

# Short-Term Exposure to Ozone Does Not Impair Vascular Function or Affect Heart Rate Variability in Healthy Young Men

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Air pollution exposure is associated with cardiovascular morbidity and mortality, yet the role of individual pollutants remains unclear. In particular, there is uncertainty regarding the acute effect of ozone exposure on cardiovascular disease. In these studies, we aimed to determine the effect of ozone exposure on vascular function, fibrinolysis, and the autonomic regulation of the heart. Thirty-six healthy men were exposed to ozone (300 ppb) and filtered air for 75 min on two occasions in randomized double-blind crossover studies. Bilateral forearm blood flow (FBF) was measured using forearm venous occlusion plethysmography before and during intra-arterial infusions of vasodilators 2–4 and 6–8 h after each exposure. Heart rhythm and heart rate variability (HRV) were monitored during and 24 h after exposure. Compared with filtered air, ozone exposure did not alter heart rate, blood pressure, or resting FBF at either 2 or 6 h. There was a dose-dependent increase in FBF with all vasodilators that was similar after both exposures at 2–4 h. Ozone exposure did not impair vasomotor or fibrinolytic function at 6–8 h but rather increased vasodilatation to acetylcholine ( $p = .015$ ) and sodium nitroprusside ( $p = .005$ ). Ozone did not affect measures of HRV during or after the exposure. Our findings do not support a direct rapid effect of ozone on vascular function or cardiac autonomic control although we cannot exclude an effect of chronic exposure or an interaction between ozone and alternative air pollutants that may be responsible for the adverse cardiovascular health effects attributed to ozone.

**Key Words:** air pollution; blood flow; endothelium; fibrinolysis; oxidative stress; ozone.

Exposure to air pollution is strongly associated with respiratory and cardiovascular morbidity and mortality. These associations are well established for exposure to fine particulate matter (PM) air pollution primarily from the combustion of fossil fuels (Brunekreef and Holgate, 2002; Miller *et al.*, 2007).

Ozone (O<sub>3</sub>) is a strongly oxidative secondary air pollutant, and epidemiological studies have shown that ozone levels

also correlate with respiratory and cardiovascular morbidity and mortality (Bell *et al.*, 2004, 2007; Brook *et al.*, 2010; Ito *et al.*, 2005; Peters *et al.*, 2004). The effects of ozone on health have been difficult to disentangle from the effects of PM in observational studies as these pollutants are often highly correlated. The difficulty in resolving the independent effects of ozone on cardiovascular mortality is illustrated by the divergent results published from the American Cancer Society Cancer Prevention Study cohort (Jerrett *et al.*, 2009; Smith *et al.*, 2009). Although Smith *et al.* (2009) report an independent association between ozone and cardiovascular death, Jerrett *et al.* (2009) found that ozone was associated only with respiratory mortality after adjustment for PM<sub>2.5</sub>. These discordant findings are not necessarily contradictory but may reflect differences in the analyses performed. Although a number of reports suggest an independent effect of ozone on mortality (de Almeida *et al.*, 2011; Gryparis *et al.*, 2004; Halonen *et al.*, 2010; Ren *et al.*, 2008; Stafoggia *et al.*, 2010), acute myocardial infarction (Halonen *et al.*, 2010; Ruidavets *et al.*, 2005), and cardiac arrest (Ensor *et al.*, 2013), it remains unclear how ozone exposure may trigger acute nonfatal and fatal cardiovascular events.

Human chamber studies have defined the effects of short-term exposure to ozone on the respiratory tract indicating an airway inflammatory response along with an alteration in antioxidant defences (Behndig *et al.*, 2009; Blomberg *et al.*, 1999; Frampton *et al.*, 1999; Stenfors *et al.*, 2002). The evidence for a direct cardiovascular effect of ozone, however, is more limited. Exposure to ozone in combination with concentrated ambient particles increases peripheral arterial tone and blood pressure (Brook *et al.*, 2002; Urch *et al.*, 2005) although subsequent studies demonstrate that this may be an effect of ambient particles rather than ozone (Brook *et al.*, 2009), and indeed ozone has been shown to reduce blood pressure in a diabetic population at the same time as particulate air pollution increased blood pressure (Hoffmann *et al.*, 2012). Ozone exposure may

influence autonomic regulation of the heart rate and has been shown to alter circulating inflammatory and fibrinolytic markers in healthy persons (Chuang *et al.*, 2007; Devlin *et al.*, 2012).

Exposure to dilute diesel exhaust has been consistently associated with important effects on vascular function, systemic hemodynamics, fibrinolysis, and thrombosis (Cosselman *et al.*, 2012; Lucking *et al.*, 2008; Mills *et al.*, 2005, 2007; Törnqvist *et al.*, 2007). Furthermore, these adverse effects can be abolished by filtration to remove ultrafine particles, suggesting that gaseous copollutants are not primarily responsible for the cardiovascular effects of air pollution (Lucking *et al.*, 2011; Mills *et al.*, 2011). The effect of exposure to ozone in isolation on microvascular endothelial and fibrinolytic function has not been explored.

We hypothesize that short-term exposure to ozone would cause acute microvasomotor and fibrinolytic dysfunction in healthy men.

## MATERIALS AND METHODS

**Subjects.** Thirty-six healthy, nonsmoking male volunteers of mean age 26 (range: 21–31) years with normal lung function were recruited (Table 1). All subjects reported no ongoing medical conditions, took no regular medication (prescribed or over the counter), and were free of the symptoms of respiratory tract infection for at least 6 weeks prior to enrollment. All subjects had a normal baseline 12-lead electrocardiogram (ECG). The local ethical review board approved the study, and written informed consent was obtained. The study was performed in accordance with Declaration of Helsinki.

