AMBIENT AIR POLLUTION, SMOKING, AND REPRODUCTIVE OUTCOMES

by

Pei-Chen Lee

B.A., Fu-Jen Catholic University, Taiwan, 2004

MS., University of Colorado at Denver, 2007

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by

Pei-Chen Lee

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and approved by

Dissertation Advisor:
Evelyn O. Talbott, Dr. P.H, Professor,
Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh

Committee Member:
James M. Roberts, M.D., Professor,
Magee-Womens Research Institute, Department of Obstetrics, Gynecology and Reproductive
Sciences, School of Medicine, University of Pittsburgh

Committee Member:
Janet M. Catov, Ph.D., Assistant Professor,
Department of Obstetrics, Gynecology and Reproductive Sciences, School of Medicine,
University of Pittsburgh

Committee Member:
Roslyn A. Stone, Ph.D., Associate Professor,
Department of Biostatistics, Graduate School of Public Health, University of Pittsburgh

Committee Member:
Richard A. Bilonick, Ph.D., Assistant Professor,
Department of Ophthalmology, School of Medicine, University of Pittsburgh

Committee Member:
Ravi K. Sharma, Ph.D., Assistant Professor,
Department of Behavioral and Community Health Sciences, Graduate School of Public
Health, University of Pittsburgh

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Pei-Chen Lee, Ph.D.

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The number of studies addressing the possible effects of air pollutants on human reproduction, especially prenatal outcomes, has grown extensively. However, the plausible biological mechanisms by which air pollutants influence prenatal outcomes remain unclear. The aims of this dissertation are (1) to determine whether ambient air pollution exposure (including particles of less than 10 μm (PM_{10}) and less than 2.5 μm diameter ($PM_{2.5}$), carbon monoxide, nitrogen dioxide, sulfur dioxide, and ozone) contributes to increased inflammatory response by measuring C-reactive protein (CRP) concentrations during early pregnancy, and (2) to examine associations between ambient air pollution exposures and blood pressure changes during pregnancy. In addition, because smoking during pregnancy is a risk factor for some adverse birth outcomes such as preterm delivery, and inflammation has been suggested to increase the risk of preterm delivery, the other aim of this dissertation was to examine whether systemic inflammation mediates the link between smoking and preterm delivery. The study population was selected from the Prenatal Exposures and Preeclampsia Prevention study (PEPP) conducted in Pittsburgh, PA between 1997 and 2001. Space-time Kriging interpolation for ambient station measures at the maternal ZIP code was performed to estimate maternal air pollution exposure. Multiple linear and logistic regressions were employed to evaluate associations between air pollution, CRP concentrations, and blood pressure changes during pregnancy. Positive associations between particulate (both $PM_{2.5}$ and PM_{10}) and ozone air pollution and elevated CRP concentrations in

non-smoking women during early pregnancy were observed. For blood pressure changes, we found that first trimester exposure to PM_{10} and ozone air pollution was associated to increases in mean systolic and diastolic blood pressure changes during pregnancy. For smoking and preterm study, no evidence that systemic inflammation mediates this association was found. Our findings provide some new evidence that associations between particulate air pollution and adverse birth outcomes may be mediated by systemic inflammation and blood pressure changes. These findings have considerable public health significance to further prevent the adverse birth outcomes associated with air pollution exposure.

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PREFACE

I would like to thank my family and friends for supporting me to complete my degree. I am heartily thankful to my advisor, Evelyn Talbott, whose guidance and support from the past couple years enabled me to develop skills and gain knowledge of this field. I would also like to thank my thesis committee members for their guidance and continuous assistance throughout this process.

1.0   INTRODUCTION

Premature birth (gestational weeks < 37) is a major cause of morbidity among neonates and is an indicator for impaired organ development that results in adverse health effects in childhood and adult life [1-3]. The incidence rate of preterm birth was 12.5 percent in 2004 in the U.S. and still remains at that level today [4]. Although significant efforts to prevent preterm birth have been made, poor understanding of underlying pathophysiology is one of the factors hampering its prevention and treatment. It has been suggested that intrauterine infection may play an important role in developing preterm birth [5,6], and low grade inflammation during pregnancy has been related to preeclampsia and preterm birth [7-13]. Some occupational and environmental activities or exposures have also been related to preterm delivery such as standing jobs [14-16], shiftwork [14-17], lead [17,18] and air pollution exposure [19-29]. The first study that connected maternal exposure to air pollutants and preterm birth was conducted in Beijing, China in 1995 [29]. This study found that mothers who lived in areas with high levels of total suspended particulates (TSP) and sulfur dioxide (SO_2) during pregnancy had an increased risk of delivering prematurity. Variables included in the model included: outdoor temperature and humidity, day of the week, season, maternal age, gender of child, and residential area. Since 1995, there have been additional studies linking air pollution to the incidence of preterm birth, indicating that exposure to air pollutants such as carbon monoxide (CO), nitrogen dioxide (NO_2), SO_2, ozone (O_3), particle diameter less than 10 μm (PM_{10}), and particle diameter less than 2.5 μm ($PM_{2.5}$) in different trimesters are

associated with an increased occurrence of prematurity [22,25-28]. However, several other studies found no such association [24]. Different methods of estimation of the concentration and outcome definition may be two of the reasons for inconsistent findings.

Although most epidemiologic studies suggest that air pollution may play a role in preterm delivery, the biological mechanism remains unknown. One hypothesis is that air pollution may induce systemic inflammation, which increases the risk of prematurity. This hypothesis has been tested in other studies for different populations; however, the results are inconsistent [30,31]. To date none of the studies has examined the association between air pollution exposure and inflammatory responses during pregnancy. The study of air pollution, prematurity and other adverse reproductive outcomes is a relatively new area of combining air pollution epidemiology with perinatal epidemiology. This area faces several challenges from both disciplines including air pollution exposure assessment, identifying an exposure time period, controlling potential confounders, and identifying effect-measure modification and selection bias [32]. To overcome these challenges, an air pollution and human reproductive workshop in Mexico City in 2007 recommended that applying a consistent analytic strategy and exposure assessment across many datasets may reconcile some of the inconsistent findings across studies. Moreover, expanding into other birth outcomes or pregnancy conditions, such as gestational hypertension or preeclampsia, can provide insights into mechanisms and other adverse outcomes [32].

1.1   AMBIENT AIR POLLUTION AND PRETERM BIRTH

Several epidemiologic studies support a link between ambient air pollutant exposure during the different trimesters of pregnancy and the risk of preterm birth (table1). Three studies conducted

in Los Angeles reported an increased risk of preterm birth for high ($\geq 75^{th}$ percentile) concentrations of CO exposure during the first month of pregnancy or six weeks before delivery [26-28]. Ritz et al. (2000) reported a 20% increase in preterm birth per an average of 50-µg increase in ambient PM_{10} levels during 6 weeks before delivery (averaged PM_{10} over the six weeks, RR_{crude}=1.20; 95%CI=1.09-1.33), and a 16% increase risk of preterm delivery over the first month of pregnancy (averaged PM_{10} over the first month of pregnancy, RR_{crude}=1.16; 95%CI=1.06-1.26). Wilhelm and Ritz (2005) studied a birth cohort including 146,972 singleton children that were born between 1994 and 2000. The study found that maternal exposure to higher levels of ambient CO (averaging CO concentrations during the first trimester ≥ 2.1 ppm) during the first trimester of pregnancy was associated with a significantly increased risk of preterm birth (adjusted relative risk = 1.26; 95% confidence interval (CI), 1.03-1.55). A third study included 58,316 infants born in 2003 in Los Angeles and found a similar association. This study indicated a 12% increase in preterm birth with higher CO exposure (averaging CO exposure concentrations ≥ 0.91 ppm) during the last 6 weeks of pregnancy [26]. Another study conducted in California reported that $PM_{2.5}$ exposure was associated with the effect on preterm birth (adjusted odds ratio=1.15, 95% CI, 1.07-1.24) but the effect was not evident in CO exposure [22]. Similar associations between $PM_{2.5}$ exposure and preterm delivery were also observed in two other studies in California [26,28].

Internationally, a study conducted by Liu et al. (2003) in Vancouver, Canada, showed a small increase in the risk of preterm birth for each 1 ppm increase of CO (odds ratio=1.08; 95% CI, 1.01-1.15) and for 5 ppb increase of SO_2 (odds ratio=1.09; 95% CI, 1.01-1.19) during the last month of pregnancy [25]. However, maternal smoking was uncontrolled in this study. Recently, a study conducted in Korea found that area-level socioeconomic status (SES) modified the effects

of PM_{10} on preterm delivery [33]. They reported that an average increase in PM_{10} per 10 µg/m³ during the second trimester of pregnancy was associated with the increased probability of preterm delivery (3.1%, 95% CI=0.17 – 6.15) for the entire population and a slightly higher probability was observed for the low income area (3.4%, 95% CI=0.31 – 6.58). Moreover, a study conducted in Spain reported women exposed to an average NO_2 concentrations above 46.2 µg/m³ during the second, third trimesters, and entire gestation were associated with an increased risk of preterm birth [34].

1.2   AMBIENT AIR POLLUTION AND BIRTH WEIGHT AND LOW BIRTH WEIGHT (LBW)

Epidemiological evidence links ambient air pollution exposure and the risk of low birth weight (LBW, birth weight <2,500g) in different trimesters of pregnancy (table2). Two studies conducted in Los Angeles reported a lower mean birth weight for babies whose mothers lived in areas of higher concentrations of CO during the third trimester of pregnancy [28,35]. In the Ritz and Yu study, the birth cohort was born between 1983 and 1993, including 125,573 singleton children. The study found that maternal exposure to higher levels of ambient CO (> 5.5 ppm) during the last trimester of pregnancy was associated with significantly increased risk of LBW (OR=1.22; 95% confidence interval (CI), 1.02-1.44). The second study included 146,972 infants born between 1994 and 2000 in Los Angeles and found the same association. This study indicated a 36% increase in LBW during high CO exposure (≥ 1.82 ppm) in the third-trimester of pregnancy [28]. However, the two studies were limited by their inability to control for maternal smoking and pregnancy weight gain, significant contributors to the risk of delivering at LBW.

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Another study conducted in the United States examined the association between ambient air pollution and LBW in the cities of Boston, Hartford, Philadelphia, Pittsburgh, Springfield, and Washington [36]. The study found that maternal exposure to both CO and SO_2 in the second and third trimesters was associated with reduced birth weight [36]. Further, the study showed that with every increase of 1ppm of CO exposure in the third trimester, the risk of LBW was increased (adjusted OR 1.31; 95% CI, 1.06-1.62).

Internationally, two studies in Seoul, Korea also found that maternal exposure to CO, SO_2, and NO_2 during the beginning or middle of pregnancy had adverse effects on birth weight [21,23]. The first study, completed by Ha et al. (2001), showed a small increase in the risk of LBW for each inter-quartile increase of CO (adjusted relative risk 1.08; 95% CI=1.04-1.12) in the first trimester. Also, this study indicated a negative relationship between birth weight and concentrations of NO_2, SO_2, and total suspended particle (TSP). This relationship was relatively linear, without thresholds for concentrations of the pollutants [21]. However, maternal smoking was uncontrolled in these two Korea studies. Another study, completed by Gouveia and colleagues, reported that a reduction of 23 g in birth weight was estimated for a 1 ppm increase in mean exposure to CO during the first trimester. Again, this study was unable to control for maternal smoking and weight gained during pregnancy.

Several studies do not support an association between ambient air pollution and LBW. Alderman et al. (1987) was the first study examining air pollution and LBW in Denver, Colorado. This study did not find an association between higher CO exposure and higher risk of LBW. The bias in this study was that the local variation in CO concentration was not incorporated from stationary monitors due to small variations [37]. They also did not control for maternal smoking. Thus, they recommended that further studies would likely require direct

measurement of total CO exposure, which includes indoor and workplace CO exposure. Another study conducted in California found that higher $PM_{2.5}$ exposure increased the risk of small for gestation age (SGA) [38]. However, they did not find a link between CO exposure and LBW which is the combination of preterm birth and IUGR. This finding varied from the two other studies described previously conducted in California [28,35]. The disparity in results could possibly be because of differences in the measures of CO exposure. Another study in Northern Nevada also did not find a link between air pollution exposure and LBW [39]. This study examined 39,338 singleton births in Washoe County from 1991 through 1999. However, CO was not found to be associated with birth weight through logistic regression after control for infant sex, city of residence, education, medical risk factors, active tobacco use, drug use, alcohol use, prenatal care, age, race, ethnicity, and maternal weight gain. In Vancouver, Canada, no association between CO exposure, and LBW was found [25]. However, preterm birth was associated with CO during the last trimester of pregnancy and the odd ratios for 1 ppm increase of CO was 1.08 (95 % CI, 1.01-1.15). Inability to control maternal smoking was the common limitation for many of these studies [37-39].

1.3 AMBIENT AIR POLLUTION, INTRAUTERINE GROWTH RETARDATION, GESTATIONAL HYPERTENSION AND PREECLAMPSIA

Most perinatal air pollution studies examined the effects of air pollution on LBW and preterm birth. However, few studies have examined the relationship between air pollution exposure and intrauterine growth retardation (IUGR), gestational hypertension and preeclampsia (table3). Small for gestational age (SGA, as a marker of IUGR) infants is defined as <10th percentile of

birth weight for gestational age and sex. Bobak et al. (2000) examined associations between SO_2, TSP, and NOx and IUGR, and found no significant associations between pollutant concentrations and IUGR after controlling for sex, parity, maternal age group, education, marital status and nationality, and month of birth (table3). Liu et al. (2003) examined the association between SO_2, NO_2, CO and O_3 air pollution and IUGR. They reported that first month of pregnancy exposure to higher SO_2 (OR=1.07, 95% CI=1.01-1.13, for a 5.0 ppb increase), NO_2 (OR=1.05, 95% CI=1.01-1.10, for a 10 ppb increase) and CO (OR=1.06, 95% CI=1.01-1.10, for a 1.0 ppm increase) was associated with IUGR.

A study assessed the relationship of hazardous air pollutants on adverse pregnancy outcomes and reported a significant protective effects of some hazardous air pollutants on preeclampsia or gestational hypertension, such as acetaldehyde (OR=0.5, p<0.01), arsenic (OR=0.6, p<0.01), benzene (OR= 0.1, p<0.0001), chromium (OR=0.6, p<0.0001), formaldehyde (OR=0.3, p<0.0001), mercury (OR=0.2, p<0.001) and nickel (OR=0.5, p<0.0001) [40]. However, Williams (2006) reported a non-significant relationship between CO air pollution and risk of pre-eclampsia (RR=1.73, 95% CI=0.91-3.27 for third tertile exposure vs. first tertile) [41].

1.4   EXPOSURE ASSESSMENT FOR AMBIENT AIR POLLUTION

Assessment of maternal exposure to air pollution has many challenges. Most of the previous studies used ZIP-code-level analysis, incorporating air monitoring station data or constant distance from the air monitor to determine levels of exposure for mothers [28,42,43]. Such methods, however, may entail certain degrees of exposure misclassification bias and such bias could ultimately lead to an overestimation or underestimation of the true effects [44]. A few studies

estimated individual exposure to ambient air pollution by modeling from air monitoring stations to a geographically identified receptor (location), through the use of an environmental transport model, or block Kriging statistical mapping technique, which can predict an average concentration over a spatial region from point locations [45,46]. These methods, which specified the individualized environmental exposures, largely reduce the misclassification bias. Therefore, they are considered better than the ZIP-code-level analysis and constant distance from the air monitoring station.

Two studies conducted in Denver and Los Angeles [35,37] used mean air pollution concentrations measured from air monitoring stations and examined the mothers who were residing in the census block or ZIP-code area within a 2-mile radius of stations to examine the associations with birth weight. The median CO exposure levels reported for Denver ranged from 0.5 to 3.6 ppm, and the 50-95-percentile exposure levels for Los Angeles were 2.2 to 5.5 ppm. Wilhelm and Ritz (2005) estimated exposure levels by calculating the distance to the nearest air monitors, and they suggested that the effects manifested during the first and second trimester as the result of CO concentrations for mothers who resided within a 1 mile distance of monitors. Another series of studies that examined associations between CO and birth weight also relied on ambient measurements of the CO air pollutant for exposure estimates [20,21,23,25,36,37,39]. These studies assigned mean concentrations of different ambient air pollutants to mothers who lived within the same cities as the stations. Mean exposure levels within each trimester were calculated by averaging daily ambient air pollution concentrations during the corresponding days.

Due to data availability and privacy issues, individual geographic location is not easily obtainable from the birth registry dataset. However, two studies conducted in California were able to obtain personal levels of information such as coordinates and addresses to identify the

residence of mothers [28,38]. In addition, both studies were able to calculate the exact distance from maternal residence to the nearest air monitoring stations. Although the studies were capable of estimating the distance to each of the stations, personal exposure concentration still could not be obtained from monitoring data.

Among modeling techniques, the environmental transport model, which is based on the Gaussian plume atmospheric transport model, was used by Rogers and Dunlop (2006) to estimate PM_{10} exposure at the scale of the individual address. The annual PM_{10} emissions were measured from air monitoring stations and industrial point sources. The Gaussian plume atmospheric transport models assume that plume concentration has independent Gaussian distributions in horizontal and vertical planes at each downwind distance [47,48]. This model is widely used to predict concentrations in the atmosphere and is recommended by the US EPA [49]. Although the environmental transport model had been validated elsewhere [50], the primary limitation involves the uncertainties in the model, such as release quantities, the transport model, ambient temperature, wind speed and direction, and stack gas temperature and velocities [46].

As another interpolation method, Kriging is a weighted average technique used to estimate a smooth surface from data points over the domain and predicts the average block based on regular grids. Additionally, cross-validation, "a technique with which each monitoring station is removed, one at a time, and the concentration at each omitted station is predicted using the concentration values observed at the other monitors," is used to evaluate the quality of the predicted values from Kriging. The first study to use the Kriging statistical mapping technique on evaluation air pollution and preterm delivery was presented by Leem et al. (2006). They used 0.17 km * 0.17 km grids to partition each dong (the administrative unit in Korea similar to a county) for each pollutant and each month. They found the Kriging technique provided

reasonable results for surface interpolation of pollutants concentrations, such as CO, SO_2, NO_2, and PM_{10}. Because this method is fairly accurate and avoids the artifacts that often result from the use of inverse distance weighted, spline, or global/local polynomials, its use for predicting the spatial distribution of air pollutants was one of the strengths of this study [27,51]. However, they still were not able to control for maternal smoking, its potential misclassification of exposure due to the use of surrogate ambient air pollution data, the uncertainty of the predicted average concentrations for the dongs, and its inability to geocode the residential addresses to point locations.

1.5   BIOLOGICAL MECHANISM OF AIR POLLUTION AND BIRTH OUTCOMES

Possible biological mechanisms by which air pollutants may influence birth outcomes have been suggested but few of them have been examined. Oxidative stress may induce DNA damage which may increase the number of placental DNA adducts [52]. Particulate air pollutants, such as PM_{10}, that are made up of absorbed polycyclic aromatic hydrocarbon (PAHs) may lead to DNA adducts, thus resulting in LBW and IUGR [53-56]. One hypothesis suggests that air pollution-induced inflammatory processes may cause preterm birth or IUGR by altering maternal immunity [57]. Maternal immunity may be altered by exposure to air pollutants which may induce inflammatory processes and increased susceptibility to infections [57]. In addition, proinflammatory cytokine genes were found to be associated with spontaneous preterm birth [58]. Although air pollution does not directly cause maternal infections, evidence on air pollution-induced inflammatory process may influence maternal immunity and increase the risk for adverse birth outcomes.

