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# **Brachial Artery Responses to Ambient Pollution, Temperature, and Humidity in People with Type 2 Diabetes: A Repeated-Measures Study**

Antonella Zanobetti,<sup>1</sup> Heike Luttmann-Gibson,<sup>1</sup> Edward S. Horton,<sup>2</sup> Allison Cohen,<sup>2</sup> Brent A. Coull,<sup>3</sup> Barbara Hoffmann,<sup>4</sup> Joel D. Schwartz,<sup>1</sup> Murray A. Mittleman,<sup>5</sup> Yongsheng Li,<sup>2</sup> Peter H. Stone,<sup>7</sup> Celine de Souza,<sup>1</sup> Brooke Lamparello,<sup>2</sup> Petros Koutrakis,<sup>1</sup> and Diane R. Gold<sup>1,6</sup>

<sup>1</sup>Harvard School of Public Health, Department of Environmental Health, Boston, Massachusetts, USA; <sup>2</sup>Joslin Diabetes Center, Boston, Massachusetts, USA; <sup>3</sup>Harvard School of Public Health, Department of Biostatistics, Boston, Massachusetts, USA; <sup>4</sup>IUF Leibniz Research Institute for Environmental Medicine and Medical School, University of Düsseldorf, Düsseldorf, Germany; <sup>5</sup>Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts, USA; <sup>6</sup>Channing Laboratory, Brigham and Women's Hospital, Department of Medicine, Harvard Medical School, Boston, Massachusetts, USA; <sup>7</sup>Brigham and Women's Hospital, Department of Medicine, Harvard Medical School, Boston, Massachusetts, USA

**Address correspondence to** Antonella Zanobetti, Department of Environmental Health, Exposure Epidemiology and Risk Program, Harvard School of Public Health, 401 Park Drive, Landmark Center, Suite 415, P.O. Box 15698, Boston, Massachusetts 02215 USA. Telephone: 617 384-8751. Fax: 617 384-8745. E-mail: [azanobet@hsph.harvard.edu](mailto:azanobet@hsph.harvard.edu)

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## Abstract

**Objectives:** Extreme weather and air pollution are associated with increased cardiovascular risk in people with diabetes. In a population with diabetes we conducted a novel assessment of vascular brachial artery responses both to ambient pollution and to weather [temperature and water vapor pressure, a measure of humidity].

**Methods:** Sixty-four 49- to 85-year old Boston residents with type 2 diabetes completed up to 5 study visits (279 repeated measures). Brachial artery diameter (BAD) was measured by ultrasound before and after brachial artery occlusion (i.e., flow-mediated dilation, FMD), and before and after nitroglycerin-mediated dilation (NMD). Ambient fine particulate mass ( $PM_{2.5}$ ), black carbon (BC), particle number (PN), sulfate ( $SO_4$ ), elemental carbon (EC) and organic carbon (OC) were measured at our monitoring site; ambient  $O_3$ , CO,  $NO_2$  data were obtained from state monitors. Particle exposure in the home and during each trip to the clinic (home/trip exposure) was measured continuously and as a 5-day integrated sample. We used linear models with fixed effects for subjects, and adjusting for date, season, temperature, and water vapor pressure on the day of each visit, to estimate associations between our outcomes and interquartile range (IQR) increases in exposure.

**Results:** Baseline BAD was negatively associated with particle pollution, including home/trip integrated BC (-0.02 mm; 95% CI: -0.04, -0.003, for a  $0.28 \mu g/m^3$  increase in BC), OC (-0.08 mm; 95% CI: -0.14, -0.03, for a  $1.61 \mu g/m^3$  increase) and  $PM_{2.5}$ ; and 5-day average ambient  $PM_{2.5}$  and BC. BAD was positively associated with ambient temperature and water vapor pressure. However, exposures were not consistently associated with FMD or NMD.

**Conclusion:** Brachial artery diameter, a predictor of cardiovascular risk, decreased in association with particle pollution and increased in association with ambient temperature in our study population of adults with type 2 diabetes.

## Introduction

Previous studies have reported that associations of cardiovascular mortality with particle pollution (Zanobetti and Schwartz 2001; Zanobetti et al. 2009) and extreme ambient temperatures during heat waves (Medina-Ramon et al. 2006; Schifano et al. 2009; Schwartz 2005) are stronger in people with diabetes than in people without diabetes. It has been hypothesized that people with diabetes are at greater risk for acute environmental perturbations of vascular (including coronary artery) function because of chronic endothelial dysfunction, autonomic dysregulation, atherosclerosis, or dysregulation of fluid balance and the renin-angiotensin system (Pop-Busui 2010; Ribeiro-Oliveira Jr. et al. 2008). Common medications like angiotensin-converting enzyme inhibitors may further block appropriate compensatory autoregulatory responses (Bell 2009) to hypertensive or to orthostatic hypotensive changes in blood pressure and vascular diameter.

