SUPPORTING INFORMATION

Ozone exposure, cardiopulmonary health, and obesity: a substantive review

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Introduction

Controlled Ozone Exposure Studies in Human Subjects

In Bennett et al. (2007), 197 non-asthmatic non-smoking young adults from North Carolina aged 18 to 35 were exposed to standardized ozone dose with exertion. Of these, 57 subjects (42 men and 15 women) were categorized as overweight or obese (BMI $\geq$ 25 kg/m^2). BMI was positively related to greater response to 0.42 ppm ozone for 90 minutes with intermittent exercise. Previous publications about this study population indicated no gender differences in response to ozone, controlling for age, and a repeatable unexplained stratification of strength of response.[1] In the 2007 Bennett et al. publication, obesity and overweight status explains some of the difference in responsiveness to ozone dose by change in standard BMI categories among all participants, and the effect modification of BMI was stronger and more significant among young women than young men. Controlling for age, BMI was negatively associated with a larger decrement of percent change FEV_1 ($\%\Delta FEV_1$) (beta -0.580 (p-value = 0.014)), percent change forced vital capacity ($\%\Delta FVC$) (beta -0.288 (p-value = 0.038)), percent change forced expiratory flow at 25–75% of FVC ($FEF_{25-75\%}$) (beta -0.952 (p-value = 0.008)), $\%\Delta FEV_1/\%\Delta FVC$ (beta -0.259 (p-value = 0.050)), $\%\Delta$ $FEF_{25-75}/\%\Delta FVC$ (beta -0.732 (p-value = 0.023)). The effect of BMI on ozone responsiveness among women was most pronounced for ΔFEF_{25-75} ($p<0.05$).

In a study designed to observe the effect of obesity on ozone responsiveness among women, Bennett et al. (2016) compared ozone-induced changes in airway function, reactivity, and inflammation in 20 obese (30 < BMI < 40 kg/m^2) and 20 normal weight (BMI < 25 kg/m^2) women.[2] Two participants were excluded due to smoking. In this double-blinded study, participants were randomized to 0.4 ppm ozone exposure and air exposure for 2 hours with intermittent light exercise. This study involved less exertion and a lower effective dose of ozone

than a previous 2007 study.[3] The health measures included spirometry and bronchial reactivity to inhaled methacholine (3 hours after exposure), inflammation and obesity markers in the blood (pre-exposure, 4 hours after exposure, and 20 hours after exposures), and induced-sputum (baseline on 24 hours pre-exposure training day (no exercise), and 4 hours after exposure). Inflammatory markers included C-reactive protein (CRP) (blood only), leptin (blood only), adiponectin, interleukin (IL) IL-6, IL-1β, and IL-8, and tumor necrosis factor alpha (TNF-α), and sputum cell differential cell counts.

The ozone-induced decrement in FVC (adjusted for the change after air exposure) was significantly greater in the obese group (mean 12.5% (7.5) vs. 8.0% (5.8), $p<0.05$) compared to the normal weight participants. Following ozone exposure, a similar degree of hyperresponsiveness was observed for the two groups: 6 obese and 6 normal weight participants responded to methacholine at ≤ 10mg/ml (the maximum dose). Both groups showed similar and significant ozone-induced increases in sputum neutrophils, whereas blood IL-6 was increased by exercise (4 hours after air exposure vs. pre-exposure) only in the obese participants. The levels returned to pre-air exposure levels at 20 hours after exposure. The authors reported no correlations between ozone-induced changes in lung function and either sputum or blood markers.

In McDonnell et al. (2009), a reanalysis of 22 combined controlled exposure human subject datasets (n=541) was consistent with Bennett et al. (2007), reporting that BMI was positively associated with enhanced ozone-induced FEV_1 decrement.[4] Sixteen controlled human ozone exposure studies were conducted in the U.S. EPA exposure facility in Chapel Hill, NC mainly during 1981-1992, and six controlled human ozone exposure studies conducted at the laboratory at the University of California at Davis, with roughly equal numbers of men and women. The

mean BMI in the combined dataset was 23.41 kg/m^2. A nonlinear random effects model was fit with and without BMI as a covariate. In the study, increasing BMI was associated with increasing FEV_1 response to ozone among 18- to 35-year old healthy males. Specifically, a one unit increase in BMI was associated with a 0.4855 (95% CI 0.1018, 0.8693) larger decrease $\%\Delta FEV_1$ at a given ozone dose (p=0.13). The addition of BMI to the model resulted in modest changes in individual predicted FEV_1, with most being within +/- 0.3 of a percentage point of FEV_1 decrement. Among young volunteers, there was no evidence of an interaction between age and BMI or confounding by other factors.