The binding of CO with hemoglobin to produce elevated levels of HbCO in smokers, decreasing the oxygen available to the fetus might be a possible mechanism for the negative impacts that smoking during pregnancy has on LBW [59]. In 1857, Claude Bernard first observed that carbon monoxide had greater affinity for binding to hemoglobin (Hb) than oxygen. As a result, the oxygen tension of blood decreases to lower than normal values. This effect may be particularly significant for the fetus since the oxygen partial pressure in its arterial blood is normally relatively low (about 20 to 30 mmHg) in the fetus as compared to adult values of about 100 mmHg [60]. In addition, fetal Hb has greater affinity for CO than maternal Hb, the half-life of carboxyhemoglobin (COHb) in fetal blood is three times longer than that of maternal blood, and the fetus has a higher rate of oxygen consumption than mothers. Thus, maternal COHb concentration has a great influence on the fetus' COHb since CO crosses the placenta by simple diffusion and binds to Hb forming COHb in the red blood cell stroma [61]. Wouters et al (1987) examined fetal outcomes and HbCO levels in the umbilical cords of 77 uneventful pregnancies. They found that HbCO levels were significantly elevated in the venous cord blood of children of smokers compared to non-smokers [62]. This research provides evidence for biological mechanisms for smoking or ambient CO exposure on LBW.

1.6   AIR POLLUTION AND INFLAMMATORY MARKERS

Epidemiological studies suggest that exposure to higher levels of air pollution, especially particulate matter (PM) air pollution, is associated with increased cardiovascular and pulmonary mortality and morbidity. Studies have investigated the underlying pathophysiology mechanisms, and one hypothesis is that inhaled particulates lead to pulmonary inflammation, which causes the

release of inflammatory mediators into the bloodstream. These mediators may influence hemostasis and increase blood coagulation, therefore, increasing the risk of cardiopulmonary disease. Markers of inflammation, such as interleukin-6 (IL-6), interleukin-8 (IL-8), tumor necrosis factor-α (TNF-α), C-reactive protein (CRP), fibrinogen, and white cell counts, have been studied to test this hypothesis (table4). CRP, an acute-phase protein and a sensitive marker of inflammation and infection, has demonstrated the strongest biomarker response to air pollution exposure and the inflammation response in humans.

Seaton et al. (1999) reported a negative association between PM_{10} exposure and fibrinogen concentration. They also observed a 147% (95%CI=20%-477%) increase in CRP for an average 3 days per 100 $\mu g/m^3$ increase in PM_{10} exposure [63]. Peter et al. (2001) reported on the impact of SO_2, CO, and total suspended particulates (TSP) air pollution on CRP in a sample of 631 men aged from 45 to 64 years [64]. They observed that the odds of increasing CRP levels were associated with both SO_2 and TSP, but not CO; however, the effects for TSP were generally stronger than SO_2. They also found that estimates from the 5 days mean exposure had slightly stronger effects on CRP, which suggest a 5 day cumulative effect of particles on CRP. The odds ratio for observing a CRP concentration above the 90th percentile (above 5.7 mg/l) for a 5 day mean of TSP (increase of 26 $\mu g/m^3$) and SO_2 were 1.46 (95%CI=1.17-1.82) and 1.24 (95%CI=1.03-1.49), respectively. Another study examined the relationship between particulates air pollution and inflammatory markers among 710 healthy men [65]. The study found a positive association between CRP and particulate air pollution, and presented that a 4.36% (95%CI=-3.25 to 11.96) increase in CRP was associated with a one standard deviation (SD) increase in 4 weeks $PM_{2.5}$ exposure.

Children, elderly, and sick people are more susceptible to the effects of air pollution exposure. Children and infants are particularly sensitive as their total lung volume is so much smaller, increased respirations result in greater exposure. Similarly, pregnancy is a period of dramatic changes in maternal anatomy, physiology, and metabolism to support the development of the fetus which may be more susceptible to air pollution. Pope et al. (2004) studied the PM's influence on cardiac autonomic function and markers of inflammation among 88 elderly subjects (age between 54-89 years) [66]. They found that $PM_{2.5}$ was not significantly associated with white or red blood cell counts, platelets, or blood viscosity, but they observed a 100 μg/m^3 increase in $PM_{2.5}$ was associated with a 0.81 mg/dL increase in CRP. Another study examined the effects of air pollution and inflammation response by studying 158 asthmatic and 50 non-asthmatic children aged from 6 to 14 years. They also reported that $PM_{2.5}$, NO_2, and O_3 were associated with inflammatory responses in both asthmatic and non-asthmatic children [67]. Two studies examined air pollution and inflammation markers by studying the participants with coronary heart disease or with a history of coronary heart disease [68,69]. They both have consistent findings of positive associations between concentration of biomarkers and air pollution exposure.

Recently, two large studies evaluated the effect of exposure to air pollution on inflammatory markers in healthy individuals. Although one study evaluated short term exposure and the other evaluated long term exposure, both reported no significant correlations between air pollution and inflammatory markers. This result may indicate that long term exposure to outdoor air pollution is not associated with cardiovascular risks, which is mediated by chronic systemic inflammation [30,31].

A recently study by Williams et al. (2009) examined the proximity to traffic and inflammation among 115 postmenopausal overweight women [70]. This study hypothesized that

living near major roads would be associated with increased inflammation. However, they did not find any increase risk of inflammation for those women who lived within 150 m of major roads. The exposure assessment in this study relied on the distance to the roads and assumed the same amount of exposure every day. This may not capture background air pollution or personal exposure, which may account for the null findings.

1.7   C-REACTIVE PROTEIN DURING PREGNANCY

CRP is an acute phase protein generated by the liver, and its levels in the blood can rise up to 1000-fold in the presence of injuries, trauma and infection [71]. Obesity, estrogen use, smoking, race, and ethnicity have been reported to be associated with subclinical elevations in serum CRP [10,72-74], but the concentrations of CRP are generally low for people without infection. In addition, pregnant women have higher CRP concentrations than non-pregnant women [75]. The increased CRP concentrations during pregnancy may support the notion that pregnancy is associated with a pro-inflammatory state and reflect maternal response to inflammation (table5).   However, the exact etiology of this increase is still unknown.

Maternal CRP concentrations are raised as early as at 4 weeks gestation [75]. Larsson et al. (2008) report that the lower (2.5 percentile) and upper limits (97.5 percentile) of CRP between 7-17 weeks were 0.32-11.91 (mg/L) [76]. The study followed 52 healthy women and showed that CRPs increased as the number of gestational weeks increased and then dropped substantially (lower and upper limits were 0.23-7.60 mg/L) within 6 days postpartum. Fialova et al. (2006) present similar findings, and demonstrating that maternal CRP levels of pregnant women gradually increase during the 1st and 2nd trimesters ($p<0.05$) [77]. However, Cicarelli et al. (2005)

observed maximum CRP concentrations at 24 hours postpartum (5.8±3.6 for vaginal delivery (VD); 11±5.3 for Cesarean section delivery (CD)), which is inconsistent with Larsson et al.'s findings [78]. One possible explanation is that both studies collected blood samples at different time periods after delivery. One measured CRP within 6 days of postpartum while the other measured CRP at 24 hours postpartum. Besides, Cicarelli et al. stratified their analysis by delivery type (vaginal delivery vs. Cesarean section) and they also found that there is a difference in CRP between VD and CD (p=0.01) after 24 hours and after 60 hours.

Maternal serum CRP has been reported to be significantly higher than newborn's [79]. Hackney et al. (2008) examined the association between maternal and fetal variation in CRP genotype and maternal plasma CRP concentrations in the first trimester among 190 mother-baby pairs. They were unable to find relationships between maternal plasma CRP and CRP genotype, and suggest that clinical factors may have more influence on maternal CRP concentrations.

1.8   CRP AND PRETERM DELIVERY

Preterm birth (defined as gestational weeks <37) is the leading cause of infant morbidity and mortality [4]. The incidence of preterm birth was 12.5 percent in 2004 in the U.S. and still increased today. One of the reasons however, is the increase in multiple births, increase in elective C-section and other medical interventions in addition to personal risk factor such as maternal age at delivery. Although significant efforts to prevent preterm birth have been made, poor understanding of underlying pathophysiology is one of the factors hampering its prevention and treatment. It has been suggested that intrauterine infection may play an important role in developing preterm birth [5,6], and low grade inflammation during pregnancy has been related to

preeclampsia and preterm birth [7-13] (table6). Pitiphat et al. (2005) examined the association between maternal plasma CRP concentrations in early pregnancy (5.3-19.3 weeks' gestation) and the risk of preterm delivery [11]. They reported an odds ratio of preterm delivery for a CRP concentration of at least 8 mg/L (vs. <8 mg/L) was 2.55 (95%CI=1.05-6.20), and the odds ratio of preterm delivery for a CRP concentration of at least 12 mg/L (vs. <12 mg/L) was 3.19 (95%CI=0.98-10.32) after controlling for gestational age at blood collection, gravidity, pregnancy BMI, genitourinary infection, maternal employment, annual household income, physical activity, and alcohol consumption during the first trimester. Subsequent studies report similar findings.

Lohsoonthorn et al. (2007) conducted a nested case-control study to examine the relationship between maternal early serum CRP (13 weeks' gestation) and preterm delivery in 146 preterm cases and 1623 term delivery controls. They presented the odds ratio of preterm delivery for highest quartile (≥7.5 vs. <2.0 mg/L) of CRP was 2.04 (95%CI=1.13-3.69) [80]. Catov et al. (2007) also reported 2.6- to 2.8-fold increase risks of preterm delivery for early pregnancy inflammation (CRP ≥ 8 µg/ml) [81]. This study also examined the relationship between dyslipidemia, inflammation and spontaneous preterm birth. They presented an independent effect of inflammation and dyslipidemia on the risk of spontaneous preterm birth. However, the study conducted by Ghezzi et al. (2002) was unable to find any association between CRP concentrations and preterm delivery [82]. The study concluded that maternal blood CRP concentrations were not associated with preterm delivery. However, they reported that women with preterm delivery at <34 weeks or <37 weeks had a higher median of amniotic fluid CRP concentrations than women with term delivery. Moreover, Boggess et al. (2005) conducted a nested case-control study among 44 cases (spontaneous abortion or fetal loss < 21 weeks

gestation) and 88 controls (full-term births), and reported a decrease risk of pregnancy loss (OR=0.20, 95%CI=0.06-0.65) for women with CRP concentrations greater than the 75th percentile [83].

1.9   SUMMARY

Over the last 15 years, numerous studies in the United States and elsewhere have reported a positive relation between air pollution and birth outcomes such as low birth weight, preterm birth, and intrauterine growth retardation. Most studies examined the association between air pollution and birth outcomes showed that ambient air pollution seems to play some role in determining birth weight or gestational week. However, the differences in study outcomes and pregnancy periods studied result in the studies undergoing continued debate [28]. One reason for this debate is because unknown biological mechanism of air pollution effects on birth outcomes.

Estimating environmental exposure to ambient air pollution is another challenge in epidemiology studies. Most of the studies rely on crude methodologies obtained from air quality monitoring data by sampling networks and assigning a crude estimate of exposure in large population areas such as cities, counties, census blocks, and postal ZIP code areas [50]. These types of ecological studies ignore the location of pollutant sources and the atmospheric influence on concentrations of pollutants. As a result, these ecological studies poorly identify the mothers as exposed or unexposed. Recently, many studies have incorporated modeling techniques, such as the environmental transport model and/or GIS spatial modeling techniques to improve the exposure assessment method and to reduce the misclassification present in previous studies, which assigned a crude estimate of exposure to large population.

Preterm birth is an important issue in reproductive health because of its influences on the fetus' health outcomes in later life. Environmental exposure may play a role in the risk of reduced birth weight and gestational period, especially air pollution exposure. Although many epidemiological studies have linked air pollution exposure to preterm birth, the evidence on plausible biological mechanisms is still unclear. It has been suggested that intrauterine infection may play an important role in developing preterm birth [5,6], and low grade inflammation during pregnancy has been related to preeclampsia and preterm birth [7-13]. CRP, proinflammatory cytokines interleukin (IL)-1, and IL-6 are the most widely studied biomarkers of inflammation [84]. Although CRP is one of the major acute-phase proteins in humans [85], it has been shown to predict cardiovascular diseases [86,87] and has been found to be associated with particulate matter (PM) air pollution [63,64,66,88]. Thus, the results of my study which examines the association between air pollutant exposure and inflammation during pregnancy may provide evidence of biological mechanisms by which in air pollution exposure in pregnancy results in adverse outcomes.

1.10   TABLES

Table 1. Ambient air pollution and preterm delivery

Author	Study Population	Outcomes definition	Air Pollution Studied	Exposure Assessment	Effect Period	Outcomes	Controlling Factors
Ritz et al. (2000)	A cohort of 97,518	• Vaginally delivered preterm births • C-Section delivered preterm births • Excluding subjects considered SGA or LBW • Restricting preterm births to SGA or LBW children	CO, PM_{10}, NO_2, and ozone	ZIP code within 2 miles of monitoring stations	• 6 weeks before delivery • First month of pregnancy	Results from crude model: A 20% increase in preterm birth per 50-µg increase in ambient PM_{10} levels over 6 weeks before delivery (RR_{crude}=1.20; 95%CI=1.09-1.33), and a 16% increase risk of preterm delivery over the first month of pregnancy (RR_{crude}=1.16; 95%CI=1.06-1.26).	Gender, prenatal care, parity, time to previous livebirth≥12 months, maternal race, education, age, tobacco use during pregnancy and pregnancy history
Wilhelm and Ritz (2005)	146,972 births	Vaginal birth <37 completed weeks gestation	CO, PM_{10}, $PM_{2.5}$, NO2, and ozone	ZIP code and personal level/various distance to monitoring stations	• 6 weeks before delivery • First month of pregnancy	Results from single pollutant model: A 26% increase in preterm birth for exposure to greater than 2.1 ppm of CO 6 weeks before delivery ($RR_{adjusted}$=1.26; 95%CI=1.03-1.55), Results from multi-pollutant model: A 27% increase risk for high (≥ 75th percentile) first trimester CO exposure.	Infant sex, maternal age, race, and education, interval since previous live birth, previous LBW or preterm birth, level of prenatal care, birth season, and parity

Table 1 (continued)

Ritz et al. (2007)	58,316 birth cohort and 2,543 women	<37 weeks of gestation	**CO,** NO$_2$, O$_3$ and PM$_{2.5}$	ZIP code level/ nearest monitoring stations	6 weeks before delivery	Results from crude model: A 12% increase in preterm birth with higher CO exposure ($\geq$ 0.91 ppm) during the last 6 weeks of pregnancy.	Birth season, parity, and mother's age, race, and education
Huynh et al. (2006)	10,673 preterm births and 32,019 term births	Between 24 and 36 weeks' gestation	CO and **PM$_{2.5}$**	5 miles of PM monitors	Entire pregnant period	PM$_{2.5}$ exposure (>22.1 μg/m^3) was associated with the effect on preterm birth (adjusted odds ratio=1.15, 95% CI, 1.07-1.24).	Maternal age, race, maternal education, marital status and parity
Bobak et al. (2000)	A cohort of 108,173	<37 weeks of gestation	**SO$_2$, TSP, NO$_x$**	District-base/ mothers living within the same area as monitoring stations	In the first trimester	Results from single pollutant model: The adjusted ORs per 50 μg/m^3 increase during the first trimester were 1.27 (95%CI=1.16-1.39) for SO$_2$; 1.18 (95%CI=1.05-1.31) for TSP; and 1.10 (95%CI=1.00-1.21) for NO$_x$.	Sex, parity, maternal age group, education, marital status and nationality, and month of birth
Xu et al. (1995)	25370 women	<37 weeks of gestation	**SO$_2$, TSP**	Four residential areas of Beijing/ mothers living within the same area as monitoring stations	Entire pregnant period	Results from single pollutant model: The adjuster ORs per one log-transform unit increase in SO2 was 1.21 (95%CI=1.01-1.46) and the adjusted ORs per 100 μg/m^3 increase in TSP was 1.10 (95%CI=1.01-1.20).	Quintiles of temperature, quintiles of humidity, day of the week, season, residential area, maternal age, and gender of child
Liu, et al. (2003)	229,085 singleton live births	<37 weeks of gestation	**SO$_2$, NO$_2$, CO** and O$_3$	Provincial census subdivision/ proximity to air monitoring stations	Last month of pregnancy	Results from multi-pollutant model: Exposure to SO$_2$ and CO in the last month of pregnancy was associated with increased risk of preterm birth (OR=1.09, 95%CI=1.01-1.20 for SO$_2$; OR=1.08, 95%CI=1.00-1.20 for CO).	Maternal age, parity, infant sex, birth weight, and season of birth

SGA: small for gestational age; LBW: low birth weight; TSP: total suspended particles

Table 2. Results of studies of ambient air pollution and low birth weight

First author (year)	Outcome and exposure measures	Exposure categories	Odds ratio/changing in birth weight (g) (95% CI)
Rite (1999)	LBW and mean concentrations of CO in third trimester of pregnancy	Divided CO mean concentrations into three categories ($< 50^{th}$, 50 to $< 95^{th}$, $\geq 95^{th}$ percentile) $<50^{th}$ vs. $>95^{th}$ percentile	 1.22 (1.03-1.44)

Ha (2001)	LBW, and mean concentrations of CO, O_3, SO_2, NO_2, and TSP in first and third trimesters of pregnancy	1.Divided CO, O_3, SO_2, NO_2, and TSP mean concentrations into quartiles, each quartile increased
		   CO
		   SO_2
		   NO_2
		   TSP
		 2.Served pollutants as continuous variables
		   CO per 100 ppb increased
		   SO_2 per 1 ppb increased
		   NO_2 per 1 ppb increased
		   TSP per 50.0 $\mu g/m^3$ increased
			
			 1.08(1.04-1.12)
			 1.06(1.02-1.10)
			 1.07(1.03-1.11)
			 1.04(1.00-1.08)
			
			 11.55(8.99-14.10)
			 8.06(5.59-10.53)
			 8.41(6.07-10.76)
			 6.06(3.85-8.27)

Maisonet (2001)	LBW and mean concentrations of CO, PM_{10}, and SO_2 in each trimester of pregnancy	Divided CO, PM_{10}, and SO_2 mean concentrations into five categories ($< 25^{th}$, 25th to $< 75^{th}$, 75th to $< 95^{th}$, $\geq 95^{th}$ percentile)
		 $< 25^{th}$ vs $\geq 95^{th}$ percentile
		   CO
		   SO_2
		 Served pollutants as continuous variables
		   CO per 1 ppm increase
			
			 1.15(0.94-1.42)
			 1.06(0.76-1.47)
			
			 1.31(1.06-1.62)

Lee (2003)	LBW and mean concentrations of CO, SO_2, NO_2, and PM_{10} in each trimester and month of pregnancy	Divided CO, O_3, SO_2, NO_2, and PM_{10} mean concentrations into quartile for each quartile increase
		   CO
		   SO_2
		   NO_2
		   PM_{10}
			
			 1.05(1.01-1.09)
			 1.14(1.04-1.24)
			 1.04(1.00-1.08)
			 1.06(1.01-1.10)

Table 2 (continued)