Baseline brachial artery diameter (BAD), and flow-mediated dilation (FMD) and nitroglycerin-mediated dilation (NMD) have been used as intermediate outcomes in studies of cardiovascular responses to pollution in controlled human exposure (Brook et al. 2002) and observational research (Moens et al. 2005). As predictors of cardiac risk and coronary artery function, BAD (Montalcini et al. 2012) and FMD (Caballero et al. 1999; Gold 2008; Liu et al. 2007; Sullivan et al. 2003) have clinical as well as physiologic relevance. FMD, a measure of the difference in BAD after brachial artery occlusion relative to baseline BAD (before occlusion), reflects the combined effects of endothelial-dependent processes that influence the production and quenching of vasodilatory nitrogen oxide (NO) and endothelial-independent processes that influence vascular smooth muscle responsiveness to NO (Ghio et al. 2000; Salvi et al. 1999;

Sorensen et al. 2003). Nitroglycerin-mediated dilation (NMD), a measure of the change in BAD before and after administration of nitroglycerin (an exogenous source of NO), reflects autonomic vascular smooth muscle responsiveness occurring independently of endothelial NO production. A decrease in FMD but not NMD suggests an effect on endothelial function specifically, whereas a decrease in both outcomes suggests that part or all of the change is due to endothelial-independent effects. People with diabetes have markedly reduced but measurable FMD (Caballero et al. 1999). In a previous cross-sectional study of Boston residents, we found that short-term increases in traffic and non-traffic pollution were associated with reduced FMD and NMD in participants with type 2 diabetes (O'Neill et al. 2005), suggesting both non-endothelial and endothelial-mediated mechanisms for pollution-related vascular dysfunction.

High humidity has been associated with hyperpyrexia, decline in physical strength and fatigue; reduction in alertness and mental capacity. High humidity often occurs when temperature is high, and the effects of the two exposures can be difficult to disentangle (Sharma et al. 1983). In Germany, Schneider and co-authors (Schneider et al. 2008b) analyzed the influence of weather parameters [including water vapor pressure (WVP)] on blood pressure, arrhythmia, and ischemia in cardiovascular patients. They noted that the associations they detected between weather parameters and ST-segment depression could be relevant to clinical cardiovascular outcomes.

In a prospective, repeated measures study, we investigated associations of BAD, FMD, and NMD with pollutant exposures, ambient temperature, and humidity in 64 adults with type 2 diabetes.

## **Method**

### **Study population and protocol**

The study population consisted of Boston residents with type 2 diabetes mellitus, who lived within 25 km of a central air monitoring site located near downtown Boston. Subjects were recruited between 2006 and 2009. If they met initial screening inclusion and exclusion criteria, they were invited to come for a baseline visit that included an interview on socio-demographic characteristics, health status, medical history, medication and lifestyle; blood and urinary analysis; and a clinical examination. This visit was used both as an opportunity for further detailed screening and also to collect baseline data on participants who after the visit were found to be eligible for the follow-up study. Exclusion criteria focused on factors that can introduce particle exposure errors (exposure to second-hand tobacco smoke at home; living beyond 25 km of the central monitoring station); on medications with acute vascular effects; on conditions with electrophysiological or vascular effects (current atrial fibrillation/flutter; history of clinically significant ventricular arrhythmias, pacemaker or implanted defibrillator; acute myocardial infarction or stent placement within the last 6 months); on clinical/biomarker parameters requiring immediate attention (e.g., uncontrolled hypertension [ $> 180$  systolic;  $> 105$  diastolic]); on markers of poor diabetes/lipid control or advanced diabetic nephropathy (serum cholesterol  $> 350$  mg/dl, serum triglycerides  $> 600$  mg/dl, hemoglobin A1c (HbA1c)  $> 10.5\%$ ; fasting blood glucose greater than 270 mg/dl; urine albumin/creatinine ratio  $> 300$   $\mu$ g/dl), and on diagnoses of other advanced diseases (solid organ transplant, active autoimmune disease, dementia, type 1 diabetes, renal failure, seizure disorder or stroke).

After entry into the follow-up study, eligible participants completed up to 5 follow-up clinical examinations scheduled two weeks apart on the same weekday in the morning. We ascertained medication use was by patient self-report at baseline and at each subsequent visit. The study protocol was approved by the institutional review boards of the Brigham & Women's Hospital, the Joslin Diabetes Clinic, and the Harvard School of Public Health. All participants provided written informed consent.

At each visit BAD, FMD, and NMD were measured by ultrasound according to standardized protocols. Participants were placed in the supine position with the right arm abducted by approximately 60 degrees and comfortably placed on a support. For the assessment of endothelial-dependent vasodilatation (FMD), a pneumatic tourniquet was placed on the right forearm 2 cm below the antecubital fossa. After a 15-minute rest period, high resolution brachial artery ultrasound was performed to measure BAD with a 10 MHz linear-array transducer and a Terason 2000 ultrasound with ECG-gated image acquisition at end diastole. After taking baseline images, the arm was immobilized and the transducer was held in a fixed position throughout the assessment. Reactive hyperemia was produced by inflating the tourniquet to approximately 50 mmHg above the individual's systolic blood pressure for 5 min and then quickly deflating it (Celermajer et al. 1992). Ultrasound picture acquisition was repeated 60 sec after sudden deflation for 10 sec.

After another 15-min rest period, endothelium-independent vasodilatation (NMD) was assessed by measuring BAD before and 3 minutes after the sublingual administration of 0.4 mg nitroglycerin. Blood pressure and heart rate were monitored in the left arm before and after the brachial artery measurements and prior to and after nitroglycerin administration. NMD was not

performed if systolic blood pressure was below 90 mm Hg. All acquired ultrasound images were analyzed centrally according to a predefined protocol using Brachial Analyzer (Medical Imaging Applications LLC, Vascular Research Tools 5, Version 5.6.12). FMD and NMD are expressed as % change in brachial artery diameter [i.e., BAD after the intervention (occlusion or nitroglycerin) minus the BAD before the intervention, divided by the BAD before the intervention].