**Study design.** All subjects were exposed to 300 ppb of ozone or filtered air in a whole-body exposure chamber of approximately 15 m<sup>3</sup> for 75 min on two occasions at least two weeks apart in a randomized double-blind crossover study (Supplementary data). During exposures, the subjects alternated moderate exercise on a bicycle ergometer to achieve an average minute ventilation of 20 l/min/m<sup>2</sup> body surface area, with rest at 15-min intervals (starting with an exercise period).

Venous occlusion forearm plethysmography before and during intra-arterial infusion of endothelium-dependent and endothelium-independent vasodilators was performed 2–4 h ( $n = 18$ ) and 6–8 h ( $n = 18$ ) after all exposures as previously described (Lucking *et al.* 2011; Mills *et al.*, 2005, 2007, 2011; Törnqvist *et al.*, 2007). A 3-lead continuous ECG was recorded from each subject during the exposure and subsequent 24-h period using a Holter monitor (model 90217, Spacelabs Healthcare, UK).

**TABLE 1**  
Baseline Characteristics of Healthy Volunteers

	2–4 h, $n = 18$	6–8 h, $n = 18$
Age, years	26 ± 1	26 ± 1
Height, cm	183 ± 3	180 ± 1
Weight, kg	77 ± 3	79 ± 2
BMI, m <sup>2</sup> /kg	23 ± 1	24 ± 1
FVC, l	5.7 ± 0.2	5.8 ± 0.2
FEV <sub>1</sub> , l	4.3 ± 0.1	4.2 ± 0.1

*Note.* Values are reported as mean ± SEM; BMI, body mass index; FVC, forced vital capacity; FEV<sub>1</sub>, forced expiratory volume in 1 s.

**Ozone exposure.** The exposures were performed in an exposure chamber described in detail elsewhere and in the Supplementary data (Blomberg *et al.*, 1999).

**Vascular studies.** Forearm blood flow (FBF) was determined during unilateral brachial artery infusion of endothelium-dependent and -independent vasodilators using venous occlusion plethysmography with mercury-silicone elastomer strain gauges, as described previously (Lucking *et al.* 2011; Mills *et al.*, 2005, 2007, 2011; Törnqvist *et al.*, 2007). Briefly, the brachial artery of one arm was cannulated using a 27-standard wire gauge steel needle, and 17 gauge venous cannulae were inserted in large veins in the antecubital fossa of both arms. Following a 30-min baseline saline infusion, acetylcholine at 5, 10, and 20 µg/min (endothelium-dependent vasodilator that does not release tissue plasminogen activator [t-PA]), bradykinin at 100, 300, and 1000 pmol/min (endothelium-dependent vasodilator that releases t-PA), and sodium nitroprusside (SNP) at 2, 4, and 8 µg/min (endothelium-independent vasodilator that does not release t-PA) were infused for 6 min at each dose with FBF measured for the last 3 min of each infusion. The infusions of the three vasodilators were given in a random order and separated by 20-min saline infusions. Due to its prolonged action, verapamil at 10, 30, and 100 µg/min (endothelium- and nitric oxide-independent vasodilator that does not release t-PA) was infused at the end of the study protocol. Blood pressure and heart rate were measured during the forearm study using a validated semiautomated oscillometric sphygmomanometer.

**Heart rate variability.** ECG recordings were analyzed by an experienced operator, blinded to exposure and the subject characteristics, using the Reynolds Medical Pathfinder Digital 700 Series Analysis System (Delmar Reynolds) (Supplementary data).

**Peripheral blood analyses.** Peripheral blood was sampled before and 2 and 6 h after each exposure for analyses of differential cell counts and fibrinolytic and inflammatory markers with samples obtained during the infusion of each dose of bradykinin for fibrinolytic markers.

**Data analysis and statistics.** FBF, t-PA release, and measures of heart rate variability (HRV) were analyzed as previously (Lucking *et al.*, 2011; Mills *et al.*, 2005, 2007, 2011; Törnqvist *et al.*, 2007) using two-way ANOVA with repeated measures or two-tailed Student's *t*-tests, where appropriate, using GraphPad Prism (GraphPad Software, Version 4 for Macintosh) and SPSS (SPSS inc., Chicago, IL, version 15). Statistical significance was defined as  $p < .05$ .

## RESULTS

There were no differences in heart rate, blood pressure, or resting FBF 2 or 6 h after exposure to ozone compared with filtered air (Table 2). Systemic inflammatory mediators were not affected by exposure to ozone or filtered air (Supplementary table S1).

### Vascular Studies

Endothelial-dependent and -independent vasodilators caused dose-dependent increases in FBF ( $p < .001$ ). There were no significant differences in forearm arm blood flow 2–4 h following exposure to ozone or filtered air (Fig. 1). At 6–8 h, FBF response to acetylcholine and sodium nitroprusside was not impaired but rather increased after exposure to ozone compared with filtered air ( $p = .02$  and  $p = .01$ , respectively). FBF responses to bradykinin and verapamil were similar after both exposures ( $p > .05$ ) (Fig. 2).