Gouveia (2004)	LBW and mean concentrations of PM_{10}, CO, SO_2, NO_2 and O_3 in each trimester of pregnancy	1.Divided PM_{10}, SO_2, and O_3 mean concentrations into quartiles (< 25th, 25th to 50th, 51st to 75th , > 75th percentile)
		\<25th vs \>75th percentile
		    PM_{10}
		    CO
		2.Served pollutants as continuous variables
		  CO per 1.0 ppm increase
		  PM_{10} per 10.0 μg/m^3 increase
			
			1.14(0.87-1.49)
			1.02(0.82-1.27)
			
			-23.1(-41.3 to -4.9)
			-13.7(-27.0 to -0.4)

Wilhelm (2005)	LBW, preterm births, and mean concentrations of CO, PM_{10}, and $PM_{2.5}$, in third trimester of pregnancy	Divided CO, PM_{10}, and $PM_{2.5}$ mean concentrations into three categories (< 25th, 25th to < 75th, and ≥ 75th percentile) Served pollutants as continuous variables CO per 1 ppm increase PM_{10} ≥ 45.1 μg/m^3	 1.12 (1.05-1.19) 1.12 (0.91-1.38)

[a] infants who are below the 10th percentile of birth weight for gestation week

Table 3. Ambient air pollution and intrauterine growth retardation (IUGR)

Author	Study Population	Outcomes definition	Air Pollution Studied	Exposure Assessment	Effect Period	Outcomes	Controlling Factors
Bobak et al. (2000)	A cohort of 108,173	<10th percentile of birth weight for gestational age and sex (SGA)	**SO$_2$, TSP, NO$_x$**	District-base	NA	No significant associations between pollutant concentrations and IUGR.	Sex, parity, maternal age group, education, marital status and nationality, and month of birth
Liu, et al. (2003)	229,085 singleton live births	<10th percentile of birth weight for gestational age and sex (SGA)	**SO$_2$, NO$_2$, CO and O$_3$**	Provincial census subdivision/ proximity to air monitoring stations	First month of pregnancy, first and second trimester	Results from single pollutant model: First month of pregnancy exposure to SO$_2$ (OR=1.07, 95% CI=1.01-1.13, for a 5.0 ppb increase), NO$_2$ (OR=1.05, 95% CI=1.01-1.10, for a 10 ppb increase) and CO (OR=1.06, 95% CI=1.01-1.10, for a 1.0 ppm increase) was associated with IUGR.	Maternal age, parity, infant sex, birth weight, and season of birth

SGA: small for gestational age
LBW: low birth weight
TSP: total suspended particles

Table 4. Research on air pollution and inflammation markers

Author	Study Population	Exposure Assessment	Markers	Air Pollutants	Results	Adjusted Variables	Including Current Smoking
Steinvil et al. (2008)	3,659 healthy subjects (exclude pregnancy women)	Within 11 km of the nearest monitoring station (short-term exposure)	WBC, Fibrinogen, and hs-CRP	PM_{10}, SO_2, NO_2, CO, and O_3	No significant correlations were found between air pollution and inflammation markers.	age, waist circumference, body mass index (BMI), complete lipid profile including low-density lipoprotein (LDL), highdensity lipoprotein (HDL), and triglycerides, diastolic and systolic blood pressure measurements, glucose concentration, alcohol consumption, sport intensity etc.[a]	Yes
Brraza-Villarreal et al. (2008)	158 asthmatic and 50 non-asthmatic children	Nearest monitoring station (short-term exposure)	Fe_{NO}, pH of exhaled breath condensate, and IL-8	$PM_{2.5}$, NO_2, and O_3	$PM_{2.5}$, NO_2, and O_3 were associated with inflammatory responses in both asthmatic and non-asthmatic children.	sex, body mass index, previous day minimum temperature and chronological time	No (but including parental smoking)
Forbes et al. (2009)	about 25,000 adults with fibrinogen measurements and 17,000 adults with CRP measurements	Air dispersion model at postcode sector level (Long-term exposure)	Fibrinogen, and CRP	PM_{10}, SO_2, NO_2, and O_3	No significant associations between concentrations of fibrinogen or CRP and outdoor air pollution (PM_{10}, SO_2, NO_2, and O_3)	age, sex, body mass index (and their interactions), social class of head of household, cigarette smoking, region, month of nurse visit, and indoor temperature (only in 1998 and 2003)	Yes
Delfino et al. (2008)	29 nonsmoking elderly subjects with a history of coronary artery disease	Direct measure indoor and outdoor pollutants (short-term exposure)	CRP, Fibrinogen, TNF-α, sTNF-RII, IL-6, IL-6sR, fibrin D-dimer, sP-selectin, sVCAM-1, sICAM-1, and MPO	Outdoor: NO_2, CO, O_3, EC, OC, BC, OC_{pri}, OC_{sec}, and PN, $PM_{0.25-10}$ Indoor: NO_2, CO, EC, OC, OC_{pri}, OC_{sec}, PN, and $PM_{0.25-10}$	Positive association for CRP, IL-6, and sTNF-RII with PM ≤ 0.25 µm, EC, OC_{pri}, BC, PN, CO and NO_2.	NA	No

Table 4 (continued)

Rückerl et al. (2006)	57 male patients with coronary heart disease	Based on one fix air monitoring station (short-term exposure)	CRP, SAA, Factor VII, vWf, Fibrinogen, prothrombin fragments 1+2, D-dimer, ICAM-1, and E-selectin	UFPs, AP, $PM_{2.5}$, PM_{10}, OC, EC, NO_2, and CO	CRP and ICAM-1 were associated with ambient air particles. The odds ratio for observing a CRP concentration above 90th percentile (above 8.5 mg/l) for a 5 days mean of PM_{10} (interquartile range) was 2.0 (95%CI=1.20-3.70).	NA	No
Williams et al. (2009)	115 postmenopausal, overweight women	Distance to major road	CRP, SAA, IL-6, NK cell cytotoxicity, and T-lymphocyte proliferation	Traffic related air pollution	CRP, SAA, IL-6, and lymphocyte proliferation did not differ according to proximity to major roads.	age, BMI, education (individual-level), season of enrollment, time spent outdoors, and median income in the census tract of residence.	No
Pope et al. (2004)	88 elderly subjects	Nearest monitoring stations from several sites (short-term exposure)	CRP, blood cell counts, and whole-blood viscosity	$PM_{2.5}$	Positive association for CRP and $PM_{2.5}$ (A 100 µg/m^3 was associated with a 0.81 mg/dL increase in CRP.)	Temperature and relative humidity	No
Peters et al. (2001)	631 healthy men	Based on one fix air monitoring station (short-term exposure)	Hs-CRP	TSP, CO, and SO_2	The odds ratio for observing a CRP concentration above 90th percentile (above 5.7 mg/l) for a 5 days mean of TSP (increase of 26 µg/m^3) and SO_2 were 1.46 (95%CI=1.17-1.82) and 1.24 (95%CI=1.03-1.49), respectively.	Age, BMI, smoking, HDL, temperature, and humidity	Yes
Seaton et al. (1999)	112 healthy people	Both personal exposure estimate based on air monitoring data with diary, and fix air monitoring station	Haemoglobin, packed cell volume, red and white cell counts, platelets, IL-6, Fibrinogen, Factor VII, and CRP	PM_{10}	The mean change of CRP for a 3 days mean of PM_{10} (per 100 µg/m^3 increase) was 147% (95%CI=20%-477%).	City, season, temperature, and repeated individual measurements	No

Table 4 (continued)

Zeka et al. (2006)	710 healthy men	Two fix monitoring stations	CRP, fibrinogen, and sediment rate	PN, BC, $PM_{2.5}$, and SO_4^{2-}	Positive association for CRP and particulate air pollution. A 7.29% (-1.63-16.21) increase of CRP was associated with one SD increase of 48 hours PN. A 4.36% (95%CI=-3.25 to 11.96) increase of CRP was associated with one SD increase of 4 weeks $PM_{2.5}$.	Age, BMI, season, temperature, humidity, and barometric pressure	Yes

WBC: white blood cell; **hs-CRP**: high-sensitivity C-reactive protein; **Fe$_{NO}$** : fractional exhaled nitric oxide; **IL-8**: interleukin-8; **TNF-α** : tumor necrosis factor-α ; **sTNF-RII**: TNF-α soluble receptor-II; **IL-6:** interleukin-6; **IL-6sR:** IL-6 soluble receptor; fibrin D-dimer; **sP-selectin**: soluble platelet selectin; **sVCAM-1**: soluble vascular cell adhesion molecule-1; **sICAM-1:** intracellular adhesion molecule-1; **MPO:** myeloperoxidase; **SAA**: serum amyloid A; **vWF**: von Willebrand factor antigen; **ICAM-1**: intercellular adhesion molecule 1; **NK**: Natural Killer

PM$_{10}$: particles diameter less than 10 μm; **SO$_2$**: sulfur dioxide; **NO$_2$**: nitrogen dioxide; **CO**: carbon monoxide; **O$_3$**: ozone; **PN**: total particle number; **EC**: fine PM elemental carbon; **OC**: organic carbon; **SOA**: estimated secondary organic aerosol; **OCpri**: primary OC from total OC; **OCsec**: secondary OC from total OC**; BC**: black carbon; **UFPs**: ultrafine particles (number concentration of particles with a size range of 0.01 to 0.1 _m in diameter); **AP**: accumulation mode particles (particles with a size range of 0.1 to 1.0 _m); **TSP**: total suspected particulates

[a] also adjusted for medications including aspirin, beta blockers, calcium channel blockers, angiotensin converting enzyme inhibitors, angiotensin II receptor blockers, statins, fibrates, oral hypoglycemics or insulin, and oral contraceptives or hormonal replacement therapy for women, cardiovascular risk factors including current and past smoking status, and family history of coronary heart disease or personal history of proven atherothrombotic event

Table 5. CRP during pregnancy

Author	Study Population	Period/Time of CRP measurement	Results	Adjusted Variables	Including Current Smoking
Cicarelli et al. (2005)	24 healthy pregnant women (full term), 12 women undergoing vaginal delivery (VD), and 12 women undergoing Cesarean section (CD)	• Admission • Delivery • 24 hours after delivery • 60 hours after delivery	• A difference of CRP between VD and CD (p=0.01) were found after 24 and 60 hours. • The maximum of CRP was observed at 24 hours postpartum (5.8±3.6 for VD; 11±5.3 for CD). • CRP was significantly higher in maternal serum than in newborn's (p<0.001).	NA	NA
Fialova et al. (2006)	86 pregnant women in the 1st trimester; 102 pregnant women in the 2nd trimester; 26 non-pregnant women	• First trimester • Second trimester	• Maternal CRP levels of pregnant women gradually increased during the 1st and 2nd trimesters (p<0.05). • Maternal CRP levels were higher in pregnant women than in non-pregnant women (p<0.05).	NA	NA
Hackney et al. (2008)	190 mother-baby pairs	• Maternal CRP genotype in the first trimester • Fetal CRP genotype • Maternal plasma CRP in the first trimester	• There was no significant relationship between maternal first trimester plasma CRP concentration and maternal or fetal CRP genotype.	Clinical variables include: BMI, smoking, race, parity and age	Yes (55% tobacco use)

Table 5 (continued)

Larsson et al. (2008)	52 healthy women	• 7-17 weeks gestation • 17-24 weeks gestation • 24-28 weeks gestation • 28-31 weeks gestation • 31-34 weeks gestation • 34-38 weeks gestation • Pre-delivery	• The lower (2.5 percentile) and upper limits (97.5 percentile) for CRP between 7-17 weeks were 0.32-11.91 (mg/L). • CRPs between 17-24 weeks were 0.40-14.02. • CRPs between 24-28 weeks were 0.43-20.28. • CRPs between 28-31 weeks were 0.43-36.98. • CRPs between 31-34 weeks were 0.33-11.92. • CRPs between 34-38 weeks were 0.64-28.26. • Prepartum CRPs were 0.38-24.75. • Postpartum CPRs were 0.23-7.60.	NA	Yes (including two smokers)
Picklesimer et al. (2008)	775 pregnant women	<26 weeks of gestation (median=14 weeks)	• Black women had higher median CRP concentrations than white women (7.68 vs. 2.59 mg/L; P<0.001).	Smoking and maternal weight	Yes (17% smoking during pregnancy)
Sacks et al. (2004)	40 pregnant women; 95 non-pregnant women	4 weeks gestation	• A mild systemic maternal inflammatory response was found in 4 weeks gestation.	NA	Yes

Table 6. CRP and preterm delivery

Author	Study Population	Period/Time of CRP measurement	Study Design	Results	Adjusted Variables	Including Current Smoking
Boggess et al. (2005)	44 cases (spontaneous abortion or fetal loss < 21 weeks gestation); 88 controls (full-term)	< 21 weeks gestation	Nested case-control	• Pregnancy is not associated with increase CRP concentrations. • Women with CRP levels greater than 75% percentile had a decrease risk of pregnancy loss (OR=0.20, 95%CI=0.06-0.65)	Maternal smoking, gestational age at blood draw, and insurance status	Yes
Catov et al. (2007)	109 cases (<34 and 34-37 gestational weeks) and 228 controls	< 21 weeks gestation	Nested case-control	• A positive linear relationship between CRP and risk of sPTB at 34-37 weeks. • Early pregnancy inflammation (CRP ≥ 8 µg/ml) increased risk of sPTB by 2.6- to 2.8-fold.	Race, BMI, periconceptional multivitamin use, and gestational age at sampling	Yes
Lohsoonthorn et al. (2007)	146 cases (<34 and 34-36 gestational weeks) and 1623 controls	13 weeks of gestation	Nested case-control	• The odds ratio of preterm delivery for highest quartile (≥7.5 vs. <2.0 mg/L) of CRP was 2.04 (95%CI=1.13-3.69).	Maternal age, race, parity, pregnancy BMI, and gestational age at blood collection	Yes
Pitiphat et al. (2005)	117 cases (delivered preterm, <37 weeks' gestation); 117 controls	5.3-19.3 weeks' gestation	Nested case-control	• The odds ratio of preterm delivery for a CRP level of at least 8 mg/L (vs. <8 mg/L) was 2.55 (95%CI=1.05-6.20). • The odds ratio of preterm delivery for a CRP level of at least 12 mg/L (vs. <12 mg/L) was 3.19 (95%CI=0.98-10.32).	Gestational age at blood collection, gravidity, pregnancy BMI, genitourinary infection, maternal employment, annual household income, physical activity, alcohol consumption during the first trimester	Yes (cases and controls were matched on age, race, and smoking status)
Ghezzi et al. (2002)	280 term delivery; 10 delivery <34 weeks; 16 delivery between 34 and 37 weeks	15-18 weeks gestation	Cohort study	• Women with preterm delivery at <34 weeks or <37 weeks had a higher median of amniotic fluid CRP concentrations than women with term delivery. • Amniotic fluid CRP and maternal blood CRP concentrations were not correlated. • Maternal blood CRP concentrations were not associated with preterm delivery.	NA	NA

2.0   SPECIFIC AIMS

The specific aims and related hypotheses are:

Specific Aim 1: Evaluate the associations between ambient air pollution (including CO, NO_2, SO_2, O_3, $PM_{2.5}$ and PM_{10}) and inflammation by measuring the CRP concentrations, during early pregnancy (gestational weeks before 22 complete weeks). We hypothesize that maternal exposure to short term (up to 1 week before blood draw) and long term (1 month before blood draw) ambient air pollutants in the early period of pregnancy is associated with inflammation.

Specific Aim 2: Evaluate the associations between smoking and inflammation and its relationship with preterm birth (gestational weeks <37). We hypothesize that maternal exposure to cigarette smoke during pregnancy is associated with inflammation, as well as increased CRP concentrations.

Specific Aim 3: Evaluate the associations between ambient air pollution and blood pressure changes. We hypothesize that maternal exposure to ambient air pollution during early pregnancy is associated with increase mean systolic and diastolic blood pressure from early to later pregnancy.

3.0 PARTICULATE AIR POLLUTION EXPOSURE AND C-REACTIVE PROTEIN DURING EARLY PREGNANCY

3.1 ABSTRACT

BACKGROUND: While it is not well understood how air pollution leads to adverse pregnancy outcomes, it has been reported that high concentrations of C-reactive protein (CRP), a biomarker of systemic inflammation, increase the risk of preterm delivery. Here, we examine whether air pollution influences serum concentrations of CRP in early pregnancy in Allegheny County, PA.

METHODS: In 1,696 women, we measured CRP concentrations in blood collected before the 22nd week of gestation from 1997-2001. We estimated levels of particles of less than 10 μm (PM_{10}) and less than 2.5 μm diameter ($PM_{2.5}$), carbon monoxide (CO), nitrogen dioxide (NO_2), sulfur dioxide (SO_2), and ozone at the maternal ZIP code using Kriging interpolation for ambient station measures. Employing logistic regression we evaluated associations between air pollution and high CRP (≥ 8 ng/ml) concentrations.

RESULTS: For non-smokers, we observed odds ratios of 1.41 (95% CI=0.99 – 2.00) and 1.47 (95% CI=1.05 – 2.06) for high CRP concentrations per 9.2 and 4.6 $\mu g/m^3$ increase in PM_{10} and $PM_{2.5}$ respectively averaged over 28 days prior to the blood sample; and an odds ratio of 1.49 (95% CI=0.75 – 2.96) per 7.9 ppb increase in ozone during summer. We saw no associations in smokers or for other air pollutants and no evidence for effect measure modification by obesity.

CONCLUSIONS: PM_{10}, $PM_{2.5}$, and ozone exposures increase the odds of high CRP in early pregnancy suggesting that these air pollutants contribute to inflammation; thereby possibly affecting pregnancy outcomes adversely.

3.2   BACKGROUND

Numerous studies have linked ambient air pollution to adverse pregnancy outcomes, such as preterm birth, low birth weight (LBW), small for gestational age (SGA, a marker of intrauterine growth retardation (IUGR)), and a few investigated preeclampsia, perinatal mortality and cardiac birth defects. [26,28,29,89-92] Positive associations have been described most consistently for increased preterm birth and particulate matter exposures especially for exposures during the first and third trimester of pregnancy. [93] Some studies relying on measures of traffic density suggested that motor vehicle exhaust may be an important source for particulates. [94,95] The biologic mechanisms explaining air pollution effects on pregnancy outcomes, however, are not well understood.

Inflammation is one pathway believed to be involved in particulate-induced adverse health outcomes including cardiovascular diseases, [96,97] and has also been hypothesized to influence birth outcomes. [52] Animal studies provide strong evidence that particulate exposure can cause localized inflammation. Both particles of less than 10 μm diameter (PM_{10}), and less than 2.5 μm diameter ($PM_{2.5}$) elicit pulmonary inflammation in rats. [97,98] Similarly, exposure to diesel exhaust particles (DEP) and ultrafine carbon particles have been reported to induce an inflammatory reaction in the lung of mice. [99] Growing epidemiologic evidence suggests that exposure to air pollution, especially particulates, is associated with not only pulmonary

inflammatory and immune responses but also with systemic inflammation measured by increased concentrations of inflammatory markers, such as interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), C-reactive protein (CRP), fibrinogen, and white cell counts. [64,65,67-69] C-reactive protein is a sensitive marker of inflammation and infection. [71] Previous studies indicated positive associations for CRP and air pollution in different populations such as healthy men, the elderly or people with coronary heart disease. [64,65,68,69,100]

Yet, to our knowledge no studies have evaluated whether air pollution exposure increases the systemic inflammatory response in pregnant women, of interest because the special state of immune response during pregnancy allows the mother to tolerate the semi-allogeneic foetus. [101] Particulate exposures during pregnancy have been hypothesized to induce acute placental inflammation. Alternatively systemic inflammatory responses may lead to alterations in maternal immunity, and in turn increase risk of adverse birth outcomes. [52] Here, we examine this hypothesis in 1,696 women enrolled in the Prenatal Exposures and Preeclampsia Prevention (PEPP) study, a cohort in which we previously demonstrated that inflammation (measured by CRP) during early pregnancy was associated with increased risk of preterm delivery. [81] The aim of our current study was to determine whether particulate air pollution (including PM_{10} and $PM_{2.5}$) exposure contributes to increased C-reactive protein (CRP) concentrations above a threshold of 8 ng/ml during early pregnancy, at which concentration our previous results showed a strong excess risk of spontaneous preterm delivery at 34-<37 weeks (OR=2.9; 95% CI=1.1 – 7.2).