### **Environmental data**

Ambient concentrations of fine particle mass (with aerodynamic diameter less than 2.5  $\mu\text{m}$ ,  $\text{PM}_{2.5}$ ), black carbon (BC), sulfate ( $\text{SO}_4^{2-}$ ), organic carbon (OC), elemental carbon (EC), and particle number concentration (PN) were measured hourly at a central monitoring site (Harvard Supersite) in Boston, MA. Hourly ambient concentrations of ozone ( $\text{O}_3$ ), carbon monoxide (CO) and nitrogen dioxide ( $\text{NO}_2$ ) were estimated by averaging data from the Massachusetts Department of Environmental Protection's Greater Boston monitoring sites. Missing hourly data for  $\text{PM}_{2.5}$  and BC were imputed using regression modeling, including a long-term time trend, day of week, hour of day, temperature, relative humidity, barometric pressure, and nitrogen dioxide as predictors. EC, OC and  $\text{SO}_4^{2-}$  were not available in 2006 and part of 2010; this resulted in approximately 20% missing values. All other exposure variables (both weather parameters and pollution) only had few missing values (< 1%) that were assumed to be missing at random.

Organic carbon can be both primary and secondary. Using published methods (Lim and Turpin 2002), we estimated primary OC (emitted by cars) by multiplying EC by 1.8, and estimated secondary OC (due to oxidation of traffic as well as biogenic emissions) as OC minus primary OC.

Pollutant exposures were averaged over 24-hour intervals (9am-9am, to correspond to the time of arrival at the study clinic for each visit). In addition we derived average exposures over the previous 1 to 6 days when at least 75% of daily data were available.

We also estimated individual PM<sub>2.5</sub> and BC exposures. Five days prior to each clinic visit fine particle samplers were placed in the study participant's home. On the day of the visit the participant brought the pollution samplers (still in operation) to the clinic. A custom-made Harvard sampling system was used to collect fine particles (PM<sub>2.5</sub>) on Teflon filters to determine PM<sub>2.5</sub> and BC mass concentration (by gravimetry and reflectance, respectively) as a measure of 5-day home/trip integrated exposures. The sampling system also included a SidePak (TSI, Inc.) that provided continuous measurements of PM<sub>2.5</sub> particle mass concentration which were calibrated using the integrated PM<sub>2.5</sub> filter to derive hourly averages, 24-hour averages, and moving averages of up to 5 days when at least 75% of daily (or hourly) data were available.

Weather parameters, including hourly temperature, barometric pressure, and dew point temperature, were obtained from the National Weather Service Station at Logan Airport, located approximately 12 km from the examination site. WVP (actual water vapor pressure), a measure of humidity defined as the amount of water vapor in a volume of air, increases as the amount of water vapor increases. WVP (hPa) was computed as  $WVP = 6.1078 * 10^{(7.5 * \text{dewpoint}) / (237.7 + \text{dewpoint})}$  (Barenbrug 1974; Buck 1981; Eaton and Kells 2009). Temperature and WVP were also evaluated as 24-hour averages (9am-9am) and as cumulative averages over the previous 1 to 5 days.

## **Statistical analysis**

We estimated the associations of air pollution and weather with the individual BAD measurements performed during each visit as separate outcomes: BAD before occlusion, BAD after occlusion, BAD before sublingual nitroglycerin administration, and BAD after sublingual nitroglycerin administration. In addition we estimated associations with FMD (i.e., the percent change in BAD in response to occlusion) and NMD (i.e., the percent change in BAD in response to nitroglycerin administration).

We fit linear models for each outcome that included fixed effects for subject and linear terms for the date of visit, season, and the average temperature on the day of each visit. The indicator variables for each subject control for correlated measurements within subjects, and for confounding by time invariant characteristics such as gender and ethnicity. Baseline and end of study glucose control (HbA1c) was measured using standard methods. When included linearly in our model was not a significant predictor of our outcomes, did not improve our model fit and therefore was not included in the final models. Model residuals were normally distributed (data not shown).

Both fixed and random effects approaches to estimation are based on the same model, so the implied correlation structure among repeated measurements is similar in the two models. Hence a fixed intercept for each subject fully accounts for the within-correlation of the repeated data for each participant. In mixed effects models, the estimated exposure effect is a blend of cross-sectional (across subject) and longitudinal (within subject) comparisons. Because of the cross-sectional component, the estimates can be subject to confounding by characteristics specific to that subject (SES, etc). In contrast, the fixed effect estimates are based purely on longitudinal

comparisons, which make them not subject to between-subject confounding. This is the primary reason we used fixed effects in this application (Fitzmaurice et al. 2004). As we did not want our reported results to be highly dependent on the chosen model, in sensitivity analyses we fit the analogous mixed effects models.

Effect estimates were scaled by the interquartile pollutant range (IQR). Estimates for pollution associations with BAD (before and after occlusion, and before and after sublingual nitroglycerin administration) are presented as change in brachial artery diameter in mm per IQR increase in pollution. Estimates for pollution associations with FMD and NMD are presented as change FMD or NMD in percent per IQR increase in pollution levels.

We focused on associations with exposures during the one-to-6 days prior to coming to the clinic because of evidence from prior studies (Hoffmann et al. 2012; O'Neill et al. 2005) and biologic priors---vascular autonomic responses to pollutants could be relatively immediate (24-hour) whereas inflammatory vascular responses would be cumulative over a week. With temperature, our previous analytic experience also suggested more immediate responses related to temperature in the 24 hours prior to the measurement, along with approximately 1-week cumulative responses that could relate effects of persistent episodes of high or low temperature (Hoffmann et al. 2012). We estimated associations of our outcomes with cumulative exposures up to 14 days, to assess how the associations varied with longer moving averages, and because participants completed clinic visits every 2 weeks. When interpreting our findings we focused on the consistency of associations among correlated pollutants (e.g., different markers of traffic-related pollution). We estimated the effects of temperature, barometric pressure, and WVP with and without adjustment for particle exposures (5-day average PM<sub>2.5</sub> and BC), using the same

model described above. We first tested the relationship between the meteorological variables and our outcomes with a penalized spline. Because the generalized cross validation (GCV) method estimated one degree of freedom for the penalized spline of the weather parameters and these showed a linear pattern, we then included the meteorological variables linearly in the models.

