 27, 2016

DOI: 10.1161/CIRCULATIONAHA.112.094359

We also show that ozone can mirror the established effects of PM and cause changes in HRV and cardiac repolarization. We observed a decrease in the HF component of HRV immediately following ozone exposure, which is consistent with findings in multiple PM studies (PM ISA). A previous controlled exposure study[29] did not find significant ozone-induced changes in HRV. However, in this study the participants were exposed to a smaller concentration of ozone (0.12 ppm) and did not exercise during exposure, which would have resulted in a smaller dose of ozone delivered to their airways. Controlled exposure studies have shown altered HRV following combined exposure to particles and ozone.[29, 30]

We also observed an increase in the duration of the QT interval immediately after exposure. A prolonged QT interval is a risk factor for ventricular tachyarrhythmias and sudden death. Some epidemiology studies have reported a positive association between ozone exposure and ventricular arrhythmias in people with implanted cardioverter defibrillators (ICD)[31-33] as well as with non-smoking adults aged 54 - 90.[34] In contrast, no evidence of an association between ozone and tachyarrhythmia events was observed in a study of 518 ICD patients.[35] Associations between ozone exposure and decreased heart rate variability (HF and LF) have been reported,[36] though the same cohort did not show any evidence of an association between ozone and QTc.[37] No associations were found between ozone and HRV in patients with congestive heart disease in Taiwan[38] or France.[39] A recent study reported associations between ozone exposure and increased heart rate, t wave flattening, and increased T wave complexity.[40]

The mechanism by which ozone can affect the cardiovascular system is not clear.. Some studies have reported that PM can translocate from the lung to the vasculature where it could directly attack vascular cells or the heart. However, we believe it is an unlikely mechanism for ozone since it is a highly reactive molecule which is consumed within seconds of inhalation.

Downloaded from http://circ.ahajournals.org/ by guest on December 27, 2016

DOI: 10.1161/CIRCULATIONAHA.112.094359

Using ozone tagged with the heavy isotope of oxygen (^{18}O) we found a small amount of heavy isotope in venous blood plasma, but the levels were too low to be statistically significant. [41] However, it has been recently shown that endogenous production of ozone by immune cells present in arterial atherosclerotic plaques can oxidize cholesterol.[42] Given the reactivity of ozone, if it were to diffuse into the circulatory system the concentration in arteries would likely be higher then in veins, raising the intriguing possibility that if small quantities of ozone were to diffuse into the blood they might contribute to oxidation of cholesterol found in the plaques. Particulate air pollution has recently been reported to be able to oxidize cholesterol, presumably by translocation of particles from the lung into the circulatory system..[43] A second proposed mechanism has been the spillover of pollutant-induced mediators from the lung into the blood, which could interact with vascular or cardiac cells. Ozone is known to cause robust increases in a number of pulmonary markers, including pro-inflammatory cytokines, eicosanoids, tPA, and fibrinogen.[21, 44, 45] These increases are much more substantial than those observed following exposure of humans to PM and they are initiated within an hour after a two hour exposure and can persist for at least 24 hours.[46, 47] A third mechanism is through modulation of the autonomic nervous system via nerve endings in the lung, which then affects cardiac and vascular function. Ozone-induced lung-function changes are also known to be mediated via C nerve fibers in the lung of humans[48] via the selective stimulation of TRPA 1 ion channels[49] and it is likely that the autonomic nervous system HRV and repolarization changes reported in this manuscript are also mediated via nerve fibers that terminate in the lung. Recent rodent studies show a significant role for autonomic mediation of heart rate variability and arrhythmia following exposure to ozone. These responses appear to involve both central nervous system and target receptors (TRPA 1). [50]

Downloaded from http://circ.ahajournals.org/ by guest on December 27, 2016

In summary, this study shows that exposure of healthy young adults to ozone causes an increase in vascular markers of inflammation, changes in fibrinolytic markers that could potentially impair fibironlysis, and changes in autonomic control of heart rate. These changes could potentially put a susceptible individual at risk for an adverse clinical event, and thus provides biological plausibility to the epidemiology studies which have reported associations between ozone exposure and mortality/morbidity.

Acknowledgements: The authors are indebted to Ms. Maryann Bassett, Ms. Jacqueline Carter, Ms. Lisa Dailey, Ms. Shirley Harder, Ms. Heidi Hiers, Ms. Tracey Montilla, Mr. Robert Silbajoris, and Ms. Joleen Soukup for their expert medical and technical assistance in the execution of this study. The research described in this article has been reviewed by the Environmental Protection Agency and approved for publication. The contents of this article do not necessarily represent Agency policy nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

Conflict of Interest Disclosures: None.

References:

1. EPA U. Air quality criteria for ozone and related photochemical oxidants. *EPA/600/R-10/076A*. 2011;Research Triangle Park.

2. EPA U. Integrated science assessment for particulate matter. *EPA/600/R-08/139F*. 2009;Research Triangle Park.

3. Bell ML, McDermott A, Zeger SL, Samet JM, Dominici F. Ozone and short-term mortality in 95 us urban communities, 1987-2000. *JAMA*. 2004;292:2372-2378

4. Bell ML, Kim JY, Dominici F. Potential confounding of particulate matter on the short-term association between ozone and mortality in multisite time-series studies. *Environ Health Perspect*. 2007;115:1591-1595.

5. Ito K, De Leon SF, Lippmann M. Associations between ozone and daily mortality: Analysis and meta-analysis. *Epidemiology*. 2005;16:446-457.