3.3　　　MATERIALS AND METHODS

3.3.1　Study Population

The study population was drawn from the Prenatal Exposures and Preeclampsia Prevention study (PEPP), which enrolled 2,211 women from clinics and private practices between 1997 and 2001. Briefly, this prospective study recruited healthy women, ages 14-44 and at less than sixteen weeks gestation and followed them to delivery. A comprehensive questionnaire was administered at baseline and postpartum. The information obtained in the baseline questionnaire included maternal demographic characteristics, socioeconomic status, active and passive cigarette smoking, consumption of alcohol, other lifestyle factors and medical history. We also obtained maternal residential ZIP code information at delivery from the hospital record. We excluded women with pre-existing medical conditions (n=37) including chronic hypertension, chronic diabetes, and HIV to avoid large variations in CRP levels due to these conditions. Since we were interested in inflammation in the first half of pregnancy, we also excluded women without a blood sample taken before 22 weeks of gestation (n=145). Additionally, since we limited our analysis to singleton births and first-time participants in the PEPP study with a maternal residence ZIP code in Allegheny County, PA, a total of 1,696 women were included in this study.

3.3.2 Biomarker Assessment

Maternal nonfasting blood samples were stored and frozen at -80 ^{0}C. We measured C-reactive protein (CRP) concentrations with a high-sensitivity enzyme-linked immunosorbent assay (ELISA) on the SpectraMax Me analyzer (Molecular Devices, USA), as described elsewhere. [102] The detection limit of the CRP assay was 0.03 ng/ml with an intra-assay variability of 7 percent.

3.3.3 Exposure Assessment

We obtained ambient air pollution data including the concentrations of carbon monoxide (CO), nitrogen dioxide (NO_2), sulfur dioxide (SO_2), ozone (O_3), PM_{10}, and $PM_{2.5}$ for Allegheny County and its neighboring counties (within 50 km of the Allegheny County boundary), from the Allegheny County Health Department (ACHD) and the Air Quality System (AQS) (http://www.epa.gov/ttn/airs/airsaqs/detaildata/downloadaqsdata.htm) of the Environmental Protection Agency (EPA) for the years 1996 to 2001. From 1999 to 2001, $PM_{2.5}$ data were collected at 23 monitoring stations including 13 monitoring stations in Allegheny County (AC) and 10 monitoring stations in its neighboring counties. PM_{10} measurements were available from 40 stations (18 monitoring stations in AC), SO_2 from 32 stations (7 monitoring stations in AC), and O_3 from 15 stations (3 monitoring stations in AC), while only 11 stations measured NO_2 and CO during the study period (3 and 2 monitoring stations respectively in AC). For each station, the data include one-hour concentrations for CO, NO_2, O_3, and SO_2; and 24-hour concentrations (some collected every day and others collected every 3rd or 6th day) for $PM_{2.5}$ and PM_{10}. Meteorologic variables including hourly temperature and daily relative humidity for the

Pittsburgh International Airport, Pittsburgh, PA monitoring station were obtained from the National Climate Data Center (http://www.ncdc.noaa.gov/oa/ncdc.html).

We employed the space-time ordinary Kriging (STOK) interpolation method to estimate daily air pollution concentrations at the ZIP code level by averaging the estimated concentration of pollution at each centroid of the grid (size of 13.4 m^2) within each ZIP code. Spatial and temporal variograms were fitted into a spherical semivariogram model separately based on temporally detrended residuals. In addition, we combined the spatial and temporal variograms to a space-time variogram by fitting a general product-sum model. [103] This modification of space-time ordinary kriging has been shown to increase mean precision compared to ordinary kriging. [104]

3.3.4   Statistical Analysis

We used logistic regression analyses to evaluate the associations between air pollution and C-reactive protein during early pregnancy. We dichotomized C-reactive protein concentrations at 8 ng/ml, a threshold we previously found associated with increased risk of preterm birth. [11,81] We also evaluated C-reactive protein as a continuous measure in linear regression models after log transformation necessary to normalize the CRP distribution. We present our results from these models as percentage changes. For all regression models, we used the robust cluster variance estimator to account for clustering of maternal residences in ZIP codes. For each air pollutant we employed continuous (per interquartile range, IQR) pollutant measures on the same day the blood was drawn (lag 0) and also up to 7 days prior to the blood draw (lag 1-lag 7) based on previous reports suggesting that systemic inflammation may increase 7 days preceding a blood draw. [63,64,100] In addition, we also calculated mean pollutant concentrations for three additional

periods prior to the blood draw (i.e. 8-day averages (Day 0-7), 22-day averages (Day 0-21), and 29-day averages (Day 0-28 before blood collection)) to allow evaluating chronic, longer term, and cumulative exposure effects.

In all models, we controlled for gestational week in which the sample was collected (weeks), maternal body mass index (BMI) at enrollment/time of blood draw (kg/m^2), maternal age (years), maternal race (White, African-American), maternal education (less than high school, high school, complete high school but did not complete college, complete college or greater), parity (first birth, second or subsequent birth), maternal cigarette exposure during early pregnancy (active smoker, non-active smoker but exposed to cigarette smoke at home or indoors elsewhere, non-active and non-passive smoker), household income (< 10,000, 10 - < 20,000, 20 - < 50,000, 50,000 or more), season of sample collection (spring, summer, fall, winter), and year entering the study (for PM$_{10}$ and other pollutants spanning: 1997-2001; for PM$_{2.5}$: 1999-2001). We also evaluated other potential confounders including marital status, alcohol intake during pregnancy, multivitamin or prenatal vitamin use, aspirin use within a year prior to pregnancy, employment, public assistance, daily mean temperature and relative humidity. Since these factors did not change the estimates for pollutants by more than 10 percent they were not included in the models we present here. [105]

Previous studies suggested that obesity and smoking status may modify the influence that air pollution has on inflammation. [65,100,106,107] Thus, we also conducted stratified analyses for obesity defined as BMI $\geq$ 30 kg/m^2 and analyses for non-smokers only. In addition, sensitivity analyses were performed such that we only included non-smokers without environmental tobacco smoke exposure (ETS in non-active smokers reporting exposure to cigarette smoke at home or indoors elsewhere). Only one monitoring station measured ozone between October and

March in the study period; thus, the analysis for ozone was restricted to the summer months (April-September) when data were available for more stations.

3.4   RESULTS

Blood samples were collected at a mean of 10.2 (std=4.0) gestational weeks. The mean BMI in early pregnancy (at baseline) was 26.5 kg/m^2 (std=6.7). In univariate and adjusted models, the crude odds (ORs) for elevated C-reactive protein concentrations (CRP $\geq$ 8 ng/ml) were higher in women for whom samples were collected later in gestation, who had higher BMI, were older, or of African-American race (Table 7), but not in women actively smoking or passively exposed to cigarette smoke during early pregnancy.

Table 8 summarizes means and correlations for mean pollutant concentrations 0 to 7 days preceding the blood collection; correlations were very similar for longer averaging periods, but means were slightly lower. Average 8-day PM_{10} concentrations were highly correlated with $PM_{2.5}$ (r = 0.9) and moderately with O_3 (r = 0.5), but very weakly correlated with CO, SO_2, and NO_2. Similarly, $PM_{2.5}$ was moderately correlated with O_3 (r = 0.5) but only weakly correlated with CO, SO_2, and NO_2.

Figure 1 and 2 represent the geographic distribution of study participants by race and ZIP code. Most of the African-American women lived in the inner city area while the majority of the White race participants lived in relatively urban areas. Figure 3 and 4 show the distribution of PM_{10} and $PM_{2.5}$ respectively by ZIP code. The mean PM_{10} exposure concentrations (8-day averages) for White race is slightly higher than the exposure concentrations for African-American (White: 25.9 $\mu g/m^3$, SD=8.3; African-American: 26.2 $\mu g/m^3$, SD=8.2). Similarly, we

also observed that African-American had higher 8-day mean exposure concentrations of $PM_{2.5}$ than White race women (African-American: 16.8 $\mu g/m^3$, SD=5.3; White: 16.0 $\mu g/m^3$, SD=5.2).

3.4.1 Ambient air pollution and C-reactive protein

Longer averaging periods, i.e., 0-21, and 0-28 day averages for PM_{10} increased the odds of having a CRP concentration above 8 ng/ml (Table 9). For an IQR increase in PM_{10} the odds ratios (ORs) of a CRP above 8 ng/ml were 1.23 (95% CI=0.97 – 1.57; 22-day average) and 1.18 (95% CI=0.91 – 1.53; 29-day average) in adjusted single-pollutant models for the entire population. $PM_{2.5}$ exposures also increased the ORs for high CRP concentrations when using longer averaging periods; i.e. the ORs were 1.32 (95% CI=1.05 – 1.67; 22-day average) and 1.26 (95% CI=0.97 – 1.63; 29-day average) per IQR increase in adjusted single-pollutant models (Table 9). When we restricted our analyses to non-smokers only, effect estimates were generally larger for both PM_{10} and $PM_{2.5}$. Per IQR increase in PM_{10} exposure the ORs for elevated CRP concentrations were 1.47 (95% CI=1.06 – 2.02; 22-day average) and 1.41 (95% CI=0.99 – 2.00; 29-day average) in adjusted single-pollutant models for non-smokers, and per IQR increase in $PM_{2.5}$ exposure they were 1.55 (95% CI=1.15 – 2.11; 22-day average) and 1.47 (95% CI=1.05 – 2.06; 29-day average) (Table 9).

Generally, effect estimates for both PM_{10} and $PM_{2.5}$ were larger in size between lag days 2 and 5. For non-smokers, a per IQR increase in PM_{10} exposure was associated with increased CRP during lag days 4 and 5, whereas $PM_{2.5}$ exposure was associated with increased CRP during lag days 1, 2, 4, and 5. However, none of these estimates achieved formal statistical significance. When we examined associations based on percentage change of log-transformed CRP in linear regression single-pollutant models 6-17% increases in CRP per IQR increase in PM_{10} or $PM_{2.5}$

were suggested for smokers but none of these results were formally statistically significant (Figure 5). For non-smokers, only a small 2% increase in CRP per IQR increase in $PM_{2.5}$ during a 22-day average period was suggested and 3-5% increases in CRP for an IQR increase in PM_{10} for longer time periods, i.e., 0-21, and 0-28 day averages. When we examined interactions between BMI and particulates, the 95% confidence intervals of the estimates included one (data not shown). No evidence of effect measure modification was observed by obesity; i.e., the effect estimates for particulates on CRP were similar in women with a BMI above 30 kg/m^2 and below 30 kg/m^2 in early pregnancy.

Positive associations were also observed between O_3 and CRP for all longer averaging periods (Table 9). Generally, the estimates for particulates (PM_{10} and $PM_{2.5}$) and O_3 air pollution were slightly larger in size for non-smokers who in addition were not exposed to ETS. However, the exclusion of those with ETS exposure from the non-smoking group greatly reduced the sample size and precision of our estimates. For CO, SO_2 and NO_2 associations were null for both the entire population and non-smokers only (e.g., per IQR increase in CO 29-day average in adjusted single-pollutant models ORs=1.05, 95% CI=0.86 − 1.30 entire population; ORs=0.95, 95% CI=0.71 − 1.27 non-smokers only).

3.5   DISCUSSION

We observed positive associations between particulate (both $PM_{2.5}$ and PM_{10}) and O_3 air pollution and elevated concentrations of a systemic inflammatory biomarker, CRP, in non-smoking women during early pregnancy. These findings support the hypothesis that these exposures during pregnancy induce an inflammatory response which may result in an increased

risk for adverse birth outcomes especially preterm birth which we have previously shown to be associated with these higher CRP concentrations (above 8 ng/ml). [81]

To date, few studies have examined associations between air pollution and inflammation in pregnant women, even though this has been studied extensively in other susceptible populations such as the elderly and children. [68,69,100,107] We found primarily medium term particulate exposures (up to 28 days prior to blood collection) to be associated with CRP concentrations above 8 ng/ml which previous studies had linked to an increase in preterm birth risk [11,81]. However, due to insufficient sample size (99 preterm births among 1,129 non-smoking women), we did not have sufficient sample size to examine whether inflammation – specifically CRP plays a mediating role for the associations between air pollutants and adverse birth outcomes. Nevertheless, our findings that particulates and O_3 increase the odds of having elevated CRP concentrations in early pregnancy provide some insight into pathways through which air pollution may influence birth outcomes.

While the gaseous combustion related air pollutants CO, NO_2, and SO_2 have also been associated with adverse birth outcomes before, [26,89,93] we did not find associations with high C-reactive protein in our study. In Allegheny County, the major sources of CO, NO_2, and particles are motor vehicles and industrial plants. With the same sources of origin, we would expect particles, CO, and NO_2 to be positively correlated. However, only very weak correlations between our particle, CO, and NO_2 measures were observed. This is most likely due to a very small number of monitoring stations that measured CO and NO_2 (2 and 3 monitoring stations respectively) in all of Allegheny County. Since CO is highly spatially heterogeneous, i.e., its distributional peaks basically follow roadways, thus, data from only two monitoring stations will not represent these sources and their spatial distribution well enough. Our findings that

particulates were associated with inflammation indicate that particles from sources such as motor vehicles or industrial plants present important adverse exposures for pregnant women and that our spatial model based on a larger number of particulate monitoring stations likely captured particulate exposures from these sources better than the monitoring for gaseous combustion products does. However, unlike other gaseous pollutants ozone is spatially more homogenous and we expect the three monitoring stations combined with our spatial interpolation model to have represented ozone exposures appropriately.

Previous studies investigated various lag times for air pollution exposures and inflammatory markers and generally assumed effects to be acute and to occur within 7 days prior to sample collection. Only a few studies examined longer time periods (i.e., months or years). [30,65,106] Our results for particulate exposures are generally consistent with other studies. Zeka et al. (2006) reported that a 7.95 $\mu g/m^3$ increase in a 4-week average $PM_{2.5}$ resulted in a 4.4% (95% CI=-3.3 to 12.0%) increase in CRP among 710 healthy men. Similarly, we observed that a 5.0 $\mu g/m^3$ increase in 29-day average $PM_{2.5}$ prior to blood collection was associated with a 3.8% increase in C-reactive protein (95% CI=-6.7 to 15.6%) for the entire population. Rückerl et al. (2006) studied the influence of air pollution on markers of inflammation and coagulation among 57 men with coronary heart disease. For a CRP concentration greater than 8.5 mg/l, they reported an odds ratio of 1.4 (95% CI=0.9 – 2.3) for an IQR (12.2 $\mu g/m^3$) increase in 5-day average $PM_{2.5}$, and an odds ratio of 2.0 (95% CI=1.2 – 3.7) for an IQR (12.8 $\mu g/m^3$) increase in 5-day average PM_{10}. In this repeated measure panel study of males the estimated effect for $PM_{2.5}$ was in the range of our finding in non-smokers (OR= 1.17; 95% CI=0.90 – 1.52 for CRP $\geq$8 ng/ml per 5.8 $\mu g/m^3$ increase in $PM_{2.5}$ 8-day average); our estimated effect size for PM_{10} however was much smaller and did not suggest an association for this short averaging period.

We also found consistent positive associations between O_3 in summer (April to September) and high CRP levels. Exposure to ozone has been associated with airway as well as systemic inflammation. [67,108] O_3 has a strong oxidative potential and is likely to generate reactive oxygen and nitrogen species and increase inflammatory reactions. [109] Ozone exposures are associated with increased levels of interleukin-8 (IL-8) and interleukin-6 (IL-6) in asthmatic children and nonsmoking adults. [67,109] Recently, a longitudinal study[110] following 40 healthy individuals reported a 5.9% (95% CI=-6.8 to 18.7%) increase in CRP for a 42 $\mu g/m^3$ increase on lag day4 O_3 exposure, and this was similar to our finding (3.6% increase; 95% CI=-8.5 to 17.3 % on lag day4 per 14.0 ppb) in the summer months for non-smokers.

We found no indication that BMI modifies the associations between particulates and C-reactive protein in pregnant women; i.e. the sizes of our effect measures were similar for normal weight and obese women. Our results are contrary to previous findings that linked air pollution to markers of inflammation in elderly and healthy male populations and reported that associations for short-term air pollution and inflammation markers (i.e., CRP and IL-6) were more pronounced in obese individuals and those with diabetes or hypertension. [65,100] These differences may be due to the fact that we excluded pregnant women with pre-existing health conditions such as chronic hypertension and chronic diabetes and that our BMI measure was inaccurate since in our study weight at baseline was measured as early as 3 and as late as 22 weeks of gestation. However, very few women had their blood collected later than 20 weeks of gestation when weight during pregnancy increases rapidly. Additionally, although we observed that African-American women exposed to higher PM_{10} and $PM_{2.5}$ concentrations than White race participants, we did not find effect modifier by race. This may due to (1) the difference of

exposure concentrations is very small (less than 1 µg/m^3) and (2) the available geographic information is in ZIP code level not individual.

Much evidence has accumulated that maternal exposure to air pollution is associated with adverse birth outcomes or pregnancy complications. CRP concentrations are generally higher in pregnant than non-pregnant women, [77] and placentally derived microparticles or soluble products from the placenta are suspected to drive systemic inflammation during normal pregnancy. [101] An increased inflammatory response during pregnancy is present in preeclampsia, small for gestational age (SGA), and preterm birth. [8,11,81,111] Two studies examined the associations between maternal plasma CRP concentrations in early pregnancy (before 21 weeks of gestation) and the risk of preterm delivery and reported 2.6- to 2.9-fold increases in risk of preterm delivery with early pregnancy inflammation (CRP $\geq$ 8 ng/ml). [11,81] Cord blood concentrations of inflammatory markers such as interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), thrombopoietin (TPO), and C-reactive protein were reported to be significantly higher in SGA infants compared with appropriate-for-gestational-age controls. [111] Thus, our findings provide some evidence that inflammation is one possible pathway through which air pollution may act to increase the risk of experiencing adverse birth outcomes such as SGA and preterm delivery.

The primary strength of our study is that we were able to examine variables that may have confounded the associations we examined, including active and passive smoking, maternal nutrition (i.e., multivitamin or prenatal vitamin use), and BMI. Our BMI information was based on measurements in early pregnancy not on self-report. Our study also has several limitations. First, we did not have maternal infection information during the period when blood was collected. Since we were unable to control for acute infections, this may have confounded our short term results (0-7 lag days). However, some infections such as respiratory infection are

likely on the causal pathways between air pollution and CRP increases and it would be inappropriate to control for such an intermediate factors. Second, we relied on residential information at time of the birth of the infant rather than at the time we collected the blood samples, and we assumed that mothers did not move during pregnancy and that the estimates of residential air pollution were representative of the woman's personal exposures all of which may have resulted in exposure measurement error. Since our study population was drawn from the longitudinal PEPP cohort, in which participants received their prenatal care and delivered in the same hospital, we believe that it is reasonable to assume that most women in our study either did not move or only moved within the same neighborhood (or ZIP code) during pregnancy as also suggested by Chen et al. (2010). [112] While we conducted a large number of statistical tests, the consistency and magnitude of our findings for particulates and O_3 air pollution and high C-reactive protein concentrations make it less likely that our results are due to chance only. Additionally, we dichotomized continuous CRP concentrations at a cut point of 8 ng/ml which may affect the statistical power and lose information. We are further exploring a new approach to calculate odds ratios for continuous CRP concentrations without dichotomizing it.