Results for weather parameters are presented as the change in BAD (in mm), FMD and NMD (in %) associated with an IQR increase in each parameter. To estimate short-term and cumulative effects of weather, we modeled individual daily averages and cumulative averages from the day of the visit up to and including 5 days before the visit.

We examined effect modification by angiotensin-converting enzyme inhibitors (ACE) and beta-blockers by including an interaction term... between the 5-day average of each exposure and the medication variables. Medication use was ascertained by patient self-report at baseline and at each subsequent visit. We also examined effect modification by patient characteristics such as BMI in 4 categories: underweight ( $BMI < 18.5$ ); normal ( $18.5 \leq BMI < 25$ ); overweight ( $25 \leq BMI < 30$ ); obese ( $BMI \geq 30$ ), sex and continuous hemoglobin A1c. Finally we examined effect modification of associations with temperature by season defined as winter (December-February), spring (March-May), summer (June-August), and autumn (September-November) by including an interaction term between temperature and season.

In two-pollutant models we included two pollutants at a time together in each model.

All analyses were performed using R 2.14.1 (The Comprehensive R Archive Network: <http://cran.r-project.org/>).

## Results

During the study period (September 2006 to July 2010) 70 participants were enrolled in the repeated measures study. Of these, 64 had complete covariate information and at least one acceptable FMD evaluation over a total of 279 visits (Table 1). A subset of 43 subjects (159 observations) consented and had BP within a range that allowed us to administer nitroglycerin and measure NMD. The subjects had an equal distribution by gender, on average a long-standing history of diabetes (mean 10 years), and an average BMI of 31 kg/m<sup>2</sup>. The baseline BAD (i.e., the BAD measurement taken prior to brachial artery occlusion) across all participants ranged between 2.5 and 6.2 mm, with a mean of 4.1 mm (range 2.5 – 5.2 mm) in women, and 5.0 mm (range 3.6 – 6.2 mm) in men. While the BAD was comparable to what we have previously measured in healthy adults without diabetes, as expected in our diabetic cohort, the FMD and NMD were markedly reduced (Brook et al. 2009).

In Table 2 we summarize the distribution of the pollutant concentrations and of temperature values for the 24-hour and 5 day averages. For most exposures, data were complete (or nearly so) for all 279 visit days included in the present analysis. However, for OC and EC, 24-hr averages measures were only available for 231 of the 279 days, and for SO<sub>4</sub>, 24-hr averages were available for 197 days (Table 2). Table S1 in the Supplemental Material shows the correlations among pollutant and meteorological variables. PM<sub>2.5</sub>, SO<sub>4</sub>, BC, EC, and OC, were highly correlated with each other but were not highly correlated with PNC, ozone, or temperature. The home/trip integrated concentrations for BC and PM<sub>2.5</sub> were not highly correlated with their respective ambient concentrations. The average 24-hour maximum PM<sub>2.5</sub> concentration (27 µg/m<sup>3</sup>) was below the National Air Quality Standard recommended upper limit of 35 µg/m<sup>3</sup>.

## **Pollution and brachial artery diameter**

Figure 1 presents the associations of the mean 5-day average concentrations of each of the pollutants with brachial artery diameter before brachial artery occlusion, and before sublingual nitroglycerin administration. We focus on results related to the 5-day moving averages because we found the largest and most consistent associations of BAD or FMD with the 4 and 5 days moving averages of pollution. A second rationale for this choice is that the indoor/trip integrated BC and PM<sub>2.5</sub> were measured from the filters that represented an integrated particle collection over a five-day period.

Baseline BAD (before brachial artery occlusion) was negatively associated with increases in particle mass and BC and OC particle components (Figure 1). Scaling for the IQR increases in pollution levels, a 0.25  $\mu\text{g}/\text{m}^3$  increase in 5-day mean ambient BC was associated with a -0.035 mm decrease (95% CI: -0.07, 0.00) in baseline BAD. Baseline BAD also decreased in association with IQR increases in 5-day average exposures to ambient PM<sub>2.5</sub> (-0.02 mm; 95% CI: -0.05, 0.01, IQR = 3.14  $\mu\text{g}/\text{m}^3$ ), ambient OC (-0.08 mm; 95% CI: -0.14, -0.03, IQR = 1.61  $\mu\text{g}/\text{m}^3$ ), indoor/trip integrated BC (-0.02 mm; 95% CI: -0.04, -0.003, IQR = 0.28  $\mu\text{g}/\text{m}^3$ ), and indoor/trip integrated PM<sub>2.5</sub> (-0.04 mm; 95% CI: -0.06, -0.01, IQR = 5.66  $\text{mg}/\text{m}^3$ ). Associations with increases in the same exposures during the previous 24 hours, or as cumulative averages over shorter or longer time periods (up to 6 days) (Supplemental Material, Figure S1) were generally weaker than associations with 5-day average values. To assess for relatively immediate responses on pollution during the trip in to the clinic, using the home/trip continuous PM<sub>2.5</sub> exposures, we also explored associations of shorter averages of PM (30, 60, and 90 minutes

before the visit) on the day of the visit, but found no associations with our outcomes (data not shown).

Associations of baseline BAD with IQR increases in 5-day mean concentrations of CO, EC and NO<sub>2</sub>, were negative but non-significant, and baseline BAD was not associated with 5-day mean SO<sub>4</sub> or O<sub>3</sub> (Supplemental Material, Figure S2).