6. Levy JI, Chemerynski SM, Sarnat JA. Ozone exposure and mortality: An empiric bayes metaregression analysis. *Epidemiology*. 2005;16:458-468.

7. Katsouyanni K, Pantazopoulou A, Touloumi G, Tselepidaki I, Moustris K, Asimakopoulos D,

Downloaded from http://circ.ahajournals.org/ by guest on December 27, 2016

Poulopoulou G, Trichopoulos D. Evidence for interaction between air pollution and high temperature in the causation of excess mortality. *Arch Environ Health*. 1993;48:235-242.

8. Chan CC, Chuang KJ, Chien LC, Chen WJ, Chang WT. Urban air pollution and emergency admissions for cerebrovascular diseases, in taipei, taiwan. *Eur Heart J*. 2006;27:1238-1244.

9. Lee IM, Tsai SS, Ho CK, Chiu HF, Yang CY. Air pollution and hospital admissions for congestive heart failure in a tropical city: Kaohsiung, taiwan. *Inhal Toxicol*. 2007;19:899-904.

10. Yang CY. Air pollution and hospital admissions for congestive heart faiulre in a subtropical city. *J Toxicol Environ Health*. 2008;71:1085-1090.

11. Foster WM, Stetkiewicz PT. Regional clearance of solute from the respiratory epithelia: 18-20 h postexposure to ozone. *J Appl Physiol.* 1996;81:1143-1149.

12. Gong H, Jr., Wong R, Sarma RJ, Linn WS, Sullivan ED, Shamoo DA, Anderson KR, Prasad SB. Cardiovascular effects of ozone exposure in human volunteers. *Am J Respir Crit Care Med*. 1998;158:538-546.

13. Chuang GC, Yang Z, Westbrook DG, Pompilius M, Ballinger CA, White CR, Krzywanski DM, Postlethwait EM, Ballinger SW. Pulmonary ozone exposure induces vascular dysfunction, mitochondrial damage, and atherogenesis. *Am J Physiol Lung Cell Mol Physiol*. 2009;297:L209-216.

14. Watkinson WP, Campen MJ, Wichers LB, Nolan JP, Costa DL. Cardiac and thermoregulatory responses to inhaled pollutants in healthy and compromised rodents: Modulation via interaction with environmental factors. *Environ Res.* 2003;92:35-47.

15. Perepu RS, Garcia C, Dostal D, Sethi R. Enhanced death signaling in ozone-exposed ischemic-reperfused hearts. *Mol Cell Biochem*. 2010;336:55-64.

16. Calderon-Garciduenas L, Mora-Tiscareno A, Chung CJ, Valencia G, Fordham LA, Garcia R, Osnaya N, Romero L, Acuna H, Villarreal-Calderon A, Devlin RB, Koren HS. Exposure to air pollution is associated with lung hyperinflation in healthy children and adolescents in southwest mexico city: A pilot study. *Inhal Toxicol*. 2000;12:537-561.

17. Xu J, Zhang Y, Fu JS, Zheng S, Wang W. Process analysis of typical summertime ozone episodes over the beijing area. *Sci Total Environ*. 2008;399:147-157.

18. Kim CS, Alexis NE, Rappold AG, Kehrl H, Hazucha MJ, Lay JC, Schmitt MT, Case M, Devlin RB, Peden DB, Diaz-Sanchez D. Lung function and inflammatory responses in healthy young adults exposed to 0.06 ppm ozone for 6.6 hours. *Am J Respir Crit Care Med*. 2011;183:1215-1221.

19. Ghio AJ, Kim C, Devlin RB. Concentrated ambient air particles induce mild pulmonary inflammation in healthy human volunteers. *Am J Respir Crit Care Med.* 2000;162:981-988.

Downloaded from http://circ.ahajournals.org/ by guest on December 27, 2016

20. McDonnell WF, Smith MV. Description of acute ozone response as a function of exposure rate and total inhaled dose. *J. Appl. Physiol.* 1994;76:2776-2784.

21. Koren H, Devlin R, Graham D, Mann R, Horstman D, Kozumbo W, Becker S, Mcdonnell W, Bromberg P. Ozone-induced inflammation in the lower airways of human subjects. *Am Rev Respir Dis.* 1988;139:407-415.

22. Frampton MW, Morrow PE, Torres A, Voter KZ, Whitin JC, Cox C, Speers DM, Tsai Y, Utell MJ. Effects of ozone on normal and potentially sensitive human subjects. Part ii: Airway inflammation and responsiveness to ozone in nonsmokers and smokers. *Res Rep Health Eff Inst*. 1997:39-72; discussion 81-99.

23. Balmes JR, Aris RM, Chen LL, Scannell C, Tager IB, Finkbeiner W, Christian D, Kelly T, Hearne PQ, Ferrando R, Welch B. Effects of ozone on normal and potentially sensitive human subjects. Part i: Airway inflammation and responsiveness to ozone in normal and asthmatic subjects. *Res Rep Health Eff Inst.* 1997:1-37; discussion 81-99.

24. Thompson AM, Zanobetti A, Silverman F, Schwartz J, Coull B, Urch B, Speck M, Brook JR, Manno M, Gold DR. Baseline repeated measures from controlled human exposure studies: Associations between ambient air pollution exposure and the systemic inflammatory biomarkers il-6 and fibrinogen. *Environ Health Perspect*. 2010;118:120-124.