3.6   CONCLUSIONS

Our study suggests that particulate matter (PM_{10} and $PM_{2.5}$) air pollution and ozone exposures during early pregnancy contribute to systemic inflammation as measured by CRP. Our findings provide some new evidence that effects of particulate air pollution on adverse birth outcomes may be mediated by systemic inflammation.

3.7 TABLES AND FIGURES

Table 7. Demographic characteristics of study population by CRP concentration (N=1,696)

	CRP < 8 ng/ml		CRP ≥ 8 ng/ml		Crude OR	Adjusted OR[a]
	Mean or no.	SD or %	Mean or no.	SD or %	(95% CI)	(95% CI)
Gestational weeks at sample collection (weeks)	10.0	3.9	11.6	4.2	1.10 (1.07 – 1.14)	1.13 (1.09 – 1.17)
Maternal BMI at baseline (kg/m²)	25.4	5.9	32.5	7.8	1.14 (1.12 – 1.17)	1.15 (1.12 – 1.18)
Maternal age (years)	24.6	5.8	26.4	6.1	1.05 (1.03 – 1.08)	1.07 (1.03 – 1.11)
Maternal race/ethnicity						
White	905	64	128	52	1	1
African American	509	36	118	48	1.64 (1.25 – 2.15)	1.31 (0.91 – 1.87)
Maternal education						
Less than high school	302	21	46	19	1.15 (0.72 – 1.83)	1.26 (0.62 – 2.55)
High school	544	37	107	43	1.48 (0.99 – 2.22)	1.46 (0.81 – 2.61)
Less than 4 years college	332	23	58	23	1.32 (0.84 – 2.05)	1.02 (0.57 – 1.84)
Above College/Bachelor	271	19	36	15	1	1
Parity						
First birth	913	63	110	45	1	1
Second or subsequent birth	536	37	137	55	2.12 (1.62 – 2.79)	0.99 (0.68 – 1.43)
Maternal cigarette exposure during early pregnancy						
Active (smokers)	480	33	73	30	0.72 (0.50 – 1.02)	0.88 (0.57 – 1. 37)
Passive (non-smokers)	610	43	97	40	0.75 (0.54 – 1.04)	0.93 (0.63 – 1.38)
None	348	24	74	30	1	1
Household income (thousands)						
Less than 10	360	25	74	30	1	1
10 to less than 20	287	20	55	22	0.93 (0.64 – 1.37)	1.02 (0.64 – 1.62)
20 to less than 50	289	20	52	21	0.88 (0.59 – 1.29)	1.06 (0.66 – 1.71)
50 or more	259	18	38	16	0.71 (0.47 – 1.09)	1.13 (0.61 – 2.11)
Unknown	254	17	28	11	0.54 (0.34 – 0.85)	0.67 (0.38 – 1.18)
Year entering the study						
1997	187	13	18	7	1	1
1998	272	19	34	14	1.30 (0.71 – 2.37)	1.21 (0.60 – 2.45)
1999	331	23	49	20	1.54 (0.87 – 2.72)	1.04 (0.53 – 2.04)
2000	396	27	92	37	2.41 (1.41 – 4.11)	1.63 (0.85 – 3.13)
2001	263	18	54	22	2.13 (1.21 – 3.75)	1.63 (0.82 – 3.24)
Season of sample collected						
Spring (March-May)	451	31	84	34	1	1
Summer (June-August)	367	25	61	25	0.89 (0.62 – 1.28)	0.73 (0.48 – 1.11)
Fall (September-November)	321	22	49	20	0.82 (0.56 – 1.20)	0.75 (0.48 – 1.19)
Winter (December-February)	310	22	53	21	0.92 (0.63 – 1.33)	0.96 (0.62 – 1.48)

[a] Adjusted for all other covariates in the table

Table 8. The descriptive statistics of air pollution (0-7 day averages)

		Percentile						Pearson Correlation					
	Mean ± SD	0th	25th	50th	75th	95th	100th	PM$_{10}$	PM$_{2.5}$	O$_3$	CO	SO$_2$	NO$_2$
PM$_{10}$ (μg/m^3)	26.1 ± 8.3	10.7	19.9	24.4	31.2	42.2	58.7	1					
PM$_{2.5}$ (μg/m^3)	16.4 ± 5.3	7.1	12.8	15.2	18.7	26.2	40.8	0.9	1				
O$_3$ [a] (ppb)	29.9 ± 7.1	4.4	25.6	30.3	34.3	41.6	51.4	0.5	0.5	1			
CO (ppm)	0.5 ± 0.2	0.0	0.3	0.5	0.6	0.9	1.4	0.1	0.0	-0.1	1		
SO$_2$ (ppb)	8.4 ± 3.3	1.5	6.1	7.9	10.1	14.4	25.4	0.1	0.2	0.1	0.3	1	
NO$_2$ (ppb)	18.8 ± 3.8	8.2	15.9	18.7	21.1	25.1	33.4	0.1	0.1	0.0	0.6	0.4	1

[a] For summer season only (April to September)

Table 9. Associations between CRP (<8 vs ≥8 ng/ml), particulates and O_3 air pollution per IQR increase by lag period for the entire population (N=1,696) and non-smokers (N=1,129)

Pollutant/lag periods	IQRs	Crude OR (95% CI)	Adjusted[a] OR (95% CI)
For the entire population			
PM_{10} (μg/m3)		N=1,696	N=1,576
Day 0-7	11.3	1.02 (0.84 – 1.24)	1.05 (0.81 – 1.36)
Day 0-21	9.8	1.06 (0.88 – 1.28)	1.23 (0.97 – 1.57)
Day 0-28	9.2	1.03 (0.85 – 1.26)	1.18 (0.91 – 1.53)
$PM_{2.5}$ (μg/m3)		N=1,189	N=1,084
Day 0-7	6.0	1.17 (1.00 – 1.38)	1.21 (1.02 – 1.43)
Day 0-21	5.2	1.19 (1.01 – 1.40)	1.32 (1.05 – 1.67)
Day 0-28	5.0	1.16 (0.96 – 1.41)	1.26 (0.97 – 1.63)
O_3[b] (ppb)		N=909	N=833
Day 0-7	8.7	1.02 (0.83 – 1.24)	1.27 (0.87 – 1.86)
Day 0-21	8.2	0.96 (0.76 – 1.23)	1.26 (0.78 – 2.03)
Day 0-28	7.7	0.91 (0.72 – 1.15)	1.05 (0.65 – 1.71)
For non-smokers			
PM_{10} (μg/m3)		N=1,129	N=1,060
Day 0-7	11.0	0.98 (0.78 – 1.24)	1.05 (0.77 – 1.44)
Day 0-21	9.9	1.13 (0.88 – 1.45)	1.47 (1.06 – 2.02)
Day 0-28	9.2	1.10 (0.86 – 1.42)	1.41 (0.99 – 2.00)
$PM_{2.5}$ (μg/m3)		N=786	N=726
Day 0-7	5.8	1.15 (0.93 – 1.43)	1.17 (0.90 – 1.52)
Day 0-21	5.2	1.29 (1.04 – 1.62)	1.55 (1.15 – 2.11)
Day 0-28	4.6	1.25 (0.98 – 1.60)	1.47 (1.05 – 2.06)
O_3[b] (ppb)		N=579	N=539
Day 0-7	9.0	1.03 (0.75 – 1.40)	1.15 (0.69 – 1.89)
Day 0-21	8.3	1.06 (0.72 – 1.58)	1.60 (0.83 – 3.09)
Day 0-28	7.9	1.05 (0.72 – 1.55)	1.49 (0.75 – 2.96)

[a] Adjusted for gestational week at sample collection, maternal BMI at enrolment, maternal age, race, education, parity, cigarette smoke exposure during early pregnancy, household income, season of sample collection (only adjusted in PM_{10} and $PM_{2.5}$ models), and year of enrolment (for PM_{10} and O_3: 1997 to 2001; for $PM_{2.5}$: 1999 to 2001); month of enrolment was also adjusted in O_3 models
[b] Restricted to participants enrolled in the study in the months of April -September only

The distribution (percentage) of
African-American by ZCTA

Legend

allzip
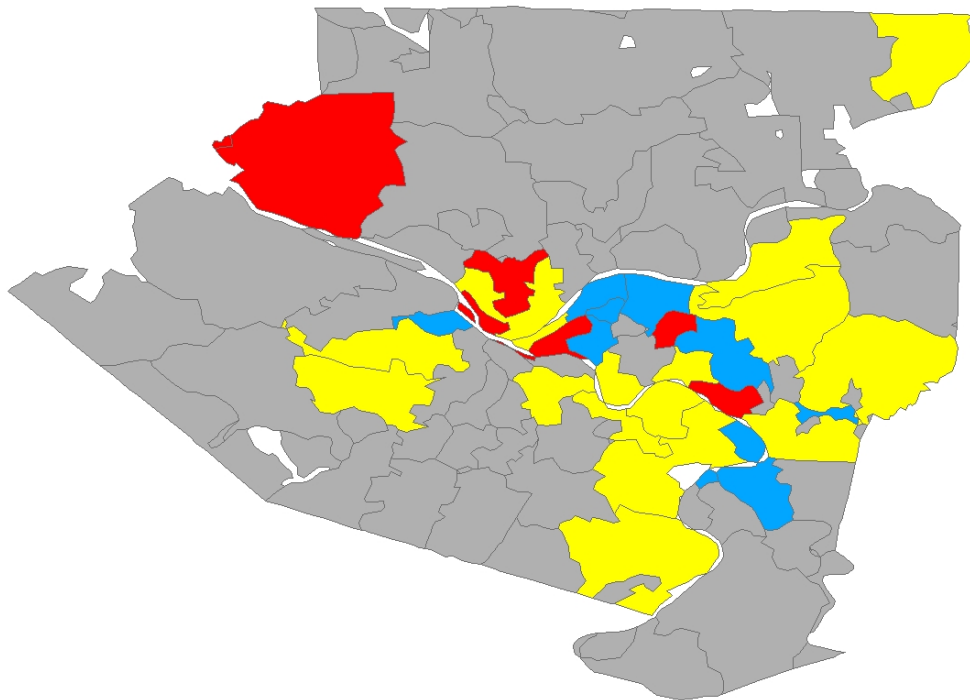
- ◻ 0.000000000 - 25
- ◻ 26 - 50
- ◻ 51 - 75
- ◻ 76 - 100
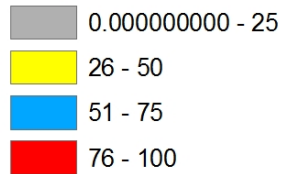
Figure 1. Geographic distribution of African-American by ZIP code

The distribution (percentage) of White race/ethnicity by ZCTA
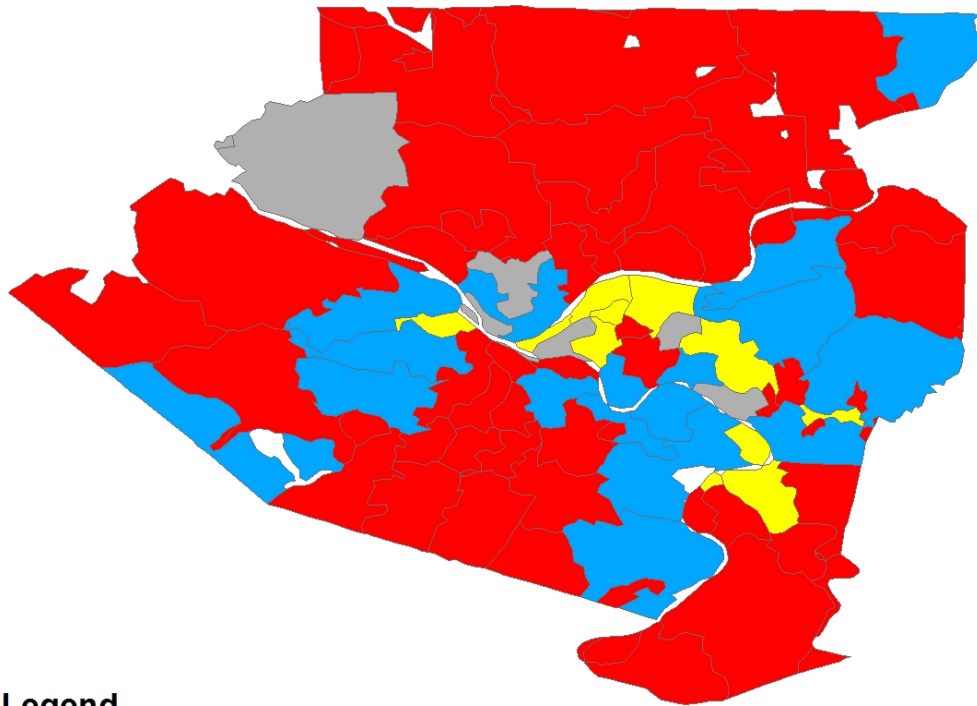
Legend

allzip

WHITE / none

- 0.000000000 - 25
- 26 - 50
- 51 - 75
- 76 - 100

Figure 2. Geographic distribution of White race by ZIP code

Figure 3. The distribution of PM_{10} by ZIP code

The distribution (quartile) of PM2.5 by ZCTA

Legend

allzip

PM25_p7 / none

⬜ (gray)	7.96 - 14.4
🟨 (yellow)	14.5 - 15.9
🟦 (blue)	16.0 - 17.6
🟥 (red)	17.7 - 27.3
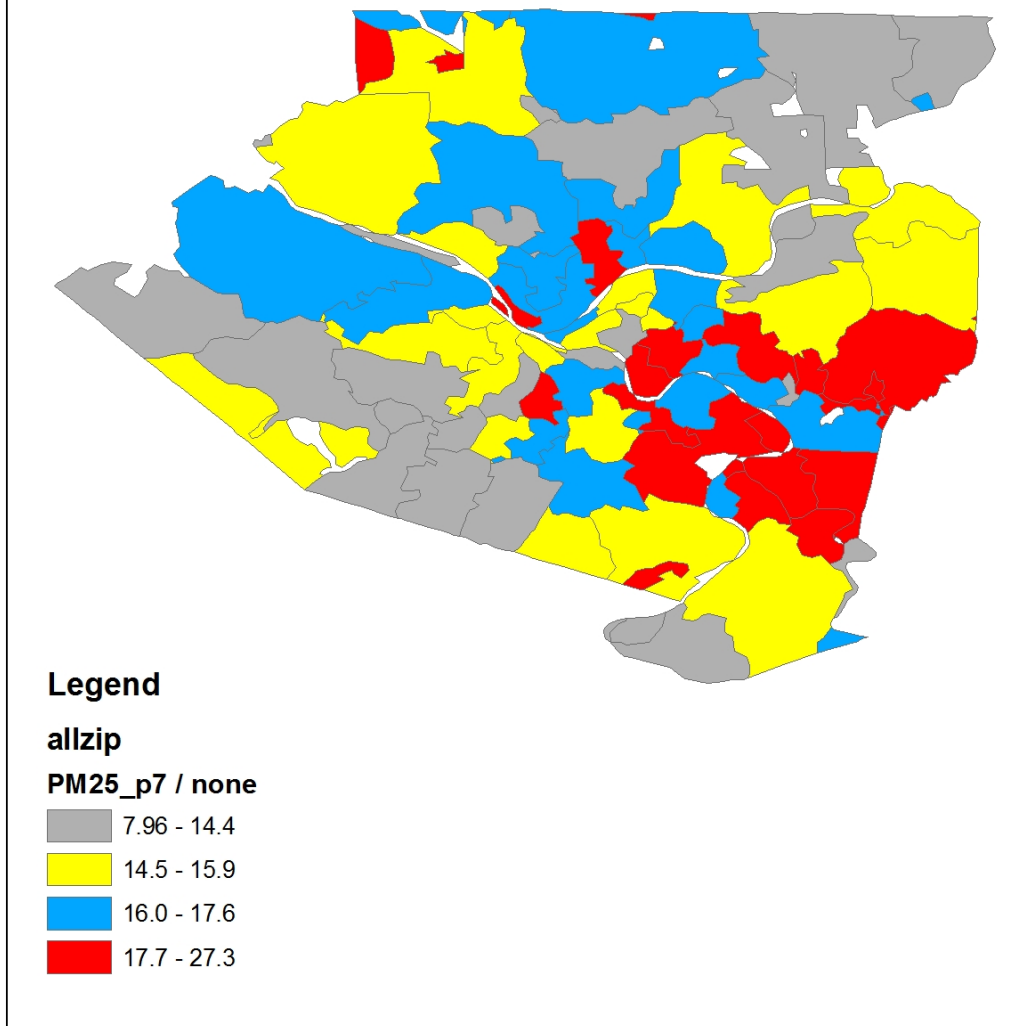
Figure 4. The distribution of PM2.5 by ZIP code
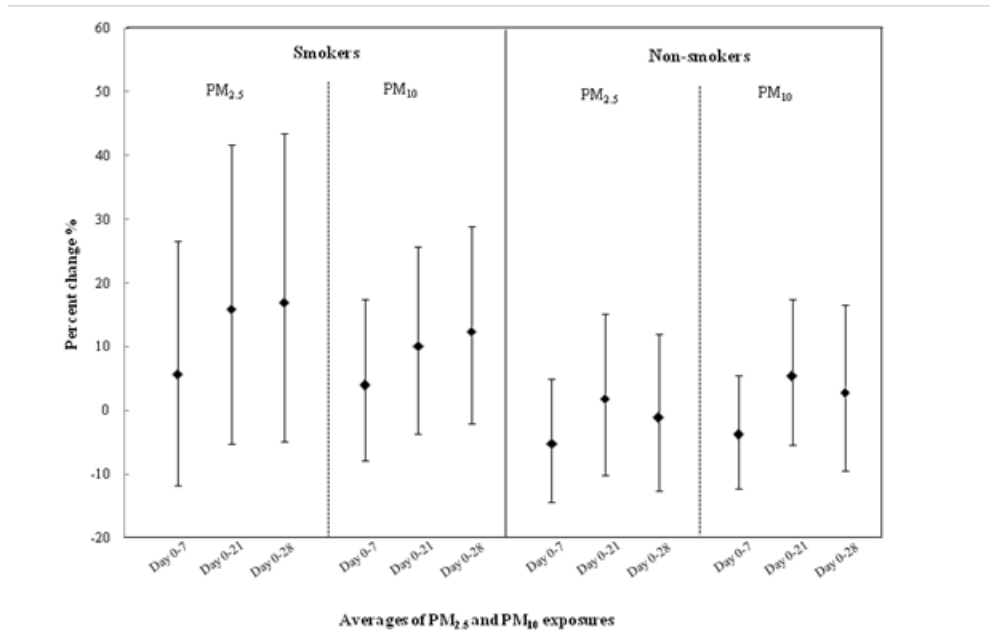
Figure 5. Percentage change for CRP with ambient PM$_{2.5}$ and PM$_{10}$ exposures (per IQR increase) in smokers and

non-smokers.