**TABLE 2**  
**Hemodynamic Variables Before Forearm Vascular Study, 2 or 6 h Postexposure to Ozone**

	Air	Ozone	<i>p</i> Value
2–4 h, <i>n</i> = 18			
Heart rate, bpm	54 ± 1	54 ± 1	.94
Systolic blood pressure, mmHg	129 ± 2	130 ± 2	.64
Diastolic blood pressure, mmHg	67 ± 2	67 ± 2	.35
Infused FBF, ml/100 ml tissue/min	2.2 ± 0.1	2.0 ± 0.1	.14
Noninfused FBF, ml/100 ml tissue/min	1.9 ± 0.1	1.8 ± 0.1	.51
6–8 h, <i>n</i> = 18			
Heart rate, bpm	63 ± 2	62 ± 2	.30
Systolic blood pressure, mmHg	142 ± 3	139 ± 4	.26
Diastolic blood pressure, mmHg	68 ± 2	67 ± 2	.45
Infused FBF, ml/100 ml tissue/min	3.2 ± 0.3	3.4 ± 0.4	.55
Noninfused FBF, ml/100 ml tissue/min	2.6 ± 0.2	2.7 ± 0.2	.86

Note. Values are reported as mean ± SEM. *p* Values from Student's paired *t*-tests.

There were no differences in plasma t-PA or plasminogen activator inhibitor-1 antigen concentrations at baseline, 2 h, or 6 h following exposure to ozone compared with filtered air (Supplementary table S2). The net release of t-PA antigen following bradykinin infusion was unaffected 6–8 h after exposure to ozone compared with filtered air (Table 3).

#### HRV

Subjects did not experience any significant rhythm disturbance during the 24-h study period although subjects' heart rate was generally higher through the ozone exposure day (at baseline and through the study) (Table 4). Exposure to ozone did not affect time or frequency domain measures of HRV over the 24-h period (Supplementary table S3). Measures of HRV averaged over a 5-min interval immediately prior to exposure were similar and were not altered at 2 and 6 h following exposure to ozone or filtered air (Table 4).

## DISCUSSION

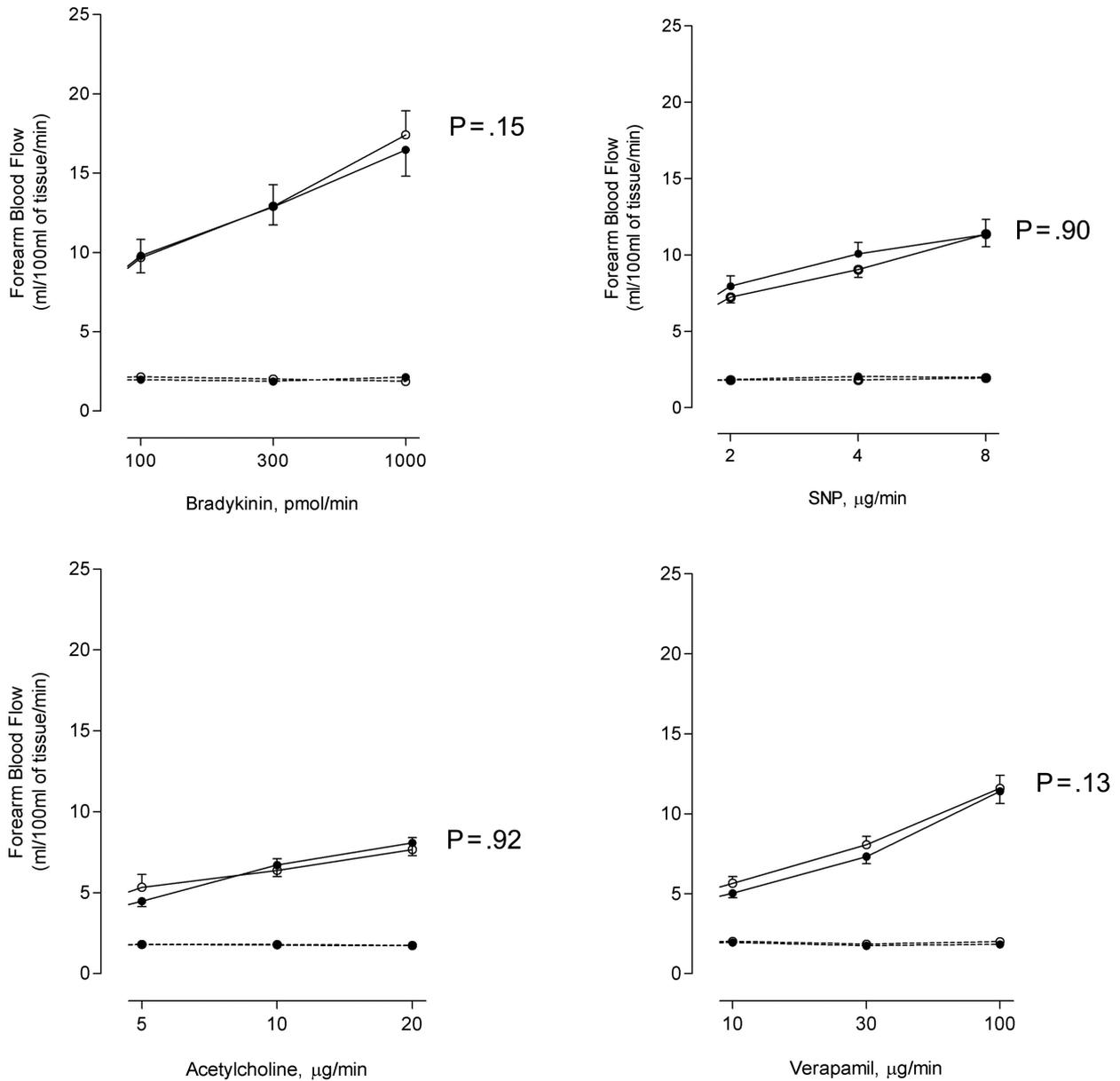
Brief exposure to ozone at a high ambient concentration does not impair vascular vasomotor or fibrinolytic function or affect HRV in young healthy subjects. Ozone exposure does not have a direct and rapid effect on the cardiovascular system, and although we cannot exclude an effect of chronic exposure, an interaction of ozone and other air pollutants increasing the toxicity of these pollutants, or an effect in more susceptible patient groups, we suggest that the association between ozone and acute cardiovascular morbidity and mortality are due to other atmospheric air pollutants rather than exposure to ozone *per se*.