25. Chuang KJ, Chan CC, Su TC, Lee CT, Tang CS. The effect of urban air pollution on inflammation, oxidative stress, coagulation, and autonomic dysfunction in young adults. *Am J Respir Crit Care Med*. 2007;176:370-376

26. Rudez G, Janssen NA, Kilinc E, Leebeek FW, Gerlofs-Nijland ME, Spronk HM, ten Cate H, Cassee FR, de Maat MP. Effects of ambient air pollution on hemostasis and inflammation. *Environ Health Perspect*. 2009;117:995-1001.

27. Steinvil A, Kordova-Biezuner L, Shapira I, Berliner S, Rogowski O. Short-term exposure to air pollution and inflammation-sensitive biomarkers. *Environ Res*. 2008;106:51-61.

28. Samet JM, Rappold A, Graff D, Cascio WE, Berntsen JH, Huang YC, Herbst M, Bassett M, Montilla T, Hazucha MJ, Bromberg PA, Devlin RB. Concentrated ambient ultrafine particle exposure induces cardiac changes in young healthy volunteers. *Am J Respir Crit Care Med*. 2009;179:1034-1042.

29. Fakhri AA, Ilic LM, Wellenius GA, Urch B, Silverman F, Gold DR, Mittleman MA. Autonomic effects of controlled fine particulate exposure in young healthy adults: Effect modification by ozone. *Environ Health Perspect*. 2009;117:1287-1292.

30. Power KL, Balmes J, Solomon C. Controlled exposure to combined particles and ozone decreases heart rate variability. *J Occup Environ Med*. 2008;50:1253-1260.

31. Rich DQ, Schwartz J, Mittleman MA, Link M, Luttmann-Gibson H, Catalano PJ, Speizer FE,

Downloaded from http://circ.ahajournals.org/ by guest on December 27, 2016

Dockery DW. Association of short-term ambient air pollution concentrations and ventricular arrhythmias. *Am J Epidemiol.* 2005;161:1123-1132.

32. Rich DQ, Kim MH, Turner JR, Mittleman MA, Schwartz J, Catalano PJ, Dockery DW. Association of ventricular arrhythmias detected by implantable cardioverter defibrillator and ambient air pollutants in the st louis, missouri metropolitan area. *Occup Environ Med*. 2006;63:591-596.

33. Anderson HR, Armstrong B, Hajat S, Harrison R, Monk V, Poloniecki J, Timmis A, Wilkinson P. Air pollution and activation of implantable cardioverter defibrillators in london. *Epidemiology*. 2010;21:405-413.

34. Sarnat SE, Suh HH, Coull BA, Schwartz J, Stone PH, Gold DR. Ambient particulate air pollution and cardiac arrhythmia in a panel of older adults in steubenville, ohio. *Occup Environ Med*. 2006;63:700-706.

35. Metzger KB, Klein M, Flanders WD, Peel JL, Mulholland JA, Langberg JJ, Tolbert PE. Ambient air pollution and cardiac arrhythmias in patients with implantable defbrillators. *Epidemiology*. 2007;18:585-592.

36. Park SK, O'Neill MS, Vokonas PS, Sparrow D, Schwartz J. Effects of air pollution on heart rate variability: The va normative aging study. *Environ Health Perspect*. 2005;113:304-309.

37. Baja ES, Schwartz JD, Wellenius GA, Coull BA, Zanobetti A, Vokonas PS, Suh HH. Traffic-related air pollution and qt interval: Modification by diabetes, obesity, and oxidative stress gene polymorphisms in the normative aging study. *Environ Health Perspect*. 2010;118:840-846.

38. Chan CC, Chuang KJ, Su TC, Lin LY. Association between nitrogen dioxide and heart rate variability in a susceptible population. *Eur J Cardiovasc Prev Rehabil*. 2005;12:580-586.

39. Ruidavets JB, Cassadou S, Cournot M, Bataille V, Meybeck M, Ferrieres J. Increased resting heart rate with pollutants in a population based study. *J Epidemiol Community Health*. 2005;59:685-693.

40. Hampel R, Breitner S, Zareba W, Kraus U, Pitz M, Geruschkat U, Belcredi P, Peters A, Schneider A. Immediate ozone effects on heart rate and repolarisation parameters in potentially susceptible individuals. *Occup Environ Med*. 2012;69:428-436.

41. Hatch GE, Slade R, Harris LP, McDonnell WF, Devlin RB, Koren HS, Costa DL, McKee J. Ozone dose and effect in humans and rats. *Am J Resp Crit Care Med.* 1994;150:676-683.

42. Wentworth P, Jr., Nieva J, Takeuchi C, Galve R, Wentworth AD, Dilley RB, DeLaria GA, Saven A, Babior BM, Janda KD, Eschenmoser A, Lerner RA. Evidence for ozone formation in human atherosclerotic arteries. *Science*. 2003;302:1053-1056.

Downloaded from http://circ.ahajournals.org/ by guest on December 27, 2016

43. Araujo JA. Particulate air pollution, systemic oxidative stress, inflammation, and atherosclerosis. *Air Qual Atmos Health*. 2010;4:79-93.

44. McGee MP, Devlin R, Saluta G, Koren H. Tissue factor and factor vii messenger rnas in human alveolar macrophages: Effects of breathing ozone. *Blood*. 1990;75:122-127.