Associations of pollution with brachial artery narrowing tended to weaken after the maneuvers designed to cause brachial artery dilation (shear stress after arterial occlusion or sublingual nitroglycerin). However, despite perturbation of vascular function by the FMD and NMD maneuvers, the previous 5-day exposures to particle pollution tended to have a relatively persistent association with brachial artery narrowing for measures of BAD conducted after occlusion, before or after sublingual nitroglycerin administration (Figure 1, Supplemental Material, Figure S3). For example, the previous 5-day's home/trip integrated PM<sub>2.5</sub> was associated with reduced BAD before and after the FMD maneuver, as well as immediately before the NMD maneuver (Supplemental Material, Figure S3).

In two-pollutant models, the magnitude and statistical significance of associations of baseline BAD with 5-day mean PM<sub>2.5</sub> and BC remained essentially unchanged, while there were no significant associations with 5-day mean values of NO<sub>2</sub> or O<sub>3</sub> based on two-pollutant models (data not shown).

Associations of 5-day mean PM<sub>2.5</sub> and BC concentrations with baseline BAD were stronger (P-value for interaction = 0.01) in participants taking angiotensin-converting enzyme (ACE) inhibitors. For example, a 3.14 mg/m<sup>3</sup> increase in 5-day average PM<sub>2.5</sub> was not associated with

baseline BAD among the 35 participants that were not taking ACE inhibitors (0.008 mm; 95% CI: -0.3, 0.04), but was significantly lower among the 29 participants that were taking ACE inhibitors (-0.06 mm; 95% CI: -0.11, -0.02). There was no evidence (P-value for interaction = 0.7) of effect modification by beta-blocker use (data not shown).

In sensitivity analyses we compared fixed to mixed effect models and found that the main results as well as the effect modification results by medications were very similar (data not shown). We also found that the associations of BAD and FMD with each pollutant were similar in the smaller group of 42 subjects who had both FMD and NMD measures compared to the larger group of 64 subjects who had FMD measures (data not shown).

### **Temperature, humidity and brachial artery diameter**

Baseline BAD (before brachial artery occlusion) increased in association with IQR increases in same day temperature (0.12 mm; 95% CI: 0.04, 0.19, IQR = 14.4 °C) and WVP (0.09 mm; 95% CI: 0.02, 0.16, IQR = 10.13 hPa) when estimated using separate models. When temperature and WVP were included in the same model, WVP was no longer significantly associated with baseline BAD (0.06 mm; 95% CI: -0.06, 0.17) and the association with temperature was attenuated (0.09 mm; 95% CI: 0.0, 0.18). However, these estimates are difficult to interpret given the high correlation between temperature and WVP ( $r = 0.88$ ). Associations between temperature and baseline BAD were similar when adjusted for 5-day  $PM_{2.5}$  or BC (Figure 2). Associations of temperature with BAD after NMD were also in a positive direction but smaller in magnitude and lower in precision. The associations of same day temperature with baseline BAD were stronger during autumn and summer and lower in spring and winter (Figure 2).

## **Pollution, temperature, humidity, FMD and NMD**

While the FMD and the NMD tended to increase with increases in pollutant concentrations, the changes with pollution were small, inconsistent, and generally non-significant (Table 3). Temperature was not associated with FMD or NMD (data not shown), and barometric pressure was not associated with baseline BAD, FMD or NMD (results not shown).

## **Discussion**

As well as demonstrating an association of elevated particle pollution with change in BAD, to the best of our knowledge this is the first longitudinal study of people with type 2 diabetes to estimate associations of BAD with temperature, now recognized in its extremes as a predictor of cardiovascular risk (Montalcini et al. 2012). Our study results suggest that for this cohort of people with diabetes, particle pollution was a brachial artery vasoconstrictor; whereas higher ambient temperature and (perhaps) high humidity were brachial artery vasodilators.

Our study demonstrating subclinical associations of pollution and weather with changes in BAD, gives us insight into potential mechanisms for the clinical effects of more extreme exposures on populations potentially at risk of particle- and temperature-related health effects. Evidence from multiple epidemiological studies suggests that people with diabetes may more susceptible to the joint and separate effects of temperature and air pollution on cardiovascular morbidity and mortality (Medina-Ramon et al. 2006; Qian et al. 2010; Schifano et al. 2009; Schwartz 2005; Zanobetti and Schwartz 2001; Zanobetti et al. 2009).

## **Potential mechanisms**

BAD is influenced by structural (e.g., atherosclerotic), endothelial dependent (e.g., NO producing), and endothelial independent (e.g., autonomic) factors. The short-term associations between environmental exposures and BAD in our study are unlikely to reflect structural effects of the exposures on the vasculature. Our study provides no evidence for endothelial dependent responses to pollution and weather, as there are no consistent FMD responses that are independent of NMD responses. In the absence of supporting evidence of endothelium-specific influences on the brachial artery vasculature, we hypothesize that environmental influences on BAD in our study are likely endothelial independent, and may, in part, reflect autonomic responses to environmental exposures.

Our data (Figure 1) suggest that the dominant effects of pollution on reduction of brachial artery diameter are relatively constant even after the FMD maneuver, particularly 15 minutes later when, before nitroglycerin administration, the brachial artery has had time to recover from the shear stress maneuver and come back towards its “baseline” diameter for the day of observation. Since the dominant and persistent influence of particle pollution appears to be to narrow the brachial artery, we do not interpret the small and inconsistent pollution-associated increases in FMD or NMD to be “beneficial”. We interpret the small, inconsistent and transient particle pollution-associated increases in FMD or NMD that we and others have seen (Liu et al. 2007; Peretz et al. 2008) to be mathematically a function of the transient increase in arterial diameter occurring after the sheer stress or NMD maneuvers, in the context of a relatively constant influences of pollution on BAD narrowing.