In contrast, a study by Todoric et al. (2014) of subjects with overweight subjects (BMI ≤ 27.5 kg/m^2) reported no significant correlation between BMI and ozone-induced decrements in FEV_1 or FVC.[5] The retrospective analysis included subjects who underwent a baseline exam followed by a 0.4-ppm ozone exposure, and a 24-hour follow up of additional spirometry, sputum induction and blood collection. Eighteen of the 40 healthy subjects (aged 20 – 28.7 years) were overweight (BMI ≥ 25 kg/m^2) and none were obese, with an average BMI of 24.1 kg/m^2 (21.8 – 27.5 kg/m^2). Baseline and post-exposure sputum and blood samples were evaluated for changes in percentage of polymorphonuclear leukocytes (%PMN), percentage of eosinophils (%Eos), IL-1β, IL-6 and IL-8. IL-1β is an important mediator of the inflammatory response. Cytokines were measured using multiplex technology. The study suggests that airway and systemic inflammation after ozone exposure, measured by IL-1β, was positively correlated with BMI in human subjects. For all subjects, controlling for sex, age, ethnicity and asthma status, a one unit increase in BMI was associated with a 20 pg/mL increase in sputum IL-1β (p=0.02). BMI was significantly associated with IL-1β but not with other measures of inflammation such as %PMN, %Eos, IL-6 or IL-8 in humans after ozone exposure. This study suggests that NLRP3 inflammasome

activation or priming with IL-1β is uniquely involved in ozone-induced BMI-related inflammation in human subjects.

Community Ozone Studies

The Normative Aging Study

As part of the Veterans Administration Normative Aging Study, started in 1963, enrolling 2 280 men in the Greater Boston area.[6] This longitudinal study included a subset of 904 mainly white elderly men whose pulmonary function and body measurements were taken every three years between 1995 and 2005. A subject was considered to have airway hyper-responsiveness (AHR) with a positive response to methacholine challenge of more than a 20% decline in FEV_1 in the most recent testing available from testing conducted from 1984 and 2000. About 23 percent of the subjects were classified as obese (BMI $\geq$ 30kg/m^2). The mean BMI was 27.8 (SD 3.7) kg/m^2. Atopy (allergy) as measured by blood eosinophil count, was associated with responsiveness to methacholine in this cohort.

Ozone concentrations were obtained from four monitoring sites in the Greater Boston area that conformed to EPA protocols. Because of observed low correlations with other pollutants such as $PM_{2.5}$, CO and NO_2, a single pollutant ozone linear mixed model was constructed, using a 48-hour average ozone exposure prior to the exam dates; the 48-hour mean ozone concentration was 24.4 ppb (SD 11.0).

The linear mixed model allows each subject to act as his own control; it accounts for intra-subject variability and allows controlling for unmeasured within-subject variation via a random subject-specific intercept. For a 15 ppb increase in ozone, the obese exhibited an enhanced FEV_1 and FVC decrement compared to non-obese subjects. The interaction term was significant for FEV_1 ($p=0.022$) but not for FVC ($p>0.05$). A three-way interaction between obesity, ozone and

AHR was investigated. For a 15 ppb increase of ozone, for both FEV_1 ($p=0.048$) and FVC ($p<0.001$), the estimated decrease in lung function associated with a 15 ppb increase in ozone was greatest among obese with AHR. Because increased inflammation is independently associated with AHR and obesity, patients with either condition may be more susceptible to the inflammatory effects of ozone, leading to lower lung function. The combination of the two conditions was associated with a larger ozone-response decrement that the size of the effect was more than additive among elderly white men. Strengths of the study include its well-characterized spirometry outcomes and the quality of the short-term air pollution measurements. Limitations of this study are that women were excluded, other racial groups are not well represented, and the subjects live in one metropolitan area, differences in long-term ozone exposures were not studied.