45. Seltzer J, Bigby BG, Stulbarg M, Holtzman MJ, Nadel JA, Ueki IF, Leikauf GD, Goetzl EJ, Boushey HA. O_3-induced change in bronchial reactivity to methacholine and airway inflammation in humans. *J Appl Physiol.* 1986;60:1321-1326.

46. Devlin RB, McDonnell WF, Becker S, Madden MC, McGee MP, Perez R, Hatch G, House DE, Koren HS. Time-dependent changes of inflammatory mediators in the lungs of humans exposed to 0.4 ppm ozone for 2 hr: A comparison of mediators found in bronchoalveolar lavage fluid 1 and 18 hr after exposure. *Toxicol Appl Pharmacol.* 1996;138:176-185.

47. Balmes Jr, Chen LL, Scannell C, Tager IB, Finkbeiner W, Christian D, Hearne PQ, Kelly T, Aris RM. Ozone-induced decrements in fev1 and fvc do not correlate with measures of inflammation. *Am J Resp Crit Care Med.* 1996;153:904-909.

48. Passannante AN, Hazucha MJ, Bromberg PA, Seal E, Folinsbee L, Koch G. Nociceptive mechanisms modulate ozone-induced human lung function decrements. *J Appl Physiol*. 1998;85:1863-1870.

49. Taylor-Clark TE, Undem BJ. Ozone activates airway nerves via the selective stimulation of trpa1 ion channels. *J Physiol*. 2010;588:423-433.

50. Farraj A, Hazari M, Winsett D, Kulukuluslani A, AP C, Coates N, Lamb C, Lappi E, Terrel D, Cascio W, Costa D. Overt and latent cardiac effects of ozone inhalation in rats: Evidence for autonomic modulation and increased myocardial vulnerability. *Environ Health Perspect*. 2012;120:348-54.

Downloaded from http://circ.ahajournals.org/ by guest on December 27, 2016

DOI: 10.1161/CIRCULATIONAHA.112.094359

Table 1. Changes in Biomarkers of Inflammation.

	Pre Air	Post Air	Follow-up Air	Pre O3	Post O3	Follow-up O3
Blood IL-1 (pg/ml)	0.29 ± 0.09	0.25 ± 0.06	0.21 ± 0.03	0.21 ± 0.05	0.24 ± 0.05	0.35 ± 0.08
Ratio		0.80 ± 0.11	0.92 ± 0.16		1.37 ± 0.23+	1.78 ± 0.22*
Blood IL-6 (pg/ml)	2.25 ± 0.26	2.18 ± 0.25	2.18 ± 0.23	2.12 ± 0.31	2.13 ± 0.27	2.09 ± 0.27
Ratio		1.00 ± 0.06	0.99 ± 0.04		1.07 ± 0.06	1.04 ± 0.06
Blood IL-8 (pg/ml)	1.15 ± 0.17	1.13 ± 0.19	1.18 ± 0.14	0.98 ± 0.14	1.56 ± 0.11	1.11 ± 0.11
Ratio		1.05 ± 0.10	1.16 ± 0.10		2.04 ± 0.27*	1.36 ± 0.21
Blood TNF (pg/ml)	4.83 ± 0.26	4.32 ± 0.23	5.27 ± 0.37	4.64 ± 0.35	4.49 ± 0.27	4.95 ± 0.31
Ratio		0.90 ± 0.03	1.09 ± 0.04		1.00 ± 0.04+	1.11 ± 0.06
Blood CRP (ng/ml)	686 ± 325	708 ± 279	760 ± 356	643 ± 148	656 ± 182	972 ± 184
Ratio		1.10 ± 0.08	1.26 ± 0.26		1.15 ± 0.11	2.62 ± 0.76*
Blood PMNs (%)	50.8 ± 2.5	59.6 ± 2.3	50.0 ± 1.8	51.7 ± 1.4	63.5 ± 2.0	50.1 ± 1.9
		1.18 ± 0.38	0.99 ± 0.02		1.22 ± 0.04	0.97 ± 0.03
BAL PMNs (%)			1.9 ± 0.34			9.6 ± 1.3*

* p< 0.025
\# p < 0.05
\+ P< 0.10

Table 2. Changes in Other Vascular Biomarkers.

	Pre Air	Post Air	Follow-up Air	Pre O3	Post O3	Follow-up O3
PAI -1 (ng/ml)	2.82 ± 0.41	2.15 ± 0.36	2.98 ± 0.50	3.04 ± 0.49	1.90 ± 0.53	2.04 ± 0.44
Ratio		0.83 ± 0.11	1.21 ± 0.19		0.62 ± 0.12#	0.73 ± 0.10#
Plasminogen (ng/ml)	162.9 ± 5.3	173.6 ± 10.2	212.9 ± 14.5	196.1 ± 12.6	201.8 ± 18.3	169.4 ± 11.3
Ratio		1.10 ± 0.08	1.34 ± 0.10		1.08 ± 0.10	0.92 ± 0.08*
tPA (ng/ml)	2.82 ± 0.41	2.15 ± 0.36	2.98 ± 0.50	3.04 ± 0.49	1.90 ± 0.53	2.04 ± 0.44
Ratio		1.11 ± 0.09	1.00 ± 0.09		1.55 ± 0.27+	1.18 ± 0.22
vWF (%)	94.6 ± 10.4	106.3 ± 13.7	89.4 ± 7.4	98.5 ± 7.2	113.1 ± 11.0	89.5 ± 10.3
Ratio		1.13 ± 0.69	1.03 ± 0.07		1.26 ± 0.09	0.93 ± 0.08
D-dimer (ng/ml)	83.8 ± 18.5	83.3 ± 16.3	88.7 ± 30.5	102.9 ± 32.1	107.1 ± 21.7	71.2 ± 9.6
Ratio		1.08 ± 0.09	0.89 ± 0.10		1.14 ± 0.11	0.89 ± 0.10
Cholesterol (mg/dL)	152 ± 6.5	149 ± 6.6	152 ± 6.3	152.3 ± 8.3	151.2 ± 7.5	148.3 ± 6.6
Ratio		1.00 ± 0.02	1.03 ± 0.02		1.01 ± 0.01	1.00 ± 0.01