Mean FMD in this diabetic population (2%) was lower than what we have measured in healthy adult populations (e.g., ~3.6%) (Brook et al. 2009); this functional impairment is well documented and makes discernment of specific influences on FMD/endothelial function in this population challenging, with a higher coefficient of variation of FMD compared to BAD. (Montalcini et al. 2012) Nevertheless, in similar populations of people with diabetes in Boston, in a placebo-controlled randomized trial of 87 people we were able to measure changes in FMD in response to troglitazone, an insulin-sensitizing agent (Caballero et al. 2003). In a smaller trial of 24 people exercise and weight reduction were associated with increased FMD (Economides et al. 2004, 2005; Hamdy et al. 2003).

Whereas most controlled human exposure chamber studies have shown concentrated ambient fine particle associations with reduction in brachial artery diameter, chamber (Brook et al. 2002, 2009; Peretz et al. 2008; Urch et al. 2005) and community-based (Briet et al. 2007; Dales et al. 2007; Schneider et al. 2008a) studies evaluating pollution effects on FMD in diabetic and non-diabetic populations have reported inconsistent findings. Differences in study design, population sensitivity, particle composition-related toxicity (Brook et al. 2009) and the higher coefficient of variation in FMD compared to BAD (Montalcini et al. 2012) have been cited as possible explanations for differing study results. In a previous Boston study we found cross-sectional (between-person) associations of  $PM_{2.5}$ , BC, and  $SO_4$  with reduced FMD and NMD in people with diabetes, but not in people without diabetes (O'Neill et al. 2005). These results supported an endothelium-independent component to the vascular responses to pollution from traffic (BC) as well as non-traffic ( $SO_4$ ) sources in people with diabetes. A Paris, France, study of healthy males breathing ambient air also found that pollution from non-traffic ( $SO_4$ ) and traffic (NO) was

associated with reduced FMD (Briet et al. 2007). But in contrast to the O'Neill study, NMD was not associated with either pollutant, supporting the possibility of endothelium-mediated mechanisms for pollution effects in this small non-diabetic cohort (N = 40). This possibility was also supported by a prospective repeated measures North Carolina study (Schneider et al. 2008a) in which people with diabetes (N = 22) had decreased FMD in association with increased PM<sub>2.5</sub>, but PM<sub>2.5</sub> was not significantly associated with NDM. Likely because of concern of potential adverse effects of NMD, many controlled human exposure and community-based studies have not measured NMD as a control, (e.g, Dales et al. 2007) making it uncertain whether an FMD response is endothelial dependent or not.

### **Sources of Pollution and BAD Narrowing**

As in some of the European and North American studies mentioned above, our data suggest that non-traffic as well as traffic sources may influence vascular outcomes. OC, BC and PM<sub>2.5</sub> had the strongest negative associations with BAD; other exposures such as particle number concentration and gaseous pollutant concentrations showed no or less consistent associations with arterial diameter. The bulk of the OC associations that we found came from secondary OC [effect estimate: -0.07 (95% CI: -0.11, -0.02)], whose sources are both oxidation of traffic and other biogenic emissions (Lim and Turpin 2002), rather than primary OC [effect estimate: -0.01 (95% CI: -0.05, 0.03)], whose primary source is motor vehicles.

Previously, in this same study we have demonstrated associations of pollution and temperature with changes in blood pressure (BP) (Hoffmann et al. 2012). However, despite the associations of pollution and temperature with both BAD and BP, these two outcomes were only weakly correlated ( $r = 0.08$  and  $r = 0.07$  for correlations of BAD and SBP or DBP, respectively). This is

not surprising, as brachial arterial diameter is measured in a medium-sized conduit artery, whereas BP is mainly determined by cardiac output X the resistance of the arterioles, which were not observed directly. Thus the constriction and dilation of these two sections of the vascular system may be subject to both shared and disparate physiologic mechanisms.

People with diabetes have impaired regulation of vascular tone in both the micro- and macrovasculature. Homeostatic vascular responses to changes in ambient temperature and other environmental stimuli may be impaired in people with diabetes due to impaired autonomic regulation, fluid shifts, and medication effects. We did not find significant departures from linearity for the relationship of temperature and BAD, which suggests that medium conduit artery vasoconstriction at lower temperatures as well as vasodilation at high temperatures. In diabetic patients either extreme may be a cardiac risk factor consistent with the U shaped relation of temperature with cardiac mortality in very cold as well as very hot weather (Wolf et al. 2009).

Our study was limited in size, limiting power to evaluate effect modification by personal characteristics of this diabetic cohort. Our study also had limitations related to the precision of outcome and exposure measurement. While estimation of “peak diameter” by evaluating post occlusion diameter at a single point in time, 60 seconds after the cuff release is a valid approach dating back to the classic work of Celermajer et al. (Celermajer et al. 1992), recent work published after this study suggests that it may result in an underestimation of FMD, with potential for type II error (Thijssen et al. 2011). Some recent studies have chosen to define the peak diameter after cuff release, allowing the time to peak diameter measure to vary (Thijssen et al. 2011). Some of our study participants worked, and were not home during the day, so that the measurements are not called “personal”, but rather home/trip measurements, and have their

limitations as estimates of personal exposure. Nevertheless, this study designed resulted in personal exposure measurements during travel to the clinic, immediately prior to outcomes measurement.