4.0   DOES SYSTEMIC INFLAMMATORY RESPONSE, MEASURED BY C-REACTIVE PROTEIN, MEDIATE THE LINK BETWEEN SMOKING AND PRETERM DELIVERY?

4.1   ABSTRACT

Maternal smoking during pregnancy is associated with several adverse pregnancy outcomes such as preterm delivery and intrauterine growth restriction but the underlying biological mechanism for this adverse effect is not well-understood. We hypothesized that systemic inflammation may play a role linking smoking and preterm delivery. We conducted a matched case-control study from the Prenatal Exposures and Preeclampsia Prevention study (PEPP) between 1997 and 2001 in Pittsburgh, PA. Smoking status was assessed by serum cotinine concentration and C-reactive protein (CRP) was measured as a biomarker of inflammation. Mediation analyses were performed to test whether inflammation mediates the relationship between smoking and preterm delivery. We observed a null association between smoking and inflammation (odds ratio=0.95, 95% CI=0.50 – 1.82) for cotinine concentrations above 100 ng/ml vs. below 5 ng/ml). We did not observe an association between elevated CRP concentrations (≥8 ng/ml) (odds ratio=1.30, 95% CI=0.76 – 2.24) and the risk of preterm delivery in the adjusted model. After adjustment for race and body mass index, smoking (odds ratio=1.72, 95% CI=1.12 – 2.64 for cotinine concentrations above 100 ng/ml vs. below 5 ng/ml) during early pregnancy were associated with

preterm delivery. Our data suggest that systemic inflammation may not be the mediating link between smoking and preterm delivery.

4.2   BACKGROUND

Preterm birth is a major cause of morbidity among neonates and is an indicator for impaired organ development, which results in adverse health effects in childhood and adult life [113,114]. Smoking is a recognized risk factor for preterm delivery and many studies have reported positive associations between maternal smoking during pregnancy and risk of preterm birth [115-118], with results from a meta-analysis reporting an odds ratio (OR) for having preterm delivery of 1.27 (95% confidence interval (CI): 1.21 – 1.33) for mothers who smoked during pregnancy compared to those who did not [119].

The mechanism to account for the increased risk of preterm delivery as a result of smoking remains unknown. Low grade inflammation, endothelial dysfunction, and carbon monoxide induce fetal hypoxia have been hypothesized to account for the role of smoking on pregnancy complications or fetal growth [117,120,121]. Luppi et al. (2007) observed an increased frequency of $CD3^+$ lymphocytes but a reduced expression of CD54 on monocytes and CD62L on granulocytes in smokers during early pregnancy[122]. Furthermore, Simhan et al. (2005) reported a dose-response relationship between cervical anti-inflammatory cytokine concentrations, i.e., interleukin (IL)-4, 10, and 13, and number of cigarette smoked during pregnancy, and the increase in anti-inflammatory cytokine concentrations was observed without an increase in pro-inflammatory cytokines including IL-1α, IL-1β, IL-6, IL-8, tumor necrosis factor-α (TNF-α), and monocyte chemotactic protein-1 (MCP-1).[123].

Recently, a growing amount of literature has documented the association between systemic inflammatory biomarkers and preterm delivery with higher risk of preterm for women with elevated inflammatory biomarker concentration [11,80,81]. However, few studies have systematically examined the association between smoking and systemic inflammation in relation to the risk of preterm birth for pregnant women. The objective of the current study was to examine whether inflammation, as indicated by the circulating concentration of serum CRP, mediates the link between smoking and preterm birth by measuring serum cotinine, and preterm delivery. CRP was selected because it has been widely used in the studies of smoking, preterm delivery, and systemic inflammation. We performed a mediational analysis recommended by Judd and Kenny [124]. A series of models were evaluated to test the effects of inflammation as a mediator in the relation between smoking and preterm delivery, including (1) smoking and preterm delivery, (2) smoking and inflammation, (3) inflammation and preterm delivery, and (4) smoking, inflammation, and preterm delivery. We hypothesized that (1) higher serum cotinine concentration is associated with increased preterm delivery, (2) higher serum cotinine concentration also is associated with higher CRP concentrations, (3) elevated CRP concentration is associated with preterm delivery, and (4) the effect size of cotinine on preterm delivery is attenuated when we control for CRP concentrations.

4.3 MATERIALS AND METHODS

4.3.1 Study Design and Study Population

This is a matched case-control study of 167 cases and 498 controls who were enrolled in the PEPP study between 1997 and 2001. The PEPP study was a prospective longitudinal study with enrollment of 2,211 healthy women, ages 14-44, who had been pregnant for less than sixteen weeks and followed to delivery. In our study, we excluded women with pre-existing medical conditions (n=37) including chronic hypertension, chronic diabetes, and HIV, as these conditions are associated with preterm delivery risk. Since we were interested in inflammation in the first half of pregnancy, we also excluded women without a blood sample taken before 22 weeks of gestation (n=145), and limited our analysis to singleton births whose mothers reside in Allegheny County, PA. Of 1,696 eligible women, 167 delivered preterm, defined as delivery before 37 gestational weeks. For each woman who delivered before 37 weeks of gestation (cases), we randomly selected 3 women with normal term delivery (controls), defined as gestational weeks at delivery $\geq$ 37 and matched maternal age (± 3 years) and gestational age (± 2 weeks) at baseline. Since only two matched controls were found for three cases, the total controls in our study were 498 women. Each participant in the PEPP study signed a consent form approved by the Institutional Review Board at the University of Pittsburgh.

4.3.2 Biomarkers Assessment

Maternal nonfasting blood samples were stored and frozen at -80 ^{0}C. We evaluated the first sample collected before 22 weeks of gestation. The mean gestational weeks at sample collection

was 11.5 (std=6.3) for cases and controls. Concentrations of CRP were measured with a high-sensitivity enzyme-linked immunosorbent assay (ELISA) on the SpectraMax Me analyzer (Molecular Devices, USA), described elsewhere [102]. The detection limit of the CRP assay was 0.03 ng/ml, with an intra-assay variability of 7 percent. Concentrations of serum cotinine were measured with the Cotinine Direct ELISA kit on the SpectraMax Me analyzer (Molecular Devices, USA). The intra- and interassay variation was smaller than 10%.

4.3.3 Covariates

Women who participated in the PEPP study underwent a basic physical exam including height and weight and an in-person interview at their first prenatal visit. The covariates that were considered include: maternal body mass index (BMI) at enrollment/time of blood draw (kg/m^2), maternal race (White, African-American), marital status (married, un-married), maternal education (less than high school, high school or greater), parity (first birth, second or subsequent birth), public assistance (yes/no), alcoholic drinks prior to the first prenatal visit (yes/no), and periconceptional multivitamin use (multivitamin use prior to the first prenatal visit).

4.3.4 Statistical Analyses

The study design was matched case-control for preterm delivery, but we also examined the continuous gestational weeks at delivery in relation to concentrations of cotinine and CRP. The residuals of cotinine concentrations and gestational weeks at delivery were not normally distributed; thus, we centered the gestational weeks at 43 and log transformed the centered gestational weeks, i.e., log(43-gestational weeks at delivery). Linear regression models were

used to examine the relationship between smoking and gestational weeks and to test the mediation of inflammation. Beta coefficients and 95% confidence intervals (95% CIs) of cotinine concentrations were reported to represent the association with gestational weeks after controlling for maternal age, race, gestational weeks at baseline, and BMI.

Odds ratios (ORs) and 95% confidence intervals of preterm delivery associated with cotinine and CRP concentrations were estimated separately using conditional logistic regression. We classified cotinine concentrations into 3 groups: <5, 5-100, and greater than 100 ng/ml to represent non-smokers, light smokers, and heavy smokers, respectively [122,125]. The residuals of cotinine concentrations and CRP concentrations were not normally distributed, so log transformation was used to examine the association between smoking and inflammation by utilizing linear regression at robust estimator. In addition to examining continuous CRP concentrations, we also categorized CRP concentrations as <8 and ≥8 ng/ml because studies have shown that elevated CRP concentration above 8 are associated with preterm delivery [11,81]. A logistic regression model with a robust estimator was fit to examine the relationship between CRP (<8, ≥8 ng/ml) and cotinine concentrations after controlling for maternal age, race, gestational weeks at baseline and BMI. Conditional logistic regression models were fit to (1) examine the relationship between CRP and preterm delivery, (2) examine the relationship between smoking and preterm delivery, and (3) test CRP as a mediator in the relationship between smoking and preterm delivery. Covariates were included only if they changed the estimate more than 10 percent. Multivariable conditional logistic regression models were adjusted for maternal race, and BMI. All the analyses were conducted using StataSE10 (Stata Corp, College Station, TX).

4.4   RESULTS

The mean gestational weeks at blood collection was 11.5 weeks (std=6.3) for cases and controls. The mean BMI in early pregnancy (at baseline) was 26.7 kg/m^2 (std=7.0) for women who smoked (cotinine concentrations >100 ng/ml) and 26.8 kg/m^2 (std=6.9) for non-smokers (cotinine concentrations <5 ng/ml). Women who were heavy smokers (cotinine concentration ≥100ng/ml) were more likely to be un-married, have second or subsequent births, and drink alcohol, and less likely to report multivitamin use, and need public assistance (Table 10).

4.4.1   Results based on gestational weeks

In the adjusted linear regression model, cotinine concentrations showed a small but significant association with gestational weeks at delivery (beta coefficient=0.0005782, 95% CI=0.0000515 – 0.001104). After untransforming the coefficient, we observed an approximately one week reduction of gestational weeks at delivery was associated with cotinine concentrations equal to 400ng/ml compared to those with zero cotinine concentrations; and when cotinine concentrations were equal to 100 ng/ml, the gestational weeks at delivery was reduced 1 day. This relationship remained the same when CRP concentration was entered in the model.

4.4.2   Results based on preterm delivery

The median CRP concentration was 3.0 ng/ml for preterm group and 2.8 ng/ml for controls. There was no strong evidence that every 1 unit increase in CRP concentration was associated with preterm delivery (OR=0.95, 95% CI=0.78 – 1.16 in the adjusted model) (Table 11).

However, an elevated although non-significant relationship was observed when we used the cut point of 8 for CRP (adjusted OR=1.30, 95% CI=0.76 – 2.24).

CRP concentrations were not correlated with cotinine concentrations (γ=-0.01, p=0.05). We observed null associations between cotinine concentrations (<5, 5-100, and above 100 ng/ml) and elevated CRP concentration (CRP below 8 ng/ml). The median cotinine concentrations were 1.59 and 2.46 ng/ml for women with elevated CRP concentration (above 8 ng/ml) and CRP below 8 ng/ml, respectively. The OR of having elevated CRP concentration for women who had cotinine concentration between 5 and 100 was 0.94 (95% CI=0.44 – 2.01) and 0.95 (95% CI=0.50 – 1.82) for women who had CRP above 100 ng/ml in the adjusted models (Table 12).

In the unadjusted model, cotinine concentrations above 100 ng/ml were significantly associated with preterm delivery (OR=1.66, 95% CI=1.10 – 2.48) compared to those with cotinine concentration below 5 ng/ml (Table 13). The relationship was similar when we controlled for maternal race and BMI (OR=1.72, 95% CI=1.12 – 2.64). When we evaluated whether CRP was a mediator of the effect of smoking on preterm delivery, we entered CRP concentration in the model with cotinine. No strong evidence of CRP as a mediator was noted. For example, the adjusted OR of preterm delivery for cotinine group in 5-100 and above 100 ng/ml were 1.15 (95% CI=0.68 – 1.99) and 1.72 (95% CI=1.12 – 2.64), respectively, compared with 1.17 (95% CI=0.69 – 2.00) and 1.76 (95% CI=1.14 – 2.70), respectively when we adjusted for CRP (Table 13).

4.5   DISCUSSION

We found that maternal smoking (measured by cotinine concentrations) and systemic inflammation (measured by CRP concentrations) during early pregnancy were independently associated with increased risk of preterm delivery. Our data, however, to not support the notion that systemic inflammatory responses mediates the link between smoking and preterm delivery.

Our results linking elevated CRP concentrations to increased risk of preterm delivery are generally consistent with previous studies, although they reported stronger associations of preterm delivery for early pregnancy inflammation (CRP≥ 8 nm/ml) [11,81]. Catov et al. (2007) examined the same cohort and reported a 2.6- to 2.8-fold increased risks of spontaneous preterm delivery for elevated CRP concentrations during early pregnancy while we only observed the odds ratio of 1.3 (95 % CI=0.76 – 2.24) for elevated CRP concentrations for preterm delivery. However, Catov et al. only included spontaneous preterm delivery while we also included women with indicated preterm birth, and we found that women with indicated preterm birth had higher CRP concentrations (median=3.3 ng/ml, range=24.3 ng/ml) than those with spontaneous preterm birth (median=2.9 ng/ml, range=18.1 ng/ml), which may have affected our results. In addition, different lot of ELISA used on measuring CRP concentrations may have also contributed to the different results.

Studies of smoking and CRP concentrations in non-pregnant populations such as the elderly and adults have reported slightly higher CRP concentrations in smokers than never or non-smokers [126-131]. However, in our current study, we observed that women who smoked had slightly lower CRP concentrations (median=2.4 ng/ml) than women who did not smoke (median CRP concentration=3.1 ng/ml). The inconsistent findings of CRP concentrations between smokers and non-smokers may be due to different data collection approaches and definitions of

smoking status. For example, most previous studies relied on self-report smoking information from interview or questionnaire and this approach may introduce information bias if women did not report their smoking status correctly. We, on the other hand, measured serum cotinine concentrations as a biomarker of smoking to define non-smokers (cotinine concentrations <5 ng/ml), light (5-100 ng/ml) and heavy (>100 ng/ml) smokers, which may improve the classification of smoking status. Furthermore, different study populations may also contribute to this inconsistent finding.

Pregnancy is a special state where dramatic changes in cardiovascular function and profound modifications of the maternal immune system occurr in order to adapt to the fetus. Luppi et al. (2007) examined the numbers of circulating leukocytes in a cohort of 198 pregnant women and they reported that smoking increased the frequency of $CD3^+$ lymphocytes but decreased the expression of CD54 on monocytes and CD62L on granulocytes, and the decreased of adhesion molecules presentation on the surface of monocytes and granulocytes may be due to an inhibitory effect of smoking on the expression of adhesion molecules on leukocytes [132]. In addition, Simhan et al. (2005) observed higher anti-inflammatory cytokines concentrations including interleukin-4, 10 and 13, in cervix among smokers compared to non-smokers, but no difference in proinflammatory cytokines concentrations was observed between smokers and non-smokers. Therefore, smoking may affect the balance of pro- and anti-inflammatory responses that may reflect the risk of preterm birth.

We found that mothers who smoked during early pregnancy had an increased risk of preterm delivery and a dose-response relationship was observed with higher cotinine concentrations. This was also reported by other studies. For example, Fantuzzi et al. (2007) in Italy showed a relationship between active smoking during pregnancy and preterm delivery

(adjusted OR= 1.53; 95% CI, 1.05-2.21). Although self-report smoking information during pregnancy was used by Fantuzzi et al., they also found a dose-response relationship for 1-10 cigarettes smoking per day and risk of preterm delivery (OR=1.54, 95% CI=1.01-2.35); for >10 cigarettes per day, the odds ratio of preterm birth (<37 weeks gestation) is 1.69 (95% CI=0.91-3.13).

The primary strength of our study is that smoking status was defined by serum cotinine concentrations not on the basis of self-report. Recently, a large population-based study estimated the percentage of nondisclosure of smoking status in US pregnant women and reported that among pregnant active smokers (defined by serum cotinine concentrations), 22.9% (95% CI=11.8 – 34.6) of them did not report smoking [133]. Similarly, in our study, we found that among 288 women with cotinine concentrations greater than 5 ng/ml, 72 (25%) of them did not report any cigarette smoked since pregnancy. Thus, our smoking information based on cotinine concentrations would reduce misclassification of smoking status due to pregnant women under- or over-reporting whether they smoked and how many cigarettes they smoked.

Our study also has several limitations. First, although CRP is an acute-phase protein and is widely used as an inflammatory biomarker on studies of smoking and inflammation [126-131], we did not examine other pro-inflammatory biomarkers such as interleukin (IL)-6 (IL-6), IL-1β and tumor necrosis factor (TNF-α) to evaluate how other systemic inflammatory bio-markers also play a role in the relationship of smoking and preterm delivery. However, cytokines IL-6, IL-1β, and TNF-α have been reported to mediate CRP mRNA transcription and they have shown a moderate significant correlation with CRP [134,135]. In addition, there was insufficient sample size to examine the relationship of smoking and inflammation on different periods of gestational

weeks at delivery among preterm births, such as gestational weeks <34, and 34-<37 weeks, or stratified the analyses on spontaneous or indicated preterm delivery status.

Numerous studies have reported on the adverse effects of smoking during pregnancy and preterm delivery. Additionally, researchers have also linked increased inflammatory response (CRP) with increased risk of preterm delivery. However, there is little evidence linking all three in a systematic fashion. The present study examined the relationship of smoking at baseline exam, CRP level and subsequent preterm delivery in a population of women in Allegheny County. The results did not demonstrate a mediated effect of smoking and preterm delivery. Consideration of multiple inflammatory biomarkers as well repeated measures during pregnancy time periods are needed to clarify the role of systemic inflammation on the pathway of smoking and preterm delivery.

4.6   TABLES

Table 10. Demographic characteristics of study participants by cotinine concentrations

	Cotinine Concentrations (ng/ml)					
	<5 n=377		5 to <100 n=110		≥100 n=178	
	N	%	N	%	N	%
Maternal race/ethnicity [a]						
White	228	61.5	57	51.8	103	59.2
African American	143	38.5	53	48.2	71	40.8
Maternal education						
Less than high school	58	15.4	27	24.5	57	32.0
Above high school	319	84.6	83	75.5	121	68.0
Marital status [a]						
Un-married	179	47.6	69	62.7	110	61.8
Married	197	52.4	41	37.3	68	38.2
Parity						
First birth	244	64.7	76	69.1	90	50.6
Second or subsequent birth	133	35.3	34	30.9	88	49.4
Alcoholic drinks during pregnancy						
Yes	39	10.3	9	8.2	32	18.0
No	338	89.7	101	91.8	146	82.0
Maternal cigarette smoked since pregnancy[b]						
Yes	9	2.4	56	50.9	160	89.9
No	368	97.6	54	49.1	18	10.1
Multivitamin/prenatal vitamin used						
Yes	188	49.9	36	32.7	73	41.0
No	189	50.1	74	67.3	105	59.0
Public assistance [a]						
Yes	65	17.2	42	38.5	64	36.0
No	312	82.8	67	61.5	114	64.0

[a] Missing observations for maternal race other than White and African-American (n=10); marital status (n=1); and public assistance (n=1).