* p<0.025
\# p< 0.05
\+ P< 0.10

Downloaded from http://circ.ahajournals.org/ by guest on December 27, 2016

DOI: 10.1161/CIRCULATIONAHA.112.094359

Table 3. Changes in Holter Monitor Variables

	Pre Air	Post Air	Follow-up Air	Pre O3	Post O3	Follow-up O3
SDNN (msec)			91.2 ± 3.6			88.2 ± 4.6
PNN50 (%)			26.2 ± 2.5			24.2 ± 2.5
# PAC			22.3 ± 8.1			23.9 ± 8.9
# PVC			5.7 ± 1.3			8.2 ± 1.8
HF (msec)	2630 ± 424	2897 ± 386	3484 ± 429	3132 ± 469	1833 ± 399	2959 ± 463
Ratio		1.37 ± 0.14	2.04 ± 0.41		0.73 ± 0.14*	1.43 ± 0.28+
LF (msec)	2678 ± 372	2576 ± 390	2620 ± 409	2857 ± 493	1833 ± 398	3218 ± 554
Ratio		1.69 ± 0.36	1.69 ± 0.38		1.49 ± 0.45	2.65 ± 0.78
HF/LF	1.06 ± 0.16	0.75 ± 0.10	0.76 ± 0.11	1.05 ± 0.14	0.72 ± 0.11	1.10 ± 0.27
Ratio		1.06 ± 0.16	0.97 ± 0.17		0.80 ± 0.10	1.31 ± 0.25
QTc	412 ± 5.2	407 ± 4.6	410 ± 5.2	408 ± 4.4	408 ± 4.9	408 ± 5.4
Ratio		0.95 ± 0.003	0.99 ± 0.007		1.00 ± 0.003*	1.00 ± 0.017
QRS Complexity	0.31 ± 0.03	0.34 ± 0.03	0.33 ± 0.03	0.34 ± 0.03	0.35 ± 0.03	0.36 ± 0.03
Ratio		1.04 ± 0.03	1.06 ± 0.02		1.03 ± 0.02*	1.24 ± 0.15
Heart Rate BPM	59.7 ± 1.7	61.6 ± 2.1	54.7 ± 1.5	58.3 ± 1.7	63.4 ± 3.1	56.1 ± 2.0
Ratio		1.04 ± 0.03	0.92 ± 0.02		1.09 ± 0.04	0.99 ± 0.06

* P < 0.025
# p < 0.05
+ P < 0.10

Circulation
Journal of the American Heart Association

Figure Legends:

Figure 1. Ozone-induced Changes in Markers of Vascular Inflammation. For analysis, the pre-exposure values are subtracted from either the post or followup values and a paired t test used to compare the differences following ozone exposure with the differences following air exposure. These differences are plotted as the mean and 95% confidence intervals (CIs). For TNF, p = 0.067; for IL-8, p < 0.001; for IL-1 post, p = 0.008 ; for IL-1 followup, p < 0.001; for CRP, p = 0.023.

Figure 2. Ozone-induced Changes in Markers of Clotting and Coagulation. For analysis, the