## **Conclusions**

Air pollution is a leading cause of mortality and morbidity (Lim et al. 2012). Observational studies have shown that rates of cardiovascular hospitalizations and death in association with particle pollution (Zanobetti and Schwartz 2001; Zanobetti et al. 2009) and extreme ambient temperatures (Medina-Ramon et al. 2006; Schifano et al. 2009; Schwartz 2005) are increased in people with diabetes compared with the general population.

Our findings suggest that in people with type 2 diabetes, particle pollution may cause vasoconstriction of medium-sized conduit arteries, whereas higher ambient temperature and WVP may cause vasodilation. Our study gives us insight into potential mechanisms for the clinical associations of more extreme environmental exposures on people with type 2 diabetes, a disease resulting in reduced ability to effectively respond to environmental perturbation of vascular responses relevant to cardiac risk.

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**Table 1.** Participant characteristics.

<b>Characteristic</b>	<b>N participants</b>	<b>N observations</b>	<b>%</b>	<b>Mean</b>	<b>Range</b>
Age, years	64	64		63.9	(45 - 81)
BMI (kg/m <sup>2</sup> )	64	64		31.5	(20.5 - 57.2)
Years of diabetes		62		10.4	(1 - 38)
Male	32		50		
Female	32		50		
Medication <sup>a</sup>					
β-blocker	24		38		
Calcium channel blocker	15		23		
ACE inhibitors	29		45		
Statin	50		78		
Insulin	9		14		
Study visits completed					
1		64	100		
2		60	94		
3		57	89		
4		54	84		
5		44	69		
Brachial artery diameter (mm) <sup>b</sup>					
Baseline	64	279		4.5	(2.5 - 6.23)
After occlusion	64	279		4.6	(2.6 - 6.3)
Before nitroglycerin	43	164		4.4	(2.6 - 6.3)
After nitroglycerin	43	159		4.9	(3.02 - 6.9)
FMD (%) <sup>c</sup>	64	279		2.0	(-4.6 - 14.2)
NMD (%) <sup>c</sup>	43	159		10.0	(-0.4 - 21.3)

<sup>a</sup>Medication use was ascertained by patient self-report at baseline and at each subsequent visit.

<sup>b</sup>Baseline refers to brachial artery diameter (BAD) measured prior to brachial artery occlusion.

BAD before and after nitroglycerine refers to measures taken before and after sublingual administration of nitroglycerine. Mean values are averaged across all study visits. <sup>c</sup>FMD = percent change in BAD after brachial artery occlusion relative to BAD before occlusion; NMD = percent change in BAD after nitroglycerine administration relative to BAD before nitroglycerine administration. Mean values are averaged across all study visits.

**Table 2.** Air pollution, temperature, and water vapor pressure among all observations (24-hour average and 5-day average values before each study visit).

	<b>No. observations</b>	<b>Mean</b>	<b>25<sup>th</sup> %ile</b>	<b>50<sup>th</sup> %ile</b>	<b>75<sup>th</sup> %ile</b>	<b>Max</b>	<b>IQR</b>
Ambient PM <sub>2.5</sub> (µg/m <sup>3</sup> )							
24-hour	278	8.37	5.52	7.38	9.58	26.69	4.06
5-day	279	8.51	6.47	7.61	9.62	21.10	3.14
Indoor continuous PM <sub>2.5</sub> (µg/m <sup>3</sup> )							
24-hour	258	7.11	3.73	5.04	7.96	56.43	4.23
5-day	260	8.93	5.02	7.11	10.51	52.88	5.49
Home/trip integrated PM <sub>2.5</sub> (µg/m <sup>3</sup> )							
5-day	269	9.18	5.05	7.74	10.70	57.28	5.66
Ambient BC (µg/m <sup>3</sup> )							
24-hour	279	0.61	0.41	0.54	0.76	2.62	0.35
5-day	279	0.60	0.48	0.57	0.73	1.25	0.25
Home/trip integrated BC (µg/m <sup>3</sup> )							
5-day	268	0.77	0.56	0.69	0.84	4.89	0.28
OC (µg/m <sup>3</sup> )							
24-hour	231	3.03	2.07	2.85	3.82	8.91	1.75
5-day	245	3.03	2.17	2.98	3.78	6.24	1.61
EC (µg/m <sup>3</sup> )							
24-hour	231	0.35	0.24	0.30	0.44	0.96	0.20
5-day	245	0.34	0.27	0.34	0.41	0.80	0.14
CO (ppm)							
24-hour	279	0.28	0.21	0.27	0.34	1.00	0.13
5-day	279	0.28	0.23	0.28	0.33	0.52	0.10
NO <sub>2</sub> (ppm)							
24-hour	279	0.015	0.011	0.015	0.018	0.033	0.006
5-day	279	0.015	0.012	0.014	0.016	0.025	0.004
O <sub>3</sub> (ppm)							
24-hour	279	0.027	0.020	0.026	0.032	0.061	0.012
5-day	279	0.028	0.022	0.028	0.033	0.047	0.010
PN (1000/cm <sup>3</sup> )							
24-hour	262	13.27	9.03	12.47	17.21	32.67	8.18
5-day	265	12.95	8.92	12.43	16.16	28.39	7.24
SO <sub>4</sub> (µg/m <sup>3</sup> )							
24-hour	197	2.13	0.95	1.61	2.41	12.34	1.47
5-day	221	2.28	1.37	1.82	2.72	7.08	1.34
Temperature (°C)							
24-hour	279	13.71	6.84	14.78	21.20	29.33	14.36
5-day	279	13.68	6.84	15.10	20.99	26.39	14.15
Water vapor pressure (hPa)							
24-hour	279	11.41	6.18	10.53	16.32	24.99	10.14
5-day	279	11.49	6.72	10.63	16.06	24.63	9.34

<sup>a</sup>Based on imputed data for missing observations.