Although ozone is an irritant gas capable of inducing oxidative stress and inflammation in the airways (Behndig *et al.*, 2009; Blomberg *et al.*, 1999; Frampton *et al.*, 1999; Stenfors *et al.*, 2002), the evidence for cardiovascular effects is less clear. Ozone rapidly reacts with low molecular weight antioxidants in

the lower respiratory tract lining fluid (Behndig *et al.*, 2009; Mudway and Kelly, 2000) and is therefore unlikely to penetrate the airway epithelium or directly cause systemic oxidant and inflammatory effects. Consistent with this we did not observe any change in circulating blood cells, inflammatory cytokines, or markers of platelet and endothelial activation. Devlin *et al.* (2012) found a marked fivefold increase in airway neutrophils following exposure to ozone at the same concentration consistent with a localized effect in the airways and reported a transient increase in systemic concentrations of interleukin-8 1 h following exposure, consistent with our own findings there were no effects on circulating interleukin-6, tumor necrosis factor, or white blood cells.

Although ozone cannot penetrate the pulmonary epithelium, it may activate receptors in the airways that can trigger an autonomic reflex to influence regulation of heart rate and vascular tone (Lai and Kou, 1998). In murine models, pulmonary C-fibers are activated by ozone, thus decreasing heart rate and cardiac output while resulting in bronchial vasodilatation (Lee and Pisarri, 2001; Taylor-Clark and Undem, 2010). Indeed, a change in cardiac autonomic control is one of the central mechanistic pathways that have been proposed to explain the cardiovascular effects of inhaled air pollutants (Brook *et al.*, 2010). Reduced HRV reflects either an increase in sympathetic drive and/or a reduction in parasympathetic tone and has been associated with increased risk of cardiovascular morbidity and mortality (Tsuji *et al.*, 1996). Chuang *et al.* (2007) found a decrease in standard deviation of successive RR intervals, root mean square difference of successive RR intervals, low-frequency power component of HRV, and high-frequency power component of HRV (HF) associated with increased ambient ozone concentrations averaged over 1–3 days in healthy students residing in a heavily polluted urban area in Taiwan. Of note, the PM<sub>10</sub> concentrations during the study period were in excess of the World Health Organization's limits of 50 µg/m<sup>3</sup>, which may be a confounding factor. There have been two recent controlled exposure studies to evaluate the effect of ozone on HRV (Brook *et al.*, 2009; Devlin *et al.*, 2012). Devlin *et al.* (2012) found no effect of exposure to 300 ppb of ozone on any measure of HRV although they report a 51% reduction in the 1 h postexposure/pre-exposure HF power ratio of uncertain significance. Brook *et al.* (2009) similarly found no effect of exposure to ozone either in isolation or in combination with concentrated ambient particles on HRV. We found no effect of exposure to ozone on any measure of HRV and suggest that any effect of ozone beyond the airways is not mediated by autonomic dysfunction.