Downloaded from http://circ.ahajournals.org/ by guest on December 27, 2016

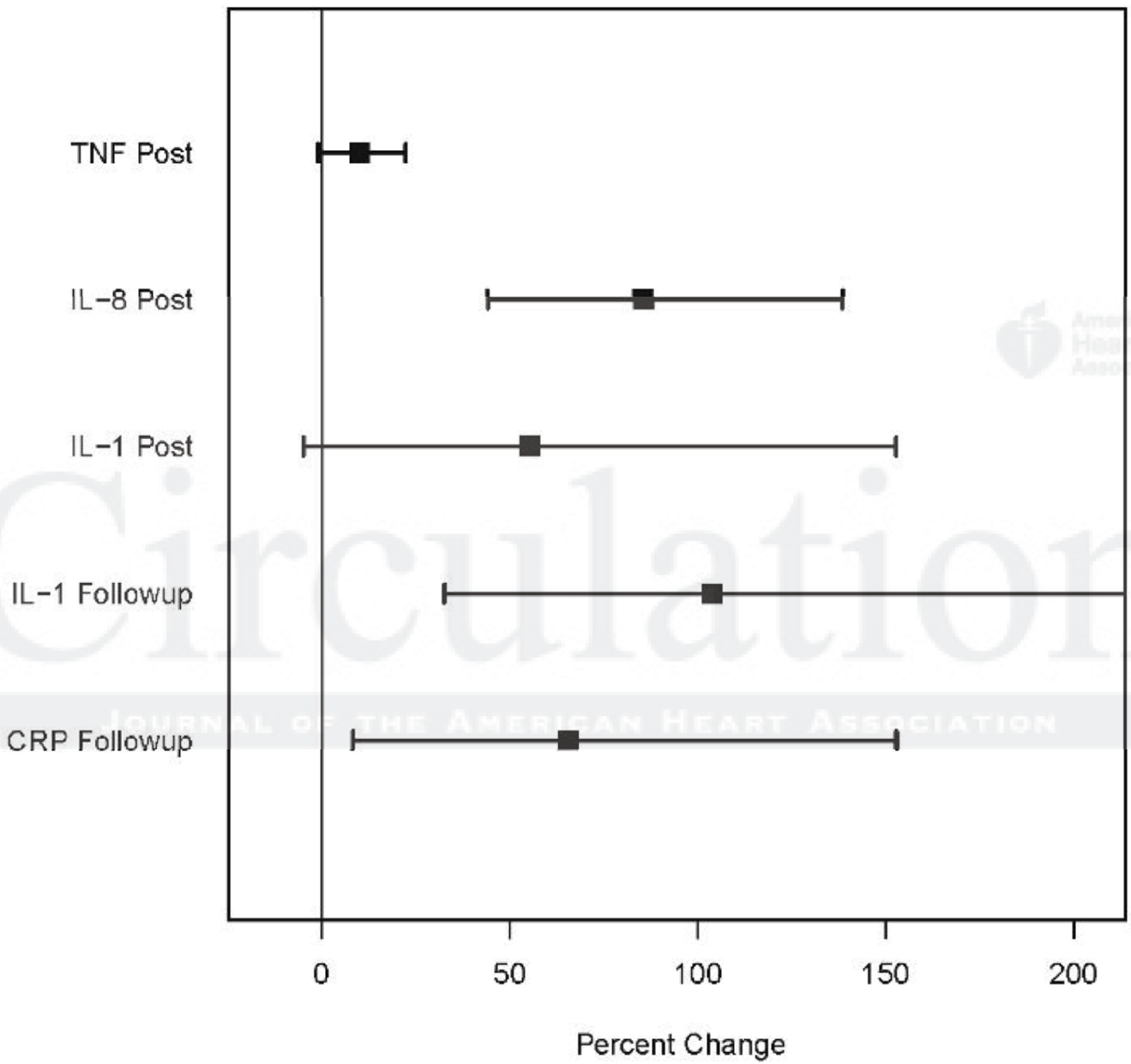
DOI: 10.1161/CIRCULATIONAHA.112.094359

pre-exposure values are divided by either the post or followup values and a paired t test used to compare the differences following ozone exposure with the differences following air exposure. These differences are plotted as the mean and 95% confidence intervals. For tPA, $p = 0.065$; for PAI-1 post, $p = 0.050$; for PAI-1 followup, $p = 0.033$; for plasminogen, $p = 0.003$.

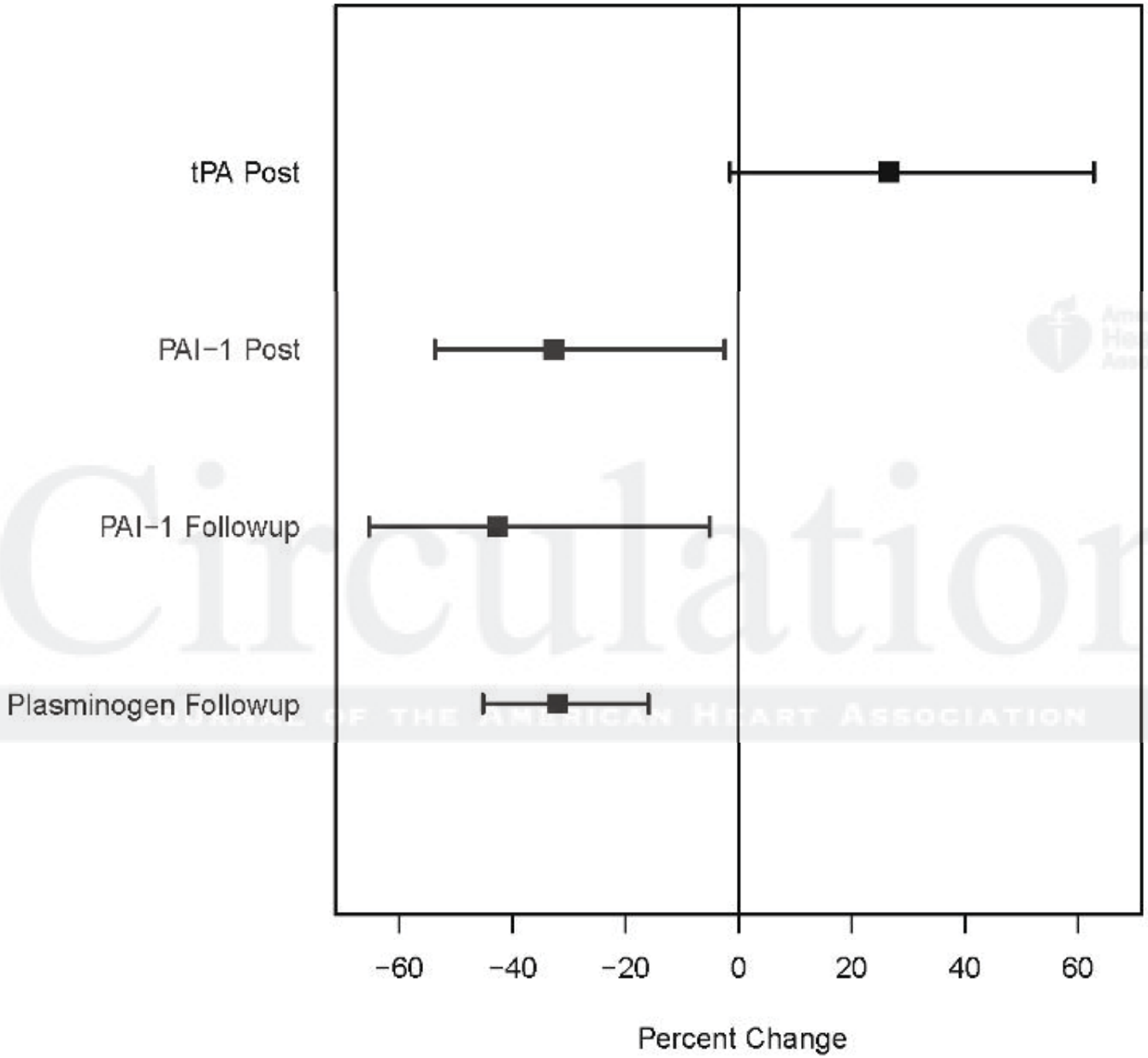
Figure 3. Ozone-induced Changes in Heart Rate Variability and Repolarization. For analysis, the pre-exposure values are divided by either the post or followup values and a paired t test used to compare the differences following ozone exposure with the differences following air exposure. These differences are plotted as the mean and 95% confidence intervals. For QRS Complexity, $p = 0.019$; for QTc, $p = 0.007$; for post HF, $p = 0.013$; for followup HF, $p = 0.002$; for LF, $p =- 0.015$; for hear rate, $p = 0.002$.