Table 11. Associations between CRP concentrations and preterm delivery

	Preterm Delivery	
	Crude ORs (95% CI)	Adjusted ORs (95% CI)[a]
C-reactive Protein		
per IQR (5.4 ng/ml)	0.96 (0.81 – 1.14)	0.95 (0.78 – 1.16)
<8	1	1
≥8	1.20 (0.75 – 1.92)	1.30 (0.76 – 2.24)

[a] Adjusted for BMI, and race

Table 12. Associations between cotinine concentrations and CRP concentrations

	CRP (<8, ≥8ng/ml)	
	Crude ORs (95% CI)	Adjusted[a] ORs (95% CI)
Cotinine (ng/ml)		
<5	1	1
5 - <100	0.77 (0.43 – 1.37)	0.94 (0.44 – 2.01)
≥100	0.87 (0.53 – 1.44)	0.95 (0.50 – 1.82)

[a] Adjusted for BMI, gestational weeks at baseline, age, and race

Table 13. Associations between cotinine concentrations and preterm delivery

	Preterm Delivery			
	Model 1	Model 2	Model 3	Model 4
	Crude ORs (95% CI)	Adjusted[a] ORs (95% CI)	Crude ORs (95% CI)	Adjusted[a] ORs (95% CI)
Cotinine (ng/ml)				
<5	1	1	1	1
5 - <100	1.30 (0.78 – 2.17)	1.15 (0.68 – 1.99)	1.32 (0.79 – 2.20)	1.17 (0.69 – 2.00)
≥100	1.66 (1.10 – 2.48)	1.72 (1.12 – 2.64)	1.68 (1.12 – 2.52)	1.76 (1.14 – 2.70)
C-reactive Protein				
<8	-	-	1	1
≥8	-	-	1.25 (0.78 – 2.02)	1.46 (0.87 – 2.47)

[a] Adjusted for BMI, and race

5.0 AMBIENT AIR POLLUTION EXPOSURE AND BLOOD PRESSURE CHANGES DURING PREGNANCY

5.1 ABSTRACT

Maternal exposure to ambient air pollution has been associated with adverse birth outcomes such as preterm delivery but only one study to date has linked air pollution to blood pressure (BP) changes during pregnancy, a period of dramatic cardiovascular function changes. The authors examined whether trimester specific exposure to particles of less than 10 μm (PM_{10}) or 2.5 μm diameter ($PM_{2.5}$), and ozone affect systolic (SBP) and diastolic (DBP) early to late pregnancy BP changes in a prospectively followed cohort of 1,684 pregnant women in Allegheny County, PA. Air pollution measures for maternal ZIP code areas were derived using Kriging interpolation. First but not second or third trimester PM_{10} and ozone exposure were associated with early to late pregnancy BP changes, most strongly in non-smokers. The authors estimated increases in SBP of 1.58 mmHg (95% CI=0.72 to 2.44) or 3.14 (95% CI=1.33 to 4.96) and in DBP of 0.53 mmHg (95% CI=0.39 to 1.45) or 1.23 (95% CI=0.29 to 2.74) per interquartile increase in PM_{10} or O_3 respectively during the first trimester in non-smokers. Our novel finding suggests that first trimester air pollution exposures may affect birth outcomes adversely via BP increases in the later part of pregnancy.

5.2   BACKGROUND

Pregnancy associated hypertension, defined as systolic blood pressure (SBP) ≥140 mmHg or diastolic blood pressure (DBP) ≥90 mmHg during the second half of pregnancy, is one of the leading causes of perinatal and maternal mortality and morbidity [136-138]. When pregnancy associated hypertension is accompanied by proteinuria after 20 weeks of gestation, the disorder is termed preeclampsia, a pregnancy complication associated with increased risk of not only preterm birth and intrauterine growth restriction (IUGR) [139,140], but also cardiovascular disease in mothers later in life [141]. Although reduced placental perfusion due to abnormal vascular remodeling is proposed as a root cause of preeclampsia, the etiology and pathophysiology of this disorder are largely unknown [137].

A growing body of research has linked changes in blood pressure (BP) to ambient air pollution, especially particulate matter (PM), among elderly persons with preexisting cardiac disease, and healthy individuals [142,143]. Some short term exposure studies have reported positive associations between PM air pollution and BP [142,143], while others have reported the reverse, i.e., increases in PM exposure associated with decreases in BP [144-146] or no association [147,148]. To date only one study has examined whether increases in particulate air pollution are associated with BP changes in women who are pregnant, even though pregnancy is a period of dramatic changes in maternal anatomy, physiology, and metabolism to support the development of the fetus.

Pregnancy hormones including progesterone and prostaglandins, and rennin-angiotensin-aldosterone system play important roles in the BP changes during pregnancy [149,150]. During normal pregnancy, blood volume as well as cardiac output starts to increase by 6 weeks of gestation to adequately perfuse and oxygenate the fetal and maternal tissues [151]. These profound cardiovascular adaptations result in changes in BP during gestation, with decreased SBP and

DBP in early pregnancy measured until approximately 20 weeks of gestation, after which BP increases and returns to non-pregnancy levels at term in normal pregnancies [152,153].

To date studies of air pollution and reproductive outcomes have focused mainly on outcomes such as preterm birth, low birth weight and small for gestational age (SGA) infants, with only three studies focusing on preeclampsia [92,154,155] and a single study suggesting a positive association with BP changes during pregnancy [156]. Positive associations have also been reported between air pollutants and preeclampsia risk. Here we examine whether and how particulate air pollution (including PM_{10} and $PM_{2.5}$) and ozone exposures are associated with BP changes in a cohort of 1,684 pregnant women followed throughout pregnancy to delivery.

5.3   MATERIALS AND METHODS

5.3.1   Study Population

Study subjects were selected from the Prenatal Exposures and Preeclampsia Prevention study (PEPP), which enrolled 2,211 healthy women from clinics and private practices, aged 14-44 in early pregnancy (<16 weeks of gestation) and followed to delivery between 1997 and 2001 in Pittsburgh, PA. Women were interviewed twice, once at the first visit and then again postpartum, to obtain specific information including sociodemographic characteristics and reproductive and medical history at baseline, and information on diet, cigarette smoking and consumption of alcohol in both interviews. Maternal residential ZIP code at the time of delivery and BP (both SBP and DBP) and maternal weight during each prenatal care visit were abstracted from hospital records. BP was taken by the clinic nursing staff with the patient seated and the cuff at the level

of the subject's heart. We excluded women with chronic hypertension and diabetes (N=32) because the BP of these women may respond differently to an exposure challenge and may have had relatively poor cardiovascular function. We also excluded women without BP measurements before 20 weeks of gestation or at least two measurements after 20 weeks (N=84). Additionally, multiparous women who were second-time participants in the PEPP study and those with a maternal residential ZIP code outside of Allegheny County, PA were excluded. A total of 1,684 women were included. This study has been approved by the Institutional Review Board at the University of Pittsburgh and written informed consent has been obtained from all participants.

5.3.2   Exposure Assessment

Maternal exposure to ambient air pollution during prenatal care was estimated based on air monitoring data, including carbon monoxide (CO), nitrogen dioxide (NO_2), sulfur dioxide (SO_2), ozone (O_3), PM_{10}, and $PM_{2.5}$ for Allegheny County (AC) and its neighboring counties (within 50 km of the AC boundary), collected by the AC Health Department (ACHD) and the US Environmental Protection Agency (EPA) between 1996 and 2001. $PM_{2.5}$ and PM_{10} were collected daily or every third or sixth day at 23 monitoring stations (including 13 monitoring stations in AC) from 1999 to 2001 and PM_{10} data at 40 stations (including 18 monitoring stations in AC) from 1997 to 2001, respectively. For gaseous pollutants, SO_2 measurements were available from 32 stations (including 7 monitoring stations in AC), and O_3 from 15 stations (including 3 monitoring stations in AC), while only 11 stations measured NO_2 and CO during the study period (with 3 and 2 monitoring stations respectively in AC). The air pollution data include one-hour concentrations for CO, NO_2, O_3, and SO_2; and 24-hour concentrations for $PM_{2.5}$ and PM_{10}.

Daily air pollution concentrations were estimated relying on the space-time ordinary Kriging (STOK) interpolation method at each centroid of a grid, sized 13.4 m^2, in AC by fitting the spatial and temporal variograms separately using a spherical semivariogram model. We also combined the individual spatial and temporal variograms into one space-time variogram by fitting a general product-sum model [157].

To calculate exposure concentrations for each woman, we first calculated the date of each prenatal visit based on gestational age (assessed by ultrasound and estimated day of delivery) at each visit and date of the delivery of her infant. We then calculated exposure concentrations for each pollutant during each trimester by averaging the estimated daily concentration for each trimester at each centroid of the grid within each ZIP code. In addition, we calculated air pollutant concentrations 0 to 7 days (lag0 – lag7) and mean concentrations for a period of 7 days (i.e., 8-day averages) prior to the BP measurement (prenatal visit date) based on maternal ZIP code to evaluate associations between short-term exposures and BP changes.

5.3.3   Statistical Analysis

We calculated SBP and DBP changes for each woman, subtracting the average of the respective BP measurements in the first 20 weeks of gestation from the average of the 2 measurements taken during the last prenatal care visits. Additionally, we calculated average pulse pressure (PP) changes for each woman, subtracting the average PP in the third trimester (average SBP-DBP in the third trimester) from the average PP in the first 20 weeks of gestation (average SBP-DBP in the first 20 weeks of gestation).   Among 1,684 women, 6% had only one SBP or DBP measurement available before 20 weeks of gestation. We coded BP measurements outside a reasonable range as missing (<1% of measurements), i.e., SBP below 40 mmHg or above 250

mmHg; DBP below 40 mmHg or above 180 mmHg. To examine associations between trimester-specific air pollution exposures and SBP and DBP changes during pregnancy, we employed multiple linear regression analyses with a robust variance estimator to account for clustering of maternal residences within ZIP codes. When examining associations between short term air pollution exposures and BP changes, we fitted linear mixed-effects models using maximum likelihood (ML) estimation with random intercepts and a spherical correlation structure (range=60 days, nugget effect=0.8) to account for correlations between visits at different intervals. We evaluated short-term (lag0 to lag7, and 8-day averages) and longer term (trimester specific averages) air pollution exposures using continuous measures, and report increases per interquartile range (IQR).

In all models, we controlled for maternal age (years), race (White, African-American), pre-pregnancy body mass index (BMI) (kg/m^2), parity (first birth, second or subsequent birth), number of cigarettes smoked during pregnancy, multivitamin or prenatal vitamin use (yes/no), and season (spring, summer, fall, winter) and year of entering the study (for PM_{10} and other pollutants spanning: 1997-2001; for $PM_{2.5}$: 1999-2001). Other potential confounders, including marital status, alcohol intake during pregnancy, maternal education, household income, public assistance, and gestational weeks, were not included because these factors did not change the estimates for pollutants by more than 10 percent when included in preliminary analyses [105].

Previous studies reported different BP patterns for smokers and non-smokers during pregnancy [158,159]. In addition, women who develop gestational hypertension and preeclampsia also may exhibit different BP patterns. Thus, we conducted sensitivity analyses restricted to only those women without pregnancy-induced hypertension (preeclampsia and gestational hypertension) and to non-smokers only. Moreover, race may modify associations between air

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pollution and BP changes. We investigated effect modification by race (mainly Africa-American and Caucasian) using interaction terms in our models and stratified analyses.

We relied on the last two measurements of BP taken during prenatal care visits that were on average 1.4 weeks apart (SD=1.2) to calculate early to late pregnancy BP changes (at 36.7 weeks (SD=2.8) mean gestation). For women who delivered preterm (i.e., before 37 gestational weeks), we averaged BP measurements from earlier gestational weeks than for women who delivered at term. We also performed sensitivity analyses in which we only included measurements for term birth (gestational weeks 37 weeks and greater).

5.4   RESULTS

The mean maternal age in our study population was 24.9 years (SD=5.9) at enrollment and a mean pre-pregnancy BMI was 25.4 kg/m^2 (Table 14). The majority of our study participants was of white race (63%), did not smoke during pregnancy (68%), and used multivitamins or prenatal vitamins (86%). Average SBP and DBP increased as gestational age increased. For example, the average SBP and DBP in the first 20 weeks of gestation were 112.5 mmHg (SD=8.3) and 68.4 mmHg (SD=6.1), respectively while the average SBP and DBP increased to 117.2 mmHg (SD=10.1) and 72.6 mmHg (SD=7.7), respectively late in the third trimester. African-American has slightly higher average SBP and DBP before 20 weeks of gestation than White (SBP: 113.2 mmHg (SD=8.2), 112.3 mmHg (SD=8.4) respectively; DBP: 68.7 mmHg (SD=6.0), 68.3 mmHg (SD=6.2) respectively).However, the average SBP and DBP in the third trimester among White are higher than African-American (SBP: 117.5 mmHg (SD=10.2), 116.8 mmHg (SD=9.9) respectively; DBP: 73.0 mmHg (SD=7.7), 71.9 mmHg (SD=7.7) respectively). Among our study

participants, 32 (2%) developed preeclampsia and 110 (7%) received a diagnosis of gestational hypertension.

Table 15 summarizes means and correlations of pollutant concentrations in the first trimester. Average first trimester PM_{10}, $PM_{2.5}$, and O_3 concentrations were strongly positively correlated with each other but negatively correlated with CO, SO_2, and NO_2.

5.4.1 Ambient air pollution and blood pressure changes

An IQR increase in PM_{10} was associated with a 1.02 mmHg increase in average SBP (95% CI=0.13 to 1.91) and a 0.40 mmHg increase in average DBP (95% CI=-0.27 to 1.06) in adjusted single-pollutant models for the entire study population (Table 16). In contrast, we found no association between first trimester $PM_{2.5}$ and early to late pregnancy SBP changes, and a weak association with DBP changes. We also saw strong associations between O_3 and changes in both SBP and DBP from early to late pregnancy. For example, an IQR increase in O_3 was associated with a 2.04 mmHg (95% CI=0.47 to 3.62) and a 0.79 mmHg (95% CI=-0.36 to 1.94) increases in SBP and DBP, respectively.

When we restricted our analyses to non-smokers, effect estimates were larger in size for PM_{10}, $PM_{2.5}$, and O_3. For non-smokers, adjusted single-pollutant models suggested a 1.58 mmHg (95% CI=0.72 to 2.44) increase in SBP and a 0.53 mmHg (95% CI=-0.39 to 1.45) increase in DBP per IQR increase in PM_{10} during the first trimester (Table 16). Similarly, associations between air pollution exposure and BP changes were stronger for O_3 in non-smokers, with 3.14 mmHg (95% CI=1.33 to 4.96) and 1.23 mmHg (95% CI=-0.29 to 2.74) increases in SBP and DBP, respectively, per IQR increase in O_3. We observed weak positive associations between second and third trimester air pollution exposure to PM_{10}, $PM_{2.5}$, and O_3 and early to late

pregnancy BP changes; all 95% confidence intervals included the null values. For example, we observed 0.46 mmHg (95% CI=-1.03 to 1.95) and 0.37 mmHg (95% CI=-0.52 to 1.26) increases in SBP per IQR increase in PM_{10} during the second and third trimester in adjusted single-pollutant models for non-smokers. For CO, SO_2 and NO_2, associations were null for both the entire population and non-smokers only (results not shown).

We observed positive associations between first trimester PM_{10} and O_3 and PP changes (Table 17). The associations were stronger when we included the analyses for non-smokers. For example, an IQR increase in PM_{10} was associated with a 0.62 mmHg increase in average PP (95% CI=-0.36 to 1.61) for the entire population and a 1.05 mmHg increase in average PP (95% CI=-0.13 to 2.24) in adjusted single-pollutant models for non-smokers.

Generally, effect estimates for PM_{10}, $PM_{2.5}$, O_3 and BP changes changed only slightly when we excluded women with preeclampsia and gestational hypertension disorders in adjusted single-pollutant models (results not shown). Sensitivity analyses examining associations only among women who delivered at term (37 weeks of gestation and greater) produced almost identical estimates (results not shown). When we stratified our results by race, first trimester PM_{10} and ozone affected DBP change only in Caucasians and not in African-Americans, while SBP increases were seen in both races. For $PM_{2.5}$ we did not observe associations between first trimester exposures and early to late pregnancy BP changes in either racial subgroup (results not shown).

None of the short term air pollution exposure measures (lag1 to lag7 and 8-day averages prior to BP measurement) was associated with SBP or DBP changes during pregnancy (results not shown).

5.5   DISCUSSION

We identified associations between first trimester exposure to PM_{10} and O_3 air pollution and increases in mean SBP and DBP in early to late pregnancy in our cohort of pregnant women. Associations between particulate and O_3 air pollution and mean BP changes generally were stronger when we restricted our analyses to non-smokers. Our study is among the first to assess the relation between air pollution exposure and BP changes among pregnant women, and our findings provide new insights on how first trimester air pollution may adversely affects birth outcomes.

A growing number of experimental studies as well as observational epidemiological studies have examined possible links between ambient air pollution and BP changes in healthy individuals, the elderly and people with cardiovascular diseases [142,143,145,146,160,161]. However, only one study has examined air pollution exposure and BP changes in pregnant women [156]. The mechanisms by which air pollution could affect BP and BP changes over time likely differ and might be more complex than those in non-pregnant populations. In most aging populations, clinical changes in BP are most commonly attributed to arterial vascular degeneration; however, in pregnant women, changes in BP result from systemic adaptation necessary to accommodate the needs and presence of the developing fetus. During normal pregnancy, maternal blood volume begins to increase in early pregnancy and increases approximately 25% to 50% in volume by 32 weeks of gestation, in order to support maternal and fetal circulation [151]. These blood volume increases are accompanied by decreases in systemic vascular resistance (SVR). The lowest SVR occurs in the first and second trimester, and increases again until term; this is reflected by both lower SBP and DBP in early pregnancy and a gradual increase during the late second and throughout the third trimester [149].

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A recent Dutch study reported associations between higher PM_{10} exposures in the second and third but not first trimester and increased SBP during the same trimester [156]. Additionally, they reported that NO_2 exposure increased SBP in all three trimesters. These results cannot easily be compared to our findings because the outcome of interest in our study was defined as early to late pregnancy BP changes while the Dutch study only examined trimester exposures in a cross-sectional manner, while we examined associations between early pregnancy (first trimester) exposures and longitudinal BP changes. Furthermore the Dutch exposure assessment relied on spatiotemporal dispersion modeling that accounts for traffic point sources including NO_2 emissions from vehicles; this detailed assessment of spatial variation due to traffic sources could not be achieved in AC.

Studies on air pollution and adverse birth outcomes have reported most consistent results for particulate air pollution and preterm birth [26,162]. Recently, a larger hospital-based study in California, USA, of local traffic-generated air pollution and preeclampsia reported odds ratios of 1.33 (95% CI=1.18 to 1.49) and 1.42 (95% CI=1.26 to 1.59) for preeclampsia in the highest exposure quartiles for NO_x and $PM_{2.5}$, respectively, during pregnancy [92]. Although it has been hypothesized that particulate air pollution may induce BP changes that result in preterm birth [57], and pregnancy-related hypertension is one of the risk factors for and key signs of preeclampsia, the insufficient sample size of our cohorts (with 142 preterm births and 32 cases of preeclampsia) precluded us from examining whether BP changes mediate the associations between air pollution and these adverse outcomes. Nevertheless, our novel findings that PM_{10} and O_3 air pollution are associated with mean increases in BP from early to late pregnancy provide some new insight into possible pathways linking air pollution and pregnancy outcomes.

Pregnancy associated hypertension has been associated with increased risks of preterm birth, low birth weight, SGA, still birth, and neonatal mortality [163,164]. In contrast, women with preeclampsia delivering after 37 weeks of gestation delivered an excess of large for gestational age infants [164]. The impact of pregnancy associated hypertension on fetal growth is multifactorial and complex. Zhang et al. (2007) reported that even in normotensive women, a rise in SBP or DBP from early pregnancy (average BP between 12 and 19 weeks of gestation) to mid third trimester (average BP between 30 and 34 weeks of gestation) is associated with spontaneous preterm birth and a higher risk of preterm birth with a greater increase in BP. They also reported that women who had late spontaneous preterm birth (defined as delivery between 34 and 36 weeks of gestation) experienced a 3.6 mmHg (95% CI=2.5 to 4.8) and 3.1 mmHg (95% CI=1.7 to 4.4) higher rise in DBP and SBP, respectively, than women with term births [165]. In our study, an IQR increase in PM_{10} and O_3 exposure in the first trimester was associated with an increase in SBP of 1.6 mmHg (95% CI=0.72 to 2.44) in all women and a 3.1 mmHg (95% CI=1.33 to 4.96) increase in non-smokers between early and late pregnancy; these increments are similar in size to those reported previously. It is possible that air pollution could be related to an increased risk for preterm delivery through such increases in BP.