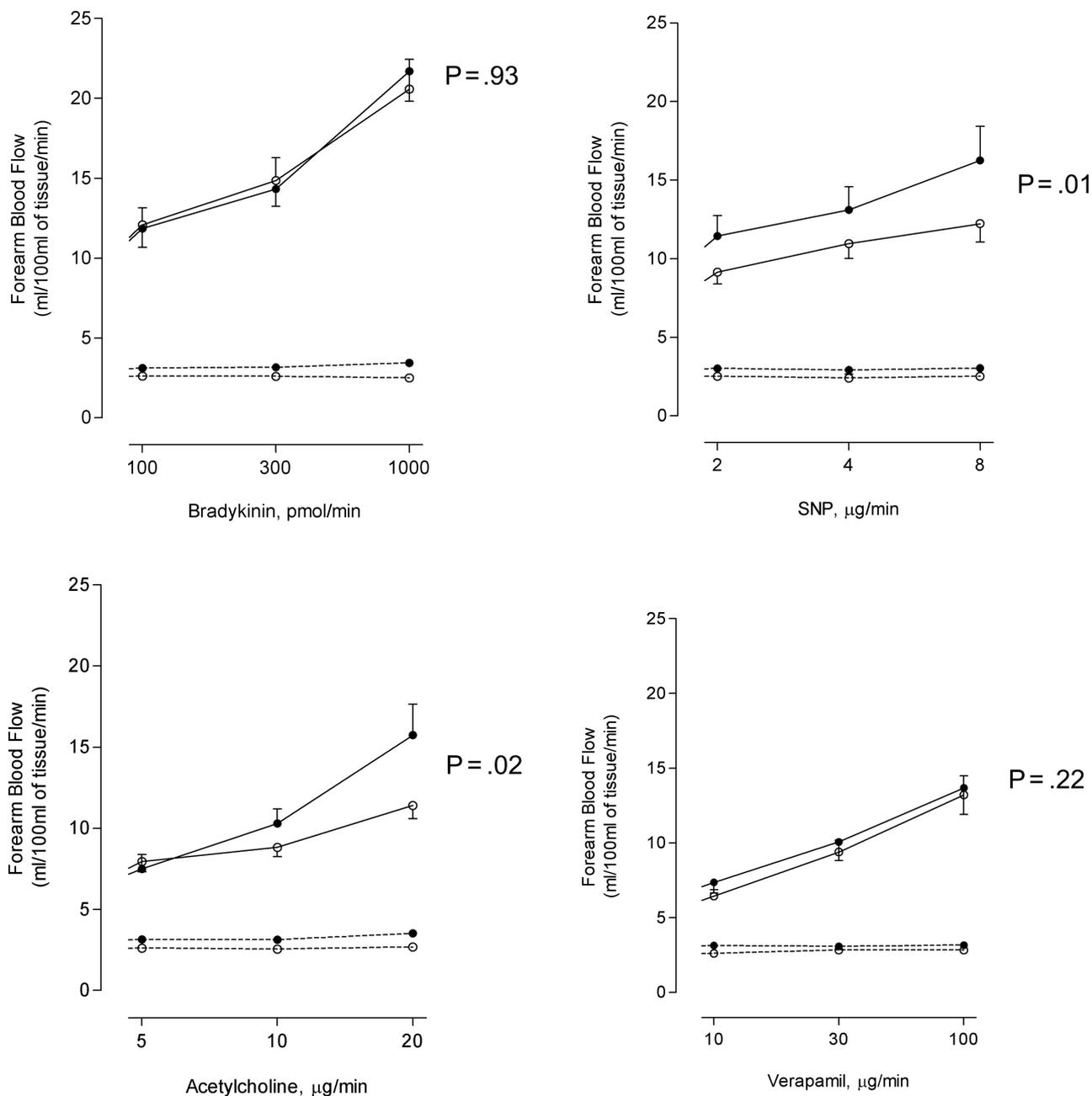
It remains possible that ozone may indirectly alter vascular function through the generation of secondary oxidized lipoproteins in the lung that are able to enter the systemic circulation (Mudway and Kelly, 2000). We assessed vascular function in the forearm using a sensitive and repeatable measure of vasomotor and fibrinolytic function. In contrast to previous studies evaluating the effects of dilute diesel exhaust on



**FIG. 1.** FBF in subjects 2–4 h after ozone (closed circles) and filtered air (open circles) exposure during unilateral intrabrachial infusion of bradykinin, acetylcholine, SNP, and verapamil. Infused arm (solid line); noninfused arm (dotted line).

vascular function (Mills *et al.*, 2005, 2007; Törnqvist *et al.*, 2007), we found no impairment in vasodilatation to either endothelial-dependent or -independent vasodilators following exposure to ozone in two separate studies. Nor did ozone affect other important aspects of vascular function with the stimulated release of t-PA antigen from the vascular endothelium unchanged following exposure. Although it is possible that we have missed an important effect of ozone beyond 8 h (and we acknowledge the findings of Devlin *et al.*, 2012 who demonstrated small late increases in plasma IL-1 and CRP concentrations 24 h after exposure to ozone), this seems unlikely given the rapid onset of most oxidation processes. If

anything, there was an apparent increase in vasodilatation in response to acetylcholine and sodium nitroprusside following ozone, perhaps suggesting enhanced nitric oxide bioavailability in the vasculature. Experimental studies have suggested that ip oxygen/ozone can upregulate endothelial nitric oxide synthase, reduce nitrotyrosine concentrations, and prevent ischaemic injury in an ischaemia-reperfusion model (Di Filippo *et al.*, 2008, 2010), adding plausibility to our observation. Previous studies have demonstrated that exposure to ozone and concentrated ambient particles together caused arterial vasoconstriction and increased blood pressure (Brook *et al.*, 2002; Urch *et al.*, 2005); however, subsequent studies



**FIG. 2.** FBF in subjects 6–8 h after ozone (closed circles) and filtered air (open circles) exposure during unilateral intrabrachial infusion of bradykinin, acetylcholine, SNP, and verapamil. Infused arm (solid line); noninfused arm (dotted line).

have established that these effects are mediated by exposure to ambient particles with no effects on arterial tone observed following exposure to ozone in isolation (Brook *et al.*, 2009).

Even though epidemiological studies have suggested an association between exposure to ozone and cardiovascular morbidity and mortality (Bell *et al.*, 2004, 2007; Ito *et al.*, 2005; Katsouyanni *et al.*, 1993; Levy *et al.*, 2005), this relationship is complex and difficult to disentangle from the effects of other major air pollutants (Bhaskaran *et al.*, 2009). Indeed, ozone has a complex relationship with PM air pollution, and in some previous analyses,

the effect of ozone on cardiovascular mortality is no longer apparent following adjustment for  $PM_{2.5}$  concentration (Smith *et al.*, 2009). There may also be a seasonal bias in some observational studies as ozone and PM are positively correlated in the warm summer months but have a negative association in the cold winter months (Langrish *et al.*, 2010). Furthermore, ozone can react with other pollutants, and therefore, increased levels may reflect changes in the concentrations of other pollutants.