Figure 4. Ozone-induced Changes in Lung Function and Pulmonary Inflammation. For analysis of FEV_1, the pre-exposure values are divided by either the post or followup values and a paired t test used to compare the differences following ozone exposure with the differences following air exposure. These differences are plotted as the mean and 95% confidence intervals. Since bronchoscopy was only performed 24 hours after each exposure, a paired t test was used to compare the changes at this time in % neutrophils following air and ozone exposure. For FEV_1, $p < 0.001$; for % neutrophils, $p < 0.001$.

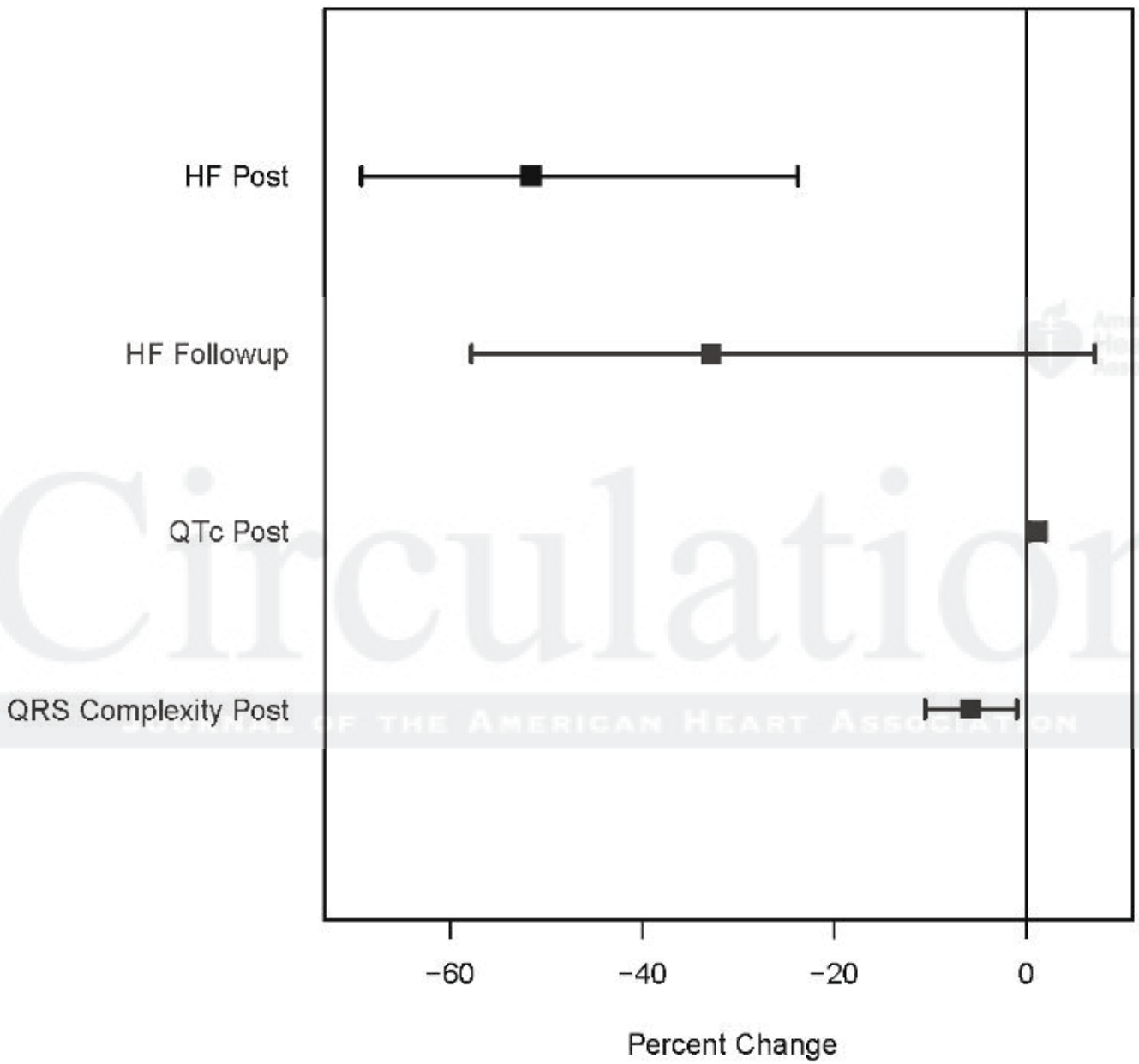
Downloaded from http://circ.ahajournals.org/ by guest on December 27, 2016