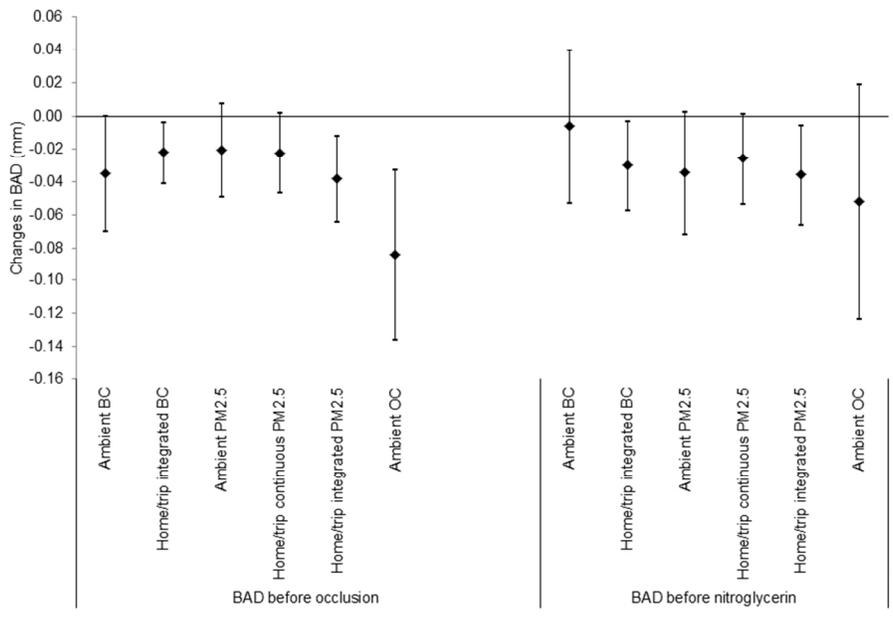
**Table 3.** Associations of FMD and NMD (percent change in BAD) with IQR increases in the 5-day mean concentrations of each pollutant.

<b>Exposure</b>	<b>IQR</b>	<b>FMD (%) change (95% CI)</b>	<b>NMD (%) change (95% CI)</b>
Ambient BC	0.25	0.41 (-0.09, 0.91)	-0.62 (-1.56, 0.31)
Home/trip integrated BC	0.28	0.38 (0.11, 0.65)	-0.08 (-0.65, 0.49)
Ambient PM <sub>2.5</sub>	3.14	0.37 (-0.04, 0.77)	0.03 (-0.74, 0.80)
Home/trip			
Continuous PM <sub>2.5</sub>	5.40	0.08 (-0.25, 0.42)	0.75 (0.20, 1.31)
Integrated PM <sub>2.5</sub>	5.66	0.24 (-0.14, 0.62)	0.62 (0.00, 1.25)
OC	1.61	1.12 (0.43, 1.80)	0.96 (-0.47, 2.38)

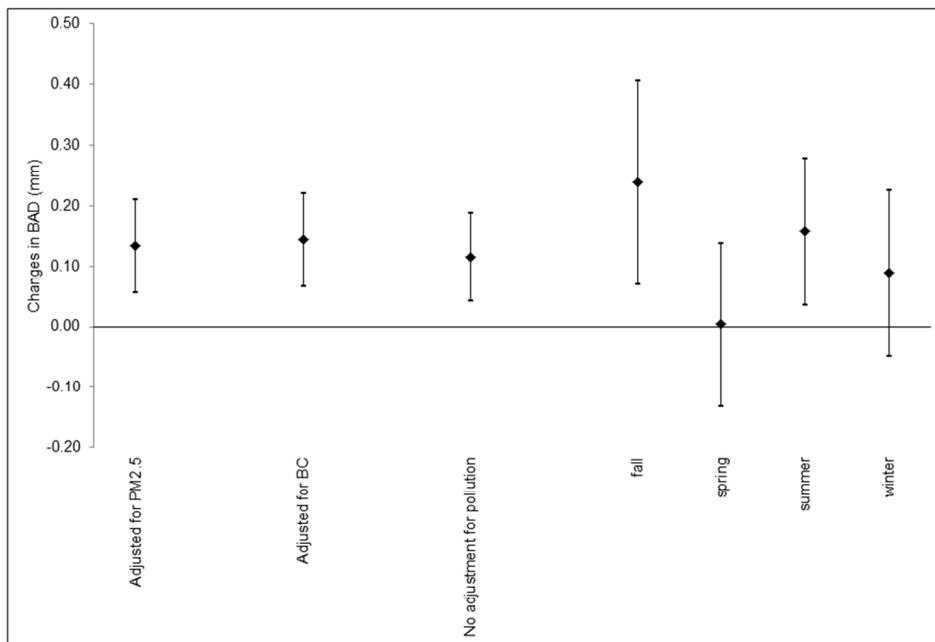
## Figure Legends

**Figure 1.** Estimated changes (95% CI) in brachial artery diameter (BAD, in mm) associated with IQR increases in mean 5-day concentrations of each pollutant, including BAD measured at baseline (before brachial artery occlusion), and before sublingual nitroglycerin administration.

**Figure 2.** Estimated changes (95% CI) in brachial artery diameter (BAD, in mm) associated with an IQR increase in average daily temperature during the 24-hours before the study visit with and without adjustment for PM<sub>2.5</sub>, or BC by season, for baseline BAD (measured before occlusion).



254x190mm (96 x 96 DPI)



254x190mm (96 x 96 DPI)