There are some important limitations to this study. Our study population included only healthy men, and an effect of ozone on

**TABLE 3**  
**Plasma t-PA Antigen Concentrations Following Ozone and Filtered Air Exposures**

6–8 h	Air				Ozone				<i>p</i> Value
	0	100	300	1000	0	100	300	1000	
Bradykinin, pmol/min									
t-PA antigen, ng/ml									
Infused arm	4.5±0.3	6.3±0.6	8.7±0.8	13.7±1.3	5.0±1.6	6.0±1.8	8.1±0.9	11.7±1.3	.16
Noninfused arm	5.0±0.4	4.5±0.4	4.9±0.4	6.1±0.4	5.2±0.6	4.8±0.4	4.8±0.4	6.1±0.5	.96
Net t-PA release, ng/100 ml of tissue/min	-1.4±0.5	12.1±2.9	34.7±5.1	104.1±14.4	-0.2±1.1	8.2±3.0	31.1±5.0	79.6±17.4	.31

*Note.* Values are reported as mean ± SEM; two-way ANOVA with repeat measures.

**TABLE 4**  
**HRV at Baseline, 2h, and 6h Following Exposure to Ozone or Filtered Air**

	Air			Ozone			Two-way ANOVA
	Baseline	2 h	6 h	Baseline	2 h	6 h	<i>p</i> Value
Heart rate, bpm	69±2	65±2	65±2	72±2	70±3	66±2	.04
SDNN, ms	91±8	79±5	67±4	90±9	76±6	65±5	.60
RMSSD, ms	54±5	58±5	48±4	52±6	49±5	48±5	.20
PNN50, %	27±4	33±4	26±4	22±4	28±5	27±4	.18
Triangular Ix	19±1	17±1	14±1	17±1	17±1	15±1	.36
HF <sub>n</sub> , ms	22±2	29±3	28±3	13±2	24±3	31±3	.07
LF <sub>n</sub> , ms	76±3	68±3	70±3	86±2	72±3	66±3	.10
HF/LF ratio	0.3±0.0	0.5±0.1	0.4±0.1	0.2±0.0	0.4±0.0	0.5±0.1	.86

*Note.* All variables are presented as mean ± SEM; two-way ANOVA filtered air versus ozone for effect of exposure; SDNN, standard deviation of successive RR intervals; RMSSD, root mean square difference of successive RR intervals; HF<sub>n</sub>, high-frequency power component of HRV expressed in normalized units; LF<sub>n</sub>, low-frequency power component of HRV expressed in normalized units; HF/LF, ratio of high frequency to low frequency components of HRV; PNN50, percentage of successive RR intervals that differ by > 50 ms.

vascular function in susceptible populations with cardiovascular disease cannot be excluded. Furthermore, we cannot exclude an effect of chronic exposure to high levels of ozone. We have investigated the effects of ozone exposure in isolation and cannot exclude the possibility that ozone may have important adverse health effects by interacting with and potentially enhancing the toxicity of other ambient air pollutants (Bosson *et al.*, 2008). Further research to evaluate the effect of ozone and diesel exhaust particles on the cardiovascular system is warranted.

### CONCLUSIONS

This study indicates that short-term exposure to ozone does not cause acute impairment in vascular function or alter the autonomic regulation of the heart in healthy men. Our findings do not support the view that ozone in isolation is an important determinant of cardiovascular health. Although we cannot exclude an effect of chronic exposure or an interaction between ozone and other air pollutants, we suggest that alternative pollutants in the ambient mixture may be responsible for the adverse cardiovascular health effects attributed to ozone in some epidemiologic studies.

### SUPPLEMENTARY DATA

Supplementary data are available online at <http://toxsci.oxfordjournals.org/>.