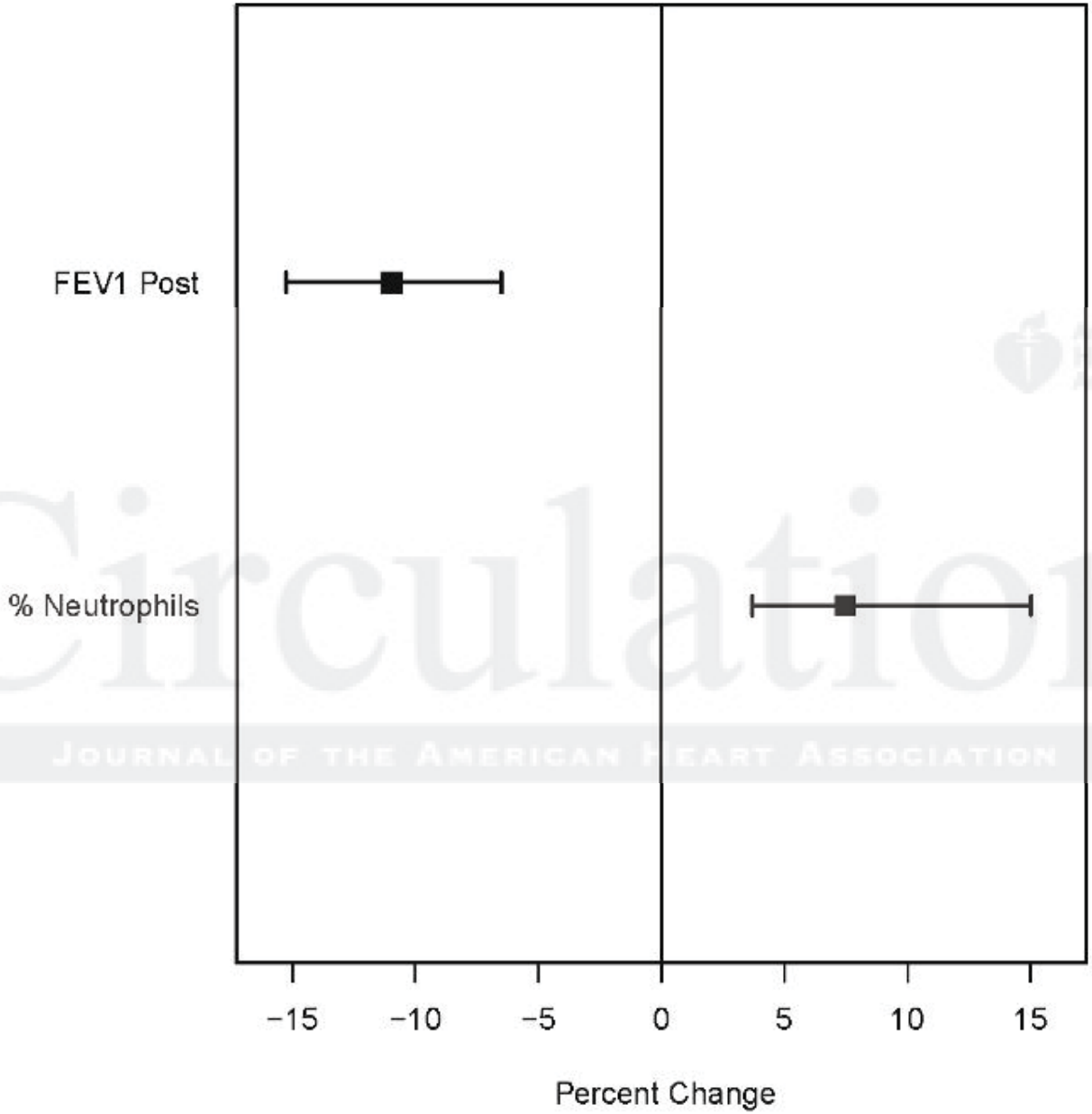
Downloaded from http://circ.ahajournals.org/ by guest on December 27, 2016



Downloaded from http://circ.ahajournals.org/ by guest on December 27, 2016



Downloaded from http://circ.ahajournals.org/ by guest on December 27, 2016



Downloaded from http://circ.ahajournals.org/ by guest on December 27, 2016

Circulation

American Heart Association®

Controlled Exposure of Healthy Young Volunteers to Ozone Causes Cardiovascular Effects

Robert B. Devlin, Kelly E. Duncan, Melanie Jardim, Michael T. Schmitt, Ana G. Rappold and David Diaz-Sanchez

Circulation. published online June 25, 2012;
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2012 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/early/2012/05/18/CIRCULATIONAHA.112.094359

Downloaded from http://circ.ahajournals.org/ by guest on December 27, 2016

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Circulation* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Circulation* is online at:
http://circ.ahajournals.org//subscriptions/