Thirty-three Communities of North China Study

Two Chinese studies examined the effect of BMI in the same population of men and women on the association of long-term annual ozone levels with cardiovascular endpoints in 11 districts in three northeastern cities with a gradient of ozone concentrations.[7,8]

The two studies used a cross-sectional design to study 24 845 randomly selected adults, aged 18 to 74 years, from three northeastern Chinese cities: Shenyang, Anshan and Jinzhou.[9] Air pollution was measured at municipal monitoring stations from 2006 to 2008 adhering to the State Environmental Protection Administration of China using ultraviolet photometry. Although there are several advantages to this ozone monitoring technique, the performance of the ozone zero air scrubber can be affected by changes in humidity as well as interferences by other compounds such as CO_2, H_2S, Hg, and aromatic hydrocarbons, which can lead to bias in measurements. Because of these shortcomings, this technique is not recommended in U.S. compliance networks.

The Chinese studies used daily 8-hour averages (10 am – 6pm) for days with at least 75% data completeness and excluded outliers in the hourly measurements.[7] The mean ozone level was 49.4 mg/m^3 (SD 14.07) (range among districts of 27 – 71 mg/m^3, IQR 22 mg/m^3). For comparison to other studies, at standard temperature and pressure, these concentrations correspond to a mean of 1.259 ppm (SD 0.3586 ppm) (district range 0.688 – 1.809 ppm).

Qin et al. (2015) used questionnaires to assess self-reported physician diagnosed cardiovascular and cerebrovascular disease. Two-level logistic regression models were used to predict the probability of CVD and stroke, controlling for age, sex, race, education, income, smoking, alcohol drinking, exercise, diet, sugar intake, family history of CVD, family history of stroke and study district. The effects of ozone on stroke were strongest in obese subjects (OR 1.47 (0.83, 2.59)), and less strong among overweight (OR 1.29 (1.05, 1.59)) and normal weight individuals (OR 0.98 (0.82, 1.18)) with a significant interaction (p=0.002). For cardiovascular disease, the OR among the obese was greater (OR 1.56 (1.02, 2.39)) than overweight (OR 1.08 (0.86, 1.35)) and normal weight (OR 1.08 (0.87, 1.35)) with a non-significant interaction (0.121). However, a cross-level interaction between BMI categorized above and below the overweight mark (BMI < 25 kg/m^2 compared to BMI ≥ 25kg/m^2) was not significant at the 95% confidence level among all subjects with stroke, (p=0.062) or with CVD (p=0.635). However, when stratified by sex, the interaction was significant among women for stroke (stroke p=0.046, CVD p=0.110), but not among men. An analysis using the obesity cut point (BMI ≥30 kg/m^2) might be a better way to examine the CVD data.

A second study using the same cohort examined the effect of air pollution and BMI on the prevalence of hypertension and blood pressure.[8] The associations between long-term ozone exposure and prevalence of hypertension were consistently stronger among overweight and

obese adults compared with normal weight individuals, controlling for the same covariates as above. Interaction terms between ozone and BMI category were significant for hypertension ($p \leq 0.001$). However, when stratified by sex, the associations were observed only among men ($p = 0.041$). Among women, ozone was not associated with hypertension among any of the BMI subgroups.

For all participants, ozone exposure was significantly associated with higher systolic and diastolic blood pressure. When stratified by sex, the associations were observed only among men, with a larger negative effect on systolic blood pressure (SBP) per interquartile range (IQR) of ozone.

Strengths of the study include its large sample size with significant numbers of overweight (n=8 764) and obese (n=1 435) participants with a broad age and air pollution range. The study controlled for known confounders. Limitations of the studies include the inability to draw causal interpretations due to the cross-sectional nature of the study and lack of temporality between the exposure and outcome; ozone exposure misclassification may bias the study toward the null; selection bias (e.g., healthy subjects are more likely to participate) and information bias (e.g., recall bias and self-reported endpoint, the use of prevalence rather than incidence of hypertension). There may also be confounding with particulate matter, which is more strongly associated with cardiovascular outcomes, due to a moderate correlation between ambient ozone and PM concentrations in the study districts.

ABBREVIATIONS

Abbreviations: body mass index (BMI); cardiovascular disease (CVD); diastolic blood pressure (DBP); forced expiratory volume in one second (FEV_1); forced vital capacity (FVC); forced expiratory flow at 25–75% of FVC ($FEF_{25–75\%}$), interleukin (IL), polymorphonuclear (PMN), eosinophils (Eos), systolic blood pressure (SBP); tumor necrosis factor alpha (TNF-α), U.S. Environmental Protection Agency (EPA); $\mu g/m^3$ micrograms per cubic meter

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