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## REFERENCES

- Behndig, A. F., Blomberg, A., Helleday, R., Duggan, S. T., Kelly, F. J., and Mudway, I. S. (2009). Antioxidant responses to acute ozone challenge in the healthy human airway. *Inhal. Toxicol.* **21**, 933–942.
- Bell, M. L., Kim, J. Y., and Dominici, F. (2007). Potential confounding of particulate matter on the short-term association between ozone and mortality in multisite time-series studies. *Environ. Health Perspect.* **115**, 1591–1595.
- Bell, M. L., McDermott, A., Zeger, S. L., Samet, J. M., and Dominici, F. (2004). Ozone and short-term mortality in 95 US urban communities, 1987–2000. *JAMA* **292**, 2372–2378.
- Bhaskaran, K., Hajat, S., Haines, A., Herrett, E., Wilkinson, P., and Smeeth, L. (2009). Effects of air pollution on the incidence of myocardial infarction. *Heart* **95**, 1746–1759.
- Blomberg, A., Mudway, I. S., Nordenhäll, C., Hedenström, H., Kelly, F. J., Frew, A. J., Holgate, S. T., and Sandström, T. (1999). Ozone-induced lung function decrements do not correlate with early airway inflammatory or antioxidant responses. *Eur. Respir. J.* **13**, 1418–1428.
- Bosson, J., Barath, S., Pourazar, J., Behndig, A. F., Sandström, T., Blomberg, A., and Adelroth, E. (2008). Diesel exhaust exposure enhances the ozone-induced airway inflammation in healthy humans. *Eur. Respir. J.* **31**, 1234–1240.
- Brook, R. D., Brook, J. R., Urch, B., Vincent, R., Rajagopalan, S., and Silverman, F. (2002). Inhalation of fine particulate air pollution and ozone causes acute arterial vasoconstriction in healthy adults. *Circulation* **105**, 1534–1536.
- Brook, R. D., Rajagopalan, S., Pope, C. A., 3rd, Brook, J. R., Bhatnagar, A., Diez-Roux, A. V., Holguin, F., Hong, Y., Luepker, R. V., Mittleman, M. A., et al.; American Heart Association Council on Epidemiology and Prevention, Council on the Kidney in Cardiovascular Disease, and Council on Nutrition, Physical Activity and Metabolism. (2010). Particulate matter air pollution and cardiovascular disease: An update to the scientific statement from the American Heart Association. *Circulation* **121**, 2331–2378.
- Brook, R. D., Urch, B., Dvonch, J. T., Bard, R. L., Speck, M., Keeler, G., Morishita, M., Marsik, F. J., Kamal, A. S., Kaciroti, N., et al. (2009). Insights into the mechanisms and mediators of the effects of air pollution exposure on blood pressure and vascular function in healthy humans. *Hypertension* **54**, 659–667.
- Brunekreef, B., and Holgate, S. T. (2002). Air pollution and health. *Lancet* **360**, 1233–1242.
- Chuang, K. J., Chan, C. C., Su, T. C., Lee, C. T., and Tang, C. S. (2007). The effect of urban air pollution on inflammation, oxidative stress, coagulation, and autonomic dysfunction in young adults. *Am. J. Respir. Crit. Care Med.* **176**, 370–376.
- Cosselman, K. E., Krishnan, R. M., Oron, A. P., Jansen, K., Peretz, A., Sullivan, J. H., Larson, T. V., and Kaufman, J. D. (2012). Blood pressure response to controlled diesel exhaust exposure in human subjects. *Hypertension* **59**, 943–948.
- de Almeida, S. P., Casimiro, E., and Calheiros, J. (2011). Short-term association between exposure to ozone and mortality in Oporto, Portugal. *Environ. Res.* **111**, 406–410.
- Devlin, R. B., Duncan, K. E., Jardim, M., Schmitt, M. T., Rappold, A. G., and Diaz-Sanchez, D. (2012). Controlled exposure of healthy young volunteers to ozone causes cardiovascular effects. *Circulation* **126**, 104–111.
- Di Filippo, C., Luongo, M., Marfella, R., Ferraraccio, F., Lettieri, B., Capuano, A., Rossi, F., and D'Amico, M. (2010). Oxygen/ozone protects the heart from acute myocardial infarction through local increase of eNOS activity and endothelial progenitor cells recruitment. *Naunyn Schmiedebergs Arch. Pharmacol.* **382**, 287–291.
- Di Filippo, C., Marfella, R., Capodanno, P., Ferraraccio, F., Coppola, L., Luongo, M., Mascolo, L., Luongo, C., Capuano, A., Rossi, F., et al. (2008). Acute oxygen-ozone administration to rats protects the heart from ischemia reperfusion infarct. *Inflamm. Res.* **57**, 445–449.
- Ensor, K. B., Raun, L. H., and Persse, D. (2013). A case-crossover analysis of out-of-hospital cardiac arrest and air pollution. *Circulation* **127**, 1192–1199.
- Frampton, M. W., Pryor, W. A., Cueto, R., Cox, C., Morrow, P. E., and Utell, M. J. (1999). Ozone exposure increases aldehydes in epithelial lining fluid in human lung. *Am. J. Respir. Crit. Care Med.* **159**(4 Pt 1), 1134–1137.
- Gryparis, A., Forsberg, B., Katsouyanni, K., Analitis, A., Touloumi, G., Schwartz, J., Samoli, E., Medina, S., Anderson, H. R., Niciu, E. M., et al. (2004). Acute effects of ozone on mortality from the “air pollution and health: A European approach” project. *Am. J. Respir. Crit. Care Med.* **170**, 1080–1087.
- Halonen, J. I., Lanki, T., Tiittanen, P., Niemi, J. V., Loh, M., and Pekkanen, J. (2010). Ozone and cause-specific cardiorespiratory morbidity and mortality. *J. Epidemiol. Community Health* **64**, 814–820.
- Hoffmann, B., Luttmann-Gibson, H., Cohen, A., Zanobetti, A., de Souza, C., Foley, C., Suh, H. H., Coull, B. A., Schwartz, J., Mittleman, M., et al. (2012). Opposing effects of particle pollution, ozone, and ambient temperature on arterial blood pressure. *Environ. Health Perspect.* **120**, 241–246.
- Ito, K., De Leon, S. F., and Lippmann, M. (2005). Associations between ozone and daily mortality: Analysis and meta-analysis. *Epidemiology* **16**, 446–457.
- Jerrett, M., Burnett, R. T., Pope, C. A., 3rd, Ito, K., Thurston, G., Krewski, D., Shi, Y., Calle, E., and Thun, M. (2009). Long-term ozone exposure and mortality. *N. Engl. J. Med.* **360**, 1085–1095.
- Katsouyanni, K., Pantazopoulou, A., Touloumi, G., Tselepidaki, I., Moustris, K., Asimakopoulos, D., Pouloupoulou, G., and Trichopoulos, D. (1993). Evidence for interaction between air pollution and high temperature in the causation of excess mortality. *Arch. Environ. Health* **48**, 235–242.
- Lai, C. J., and Kou, Y. R. (1998). Stimulation of vagal pulmonary C fibers by inhaled wood smoke in rats. *J. Appl. Physiol.* **84**, 30–36.
- Langrish, J. P., Mills, N. L., Donaldson, K., and Newby, D. E. (2010). Response to Peter Joseph. *Heart* **96**, 472–473.
- Lee, L. Y., and Pisarri, T. E. (2001). Afferent properties and reflex functions of bronchopulmonary C-fibers. *Respir. Physiol.* **125**, 47–65.
- Levy, J. I., Chemerynski, S. M., and Sarnat, J. A. (2005). Ozone exposure and mortality: An empiric bayes metaregression analysis. *Epidemiology* **16**, 458–468.
- Lucking, A. J., Lundbäck, M., Barath, S. L., Mills, N. L., Sidhu, M. K., Langrish, J. P., Boon, N. A., Pourazar, J., Badimon, J. J., Gerlofs-Nijland, M. E., et al. (2011). Particle traps prevent adverse vascular and prothrombotic effects of diesel engine exhaust inhalation in men. *Circulation* **123**, 1721–1728.
- Lucking, A. J., Lundback, M., Mills, N. L., Faratian, D., Barath, S. L., Pourazar, J., Cassee, F. R., Donaldson, K., Boon, N. A., Badimon, J. J., et al. (2008). Diesel exhaust inhalation increases thrombus formation in man. *Eur. Heart J.* **29**, 3043–3051.
- Miller, K. A., Siscovick, D. S., Sheppard, L., Shepherd, K., Sullivan, J. H., Anderson, G. L., and Kaufman, J. D. (2007). Long-term exposure to air pollution and incidence of cardiovascular events in women. *N. Engl. J. Med.* **356**, 447–458.
- Mills, N. L., Miller, M. R., Lucking, A. J., Beveridge, J., Flint, L., Boere, A. J., Fokkens, P. H., Boon, N. A., Sandstrom, T., Blomberg, A., et al. (2011). Combustion-derived nanoparticulate induces the adverse vascular effects of diesel exhaust inhalation. *Eur. Heart J.* **32**, 2660–2671.
- Mills, N. L., Törnqvist, H., Gonzalez, M. C., Vink, E., Robinson, S. D., Söderberg, S., Boon, N. A., Donaldson, K., Sandström, T., Blomberg, A., et al. (2007). Ischemic and thrombotic effects of dilute diesel-exhaust inhalation in men with coronary heart disease. *N. Engl. J. Med.* **357**, 1075–1082.
- Mills, N. L., Törnqvist, H., Robinson, S. D., Gonzalez, M., Darnley, K., MacNee, W., Boon, N. A., Donaldson, K., Blomberg, A., Sandstrom, T., et al. (2005). Diesel exhaust inhalation causes vascular dysfunction and impaired endogenous fibrinolysis. *Circulation* **112**, 3930–3936.

- Mudway, I. S., and Kelly, F. J. (2000). Ozone and the lung: A sensitive issue. *Mol. Aspects Med.* **21**, 1–48.
- Peters, A., von Klot, S., Heier, M., Trentinaglia, I., Hörmann, A., Wichmann, H. E., and Löwel, H.; Cooperative Health Research in the Region of Augsburg Study Group. (2004). Exposure to traffic and the onset of myocardial infarction. *N. Engl. J. Med.* **351**, 1721–1730.
- Ren, C., Williams, G. M., Morawska, L., Mengersen, K., and Tong, S. (2008). Ozone modifies associations between temperature and cardiovascular mortality: Analysis of the NMMAPS data. *Occup. Environ. Med.* **65**, 255–260.
- Ruidavets, J. B., Cournot, M., Cassadou, S., Giroux, M., Meybeck, M., and Ferrières, J. (2005). Ozone air pollution is associated with acute myocardial infarction. *Circulation* **111**, 563–569.
- Smith, K. R., Jerrett, M., Anderson, H. R., Burnett, R. T., Stone, V., Derwent, R., Atkinson, R. W., Cohen, A., Shonkoff, S. B., Krewski, D., *et al.* (2009). Public health benefits of strategies to reduce greenhouse-gas emissions: Health implications of short-lived greenhouse pollutants. *Lancet* **374**, 2091–2103.
- Stafoggia, M., Forastiere, F., Faustini, A., Biggeri, A., Bisanti, L., Cadum, E., Cernigliaro, A., Mallone, S., Pandolfi, P., Serinelli, M., *et al.*; EpiAir Group. (2010). Susceptibility factors to ozone-related mortality: A population-based case-crossover analysis. *Am. J. Respir. Crit. Care Med.* **182**, 376–384.
- Stenfors, N., Pourazar, J., Blomberg, A., Krishna, M. T., Mudway, I., Helleday, R., Kelly, F. J., Frew, A. J., and Sandström, T. (2002). Effect of ozone on bronchial mucosal inflammation in asthmatic and healthy subjects. *Respir. Med.* **96**, 352–358.
- Taylor-Clark, T. E., and Udem, B. J. (2010). Ozone activates airway nerves via the selective stimulation of TRPA1 ion channels. *J. Physiol. (Lond.)* **588**(Pt 3), 423–433.
- Törnqvist, H., Mills, N. L., Gonzalez, M., Miller, M. R., Robinson, S. D., Megson, I. L., Macnee, W., Donaldson, K., Söderberg, S., Newby, D. E., *et al.* (2007). Persistent endothelial dysfunction in humans after diesel exhaust inhalation. *Am. J. Respir. Crit. Care Med.* **176**, 395–400.
- Tsuji, H., Larson, M. G., Venditti, F. J., Jr, Manders, E. S., Evans, J. C., Feldman, C. L., and Levy, D. (1996). Impact of reduced heart rate variability on risk for cardiac events. The Framingham Heart Study. *Circulation* **94**, 2850–2855.
- Urch, B., Silverman, F., Corey, P., Brook, J. R., Lukic, K. Z., Rajagopalan, S., and Brook, R. D. (2005). Acute blood pressure responses in healthy adults during controlled air pollution exposures. *Environ. Health Perspect.* **113**, 1052–1055.