We did not find associations between mean BP changes and gaseous combustion related air pollutants, i.e., CO, NO_2, and SO_2, which previous studies have linked to adverse birth outcomes [26,89]. Our null findings for these pollutants and BP are most likely due to our inability to sufficiently capture the spatial distribution of these pollutants with a small number of monitoring stations measuring CO and NO_2 (2 and 3 monitoring stations, respectively) in AC. For pollutants that are highly spatially heterogeneous, such as CO, a few monitoring stations will not be able to represent local sources adequately, resulting in poor spatial resolution.

Furthermore, while we observed a positive association of $PM_{2.5}$ with mean BP changes, the estimated effect size was much smaller than for PM_{10}; due to fewer years of monitoring for fine particles, our small sample size limited our analyses.

Trimester specific air pollution exposures have been evaluated in a number of air pollution and birth outcomes studies, and the most consistent evidence points to the importance of first and third trimester exposures for preterm and low birth weight outcomes [26,45,89,162]. Clinical studies have reported that first trimester growth restriction (determined by crown to rump length) is significantly related to increased risks of preterm birth, SGA at birth, and low birth weight [166-168]. Further it is in the first half of pregnancy that the vascular remodeling of maternal vessels supplying the intervillus space is completed. A failure of these adaptations to occur normally is associated with IUGR, preeclampsia and preterm birth. Our findings that first trimester particulate and O_3 air pollution exposures were associated with mean BP increase during pregnancy, with little or no indication of association in either the second or third trimester. This suggests that air pollution exposure in the first trimester may be more relevant than exposures later in pregnancy.

A limitation of our study is that BP measurements may have been subject to other factors that might increase BP in the short term, such as anxiety and caffeine consumption at the time of a prenatal visit. However, our analyses are based on averages of BP measurements at several visits for most women during the first 20 weeks of gestation and one measurement each at two visits late in the third trimester. We expect this approach to have reduced variability of BP due to short term factors. In addition, we did not have maternal residential history during pregnancy and relied on residential information at birth to assess air pollution exposure, which may have resulted in exposure measurement error. However, because participants in this longitudinal study

received their prenatal care and delivered in the same hospital, we believe that our assumption is reasonable that most women in our study either did not move or moved only within the same neighborhood (or ZIP code) during pregnancy, as also suggested by Chen et al. (2010) [112].

5.6   CONCLUSIONS

In summary, first trimester exposure to PM_{10} and ozone air pollution during pregnancy was positively associated with mean SBP and DBP changes, and these associations were stronger when we restricted our analyses to non-smokers. Our results suggest that BP changes may play a role in mediating reported associations between air pollution and adverse birth outcomes.

5.7 TABLES

Table 14. Demographics and major risk factors in the study population (N=1,684)

Continuous Measures	Mean	std
Pre-pregnancy BMI (kg/m^2)	25.4	6.4
Maternal age (years)	24.9	5.9
Average SBP (mm-Hg) in the first 20 weeks of gestation	112.5 (range=70-155)	8.3
Average DBP (mm-Hg) in the first 20 weeks of gestation	68.4 (range=40-94)	6.1
Average SBP (mm-Hg) in the last two blood pressure measurements	117.2 (range=79-170)	10.1
Average DBP (mm-Hg) in the last two blood pressure measurements	72.6 (range=50-130)	7.7
Categorical Measures	**N**	**%**
Maternal race/ethnicity		
White	1,040	63
African American	610	37
Parity		
First birth	1,033	61
Second or subsequent birth	651	39
Smoking status during pregnancy		
Yes	527	32
No	1,104	68
Multivitamin or prenatal vitamin used		
Yes	1,440	86
No	143	8
Missing	101	6
Year entering the study		
1997	197	12
1998	299	18
1999	371	22
2000	498	29
2001	319	19
Season entering the study		
Spring (March-May)	523	31
Summer (June-August)	428	25
Fall (September-November)	373	22
Winter (December-February)	360	22
Preeclampsia		
Yes	32	2
No	1,652	98
Gestational Hypertension		
Yes	110	7
No	1,574	93

Table 15. The descriptive statistics of first trimester air pollution exposures

Pollutants	Mean ± SD	IQR	Percentile							Pearson Correlation					
			0th	25th	50th	75th	95th	100th		PM_{10}	$PM_{2.5}$	O_3	NO_2	SO_2	CO
PM_{10} ($\mu g/m^3$)	26.1 ± 4.8	7.3	13.8	22.4	25.4	29.6	34.7	40.0		1					
$PM_{2.5}$ ($\mu g/m^3$)[a]	16.5 ± 2.7	3.8	10.8	14.3	16.2	18.1	21.6	24.7		0.9	1				
O_3 (ppb)	22.7 ± 8.6	15.3	6.3	14.8	22.9	30.1	35.6	42.7		0.7	0.5	1			
NO_2 (ppb)	18.7 ± 2.9	4.0	9.0	16.7	18.8	20.7	23.3	26.1		-0.3	-0.5	-0.5	1		
SO_2 (ppb)	8.6 ± 2.4	3.6	3.3	6.7	8.1	10.3	12.8	17.5		-0.3	-0.3	-0.6	0.3	1	
CO (ppm)	0.5 ± 0.2	0.3	0.1	0.3	0.4	0.6	0.8	1.1		-0.1	-0.2	-0.4	0.7	0.3	1

[a] For years of 1999, 2000, and 2001

Table 16. Increase in average blood pressure (in mmHg)[a] per IQR increase in first trimester air pollution exposure[a]

	N	Change in BP (95% CI)	
		Crude	**Adjusted[a]**
For the entire population			
PM$_{10}$ (μg/m^3)			
SBP	1,684	0.88 (0.30 to 1.45)	1.02 (0.13 to 1.91)
DBP	1,684	0.38 (-0.16 to 0.92)	0.40 (-0.27 to 1.06)
PM$_{2.5}$ (μg/m^3)			
SBP	1,128	0.12 (-0.71 to 0.94)	0.01 (-1.08 to 1.11)
DBP	1,128	0.23 (-0.42 to 0.87)	0.29 (-0.49 to 1.07)
O$_3$ (ppb)			
SBP	1,684	1.33 (0.57 to 2.09)	2.04 (0.47 to 3.62)
DBP	1,684	0.75 (-0.05 to 1.55)	0.79 (-0.36 to 1.94)
For non-smokers			
PM$_{10}$ (μg/m^3)			
SBP	1,104	0.91 (0.26 to 1.56)	1.58 (0.72 to 2.44)
DBP	1,104	0.33 (-0.30 to 0.95)	0.53 (-0.39 to 1.45)
PM$_{2.5}$ (μg/m^3)			
SBP	726	0.17 (-0.83 to 1.17)	0.44 (-0.73 to 1.61)
DBP	726	0.33 (-0.37 to 1.02)	0.50 (-0.27 to 1.28)
O$_3$ (ppb)			
SBP	1,104	1.41 (0.51 to 2.32)	3.14 (1.33 to 4.96)
DBP	1,104	0.65 (-0.30 to 1.60)	1.23 (-0.29 to 2.74)
For smokers			
PM$_{10}$ (μg/m^3)			
SBP	527	0.71 (-0.38 to 1.79)	0.19 (-1.64 to 2.01)
DBP	527	0.25 (-0.57 to 1.08)	0.37 (-0.91 to 1.65)
PM$_{2.5}$ (μg/m^3)			
SBP	357	-0.31 (-1.49 to 0.87)	-0.50 (-2.24 to 1.25)
DBP	357	-0.26 (-1.66 to 1.14)	0.11 (-1.94 to 2.17)
O$_3$ (ppb)			
SBP	527	1.26 (-0.15 to 2.66)	0.29 (-2.00 to 2.59)
DBP	527	0.87 (-0.36 to 2.09)	0.47 (-1.85 to 2.79)

[a] All models were adjusted for: maternal age, race, parity, number of cigarettes smoked during pregnancy (for the entire population only), multivitamin or prenatal vitamin used during pregnancy, maternal pre-pregnancy BMI, and season and year of enrolment (for PM$_{10}$ and O$_3$: 1997 to 2001; for PM$_{2.5}$: 1999 to 2001)

Table 17. Increase in average pulse pressure (PP) (in mmHg) per IQR increase in the first trimester air pollution

exposure

	N	Change in PP (95% CI)	
		Crude	**Adjusted[a]**
For the entire population			
PM_{10} ($\mu g/m^3$)	1683	0.50 (-0.05 to 1.05)	0.62 (-0.36 to 1.61)
$PM_{2.5}$ ($\mu g/m^3$)	1127	-0.12 (-0.91 to 0.68)	-0.28 (-1.44 to 0.88)
O_3 (ppb)	1683	0.58 (-0.09 to 1.25)	1.25 (-0.16 to 2.67)
For non-smokers			
PM_{10} ($\mu g/m^3$)	1103	0.58 (-0.07 to 1.23)	1.05 (-0.13 to 2.24)
$PM_{2.5}$ ($\mu g/m^3$)	725	-0.16 (-1.0 to 0.68)	-0.06 (-1.27 to 1.15)
O_3 (ppb)	1103	0.75 (-0.07 to 1.58)	1.92 (0.14 to 3.70)
For smokers			
PM_{10} ($\mu g/m^3$)	527	0.46 (-0.54 to 1.45)	-0.19 (-1.88 to 1.51)
$PM_{2.5}$ ($\mu g/m^3$)	357	-0.05 (-1.55 to 1.45)	-0.61 (-3.15 to 1.93)
O_3 (ppb)	527	0.39 (-0.96 to 1.73)	-0.18 (-2.71 to 2.36)

[a] All models were adjusted for: maternal age, race, parity, number of cigarettes smoked during pregnancy (for the entire population only), multivitamin or prenatal vitamin used during pregnancy, maternal pre-pregnancy BMI, and season and year of enrolment (for PM_{10} and O_3: 1997 to 2001; for $PM_{2.5}$: 1999 to 2001)

6.0   SUMMARY AND CONCLUSIONS

This project has been developed to explore several possible mechanisms link ambient air pollution and adverse birth outcomes. In addition, the project has also examined the role of systemic inflammation in the pathway of smoking and preterm birth. The results showed positive associations between (1) particulate (both $PM_{2.5}$ and PM_{10}) and ozone air pollution and elevated CRP concentrations in non-smoking women during early pregnancy, (2) first trimester exposure to PM_{10} and ozone air pollution contributed to increases in mean systolic and diastolic early to late pregnancy blood pressure, and (3) no evidence that systemic inflammation mediates the link between smoking and preterm delivery. These findings provide some new evidence that associations between particulate air pollution and adverse birth outcomes may be mediated by systemic inflammation and blood pressure changes, and further studies with larger study population are needed to clarify the role of systemic inflammation on the pathway of smoking and preterm delivery.

APPENDIX

SPACE-TIME KRIGING

Space-time ordinary Kriging (STOK) interpolation method to estimate daily air pollution concentrations at the zip code level by averaging the estimated concentration of pollution at each centroid of the grid (size of 13.4 m^2) within each zip code was performed. Spatial and temporal variograms were fitted into a spherical semivariogram model separately based on temporally detrended residuals (below A.1 to A.6). In addition, we combined the spatial and temporal variograms to a space-time variogram by fitting a general product-sum model. This modification of space-time ordinary kriging has been shown to increase mean precision compared to ordinary kriging.

A.1   SPACE-TIME VARIOGRAM-PM$_{10}$

|	
Distribution of PM$_{10}$ monitoring sites	**Semi-variogram: Spherical model** • If h=0 then $\gamma(h;\theta)=0$; • If $0 < h \le a_s$ then $\gamma(h;\theta)=C_0+C_s[(3/2)(h/a_s)-(1/2)(h/a_s)^3]$; • If $h > a_s$ then $\gamma(h;\theta)=C_0+C_s$
Spatial Semivariogram	**Spatial Semivariogram (Cont.)**
Spatial Semivariogram (Cont.)	**Temporal Semivariogram**

Temporal Semivariogram (Cont.)

90 – Time
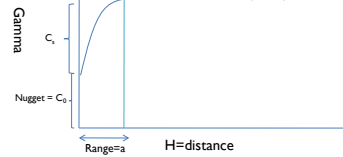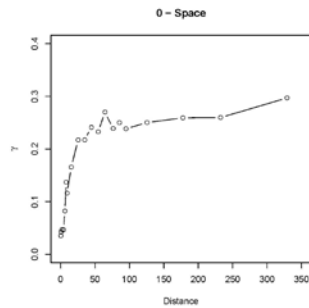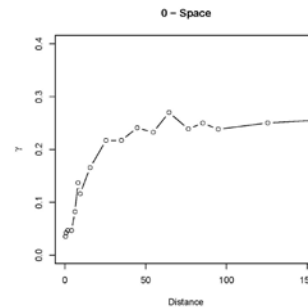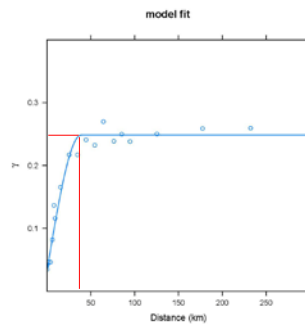
Temporal Semivariogram (Cont.)

model fit

Results from model fit:
```
  model      psill        range
1 Nug   0.06190351  0.000000
2 Sph   0.20594041  3.649337
```

Nugget effect= 0.06190351
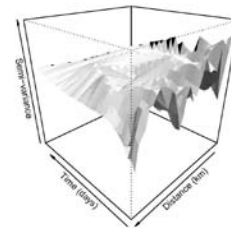Sill= 0.0619035 + 0.2059404
 =0.2678
Range=3.649 (days)

Spatial-Temporal Semi-variogram (contour)
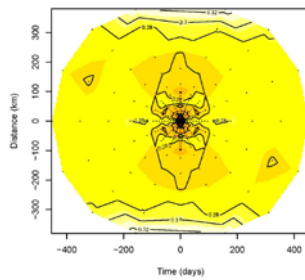
Spatial-Temporal Semi-variogram

Sill=0.28
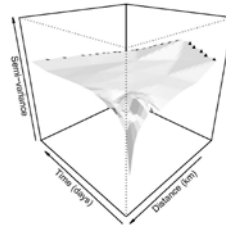
Spatiotemporal variogram model:

- Coefficients

$k1 = [Cs(0) + Ct(0) − Cst(0; 0)]/Cs(0)Ct(0);$

$k2 = [Cst(0; 0) − Ct(0)]/Cs(0);$

$k3 = [Cst(0; 0) − Cs(0)]/Ct(0):$

*here Cs means sill

- Product Sum Model:

$Cst(hs; ht) = k1Cs(hs)Ct(ht) + k2Cs(hs) + k3Ct(ht)$

$\gamma st(hs; ht) = [k2 + k1Ct(0)]\gamma s(hs) + [k3 + k1Cs(0)]\gamma t(ht) − k1\gamma s(hs)\gamma t(ht)$

*Assume: $Ks=1=(K2+K1Ct(0))$
        $Kt=1=(K3+K1Cs(0))$

Variograms

- Semi-variogram (temporal):
  - $\gamma (h;\theta)=C_0+C_s[(3/2)(h/a_s)-(1/2)(h/a_s)^3]$
    $=0.0619 +0.20594[(3/2)(h/3.649)-(1/2)(h/3.649)^3]$

- Semi-variogram (Spatial):
  - $\gamma (h;\theta)=C_0+C_s[(3/2)(h/a_s)-(1/2)(h/a_s)^3]$
    $= 0.03245+0.21590[(3/2)(h/38.83)-(1/2)(h/38.83)^3]$
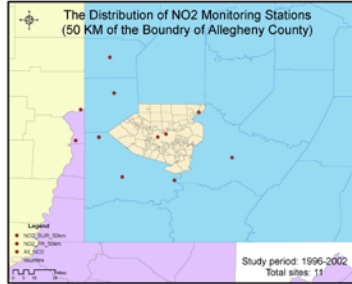
- Step2: Calculate Product Sum Model:
  - $\gamma st(hs; ht) = [k2 + k1Ct(0)]\gamma s(hs) + [k3 + k1Cs(0)]\gamma t(ht) − k1\gamma s(hs)\gamma t(ht)$
    $= \gamma s(hs) + \gamma t(ht) − 3.5507 \gamma s(hs)\gamma t(ht)$

A.2   SPACE-TIME VARIOGRAM-PM$_{2.5}$

Distribution of PM$_{2.5}$ monitoring sites
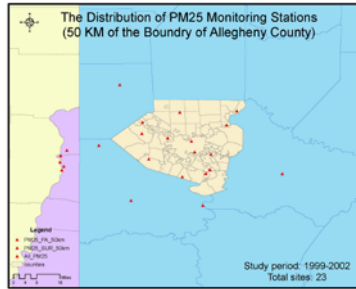
Semi-variogram: Spherical model

- If h=0 then γ (h;θ)=0;
- If 0< h $\leq$ a$_s$ then γ (h;θ)=C$_0$+C$_s$[(3/2)(h/a$_s$)-(1/2)(h/a$_s$)^3];
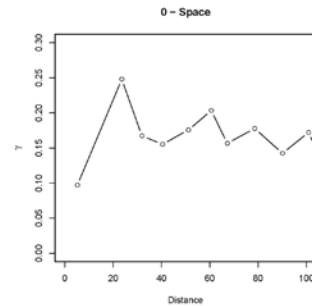- If h > a$_s$ then γ (h;θ)=C$_0$+C$_s$
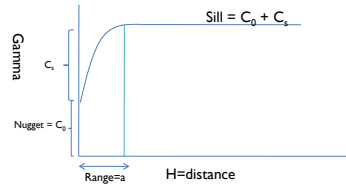
Spatial Semivariogram
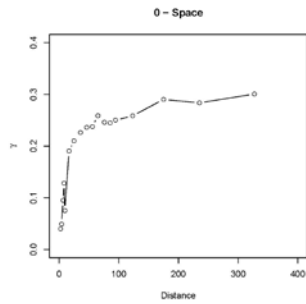
Spatial Semivariogram (Cont.)
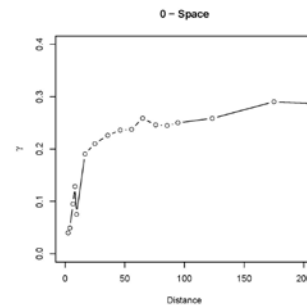
Spatial Semivariogram (Cont.)

Results from model fit:
```
  model      psill        range
1  Nug  0.01535773   0.00000
2  Sph  0.23274368  31.97886
```

Nugget effect=0.07657
Sill=(0.01536+0.2327)=0.24806
Range=31.97886 (Km)
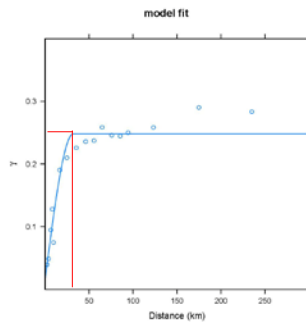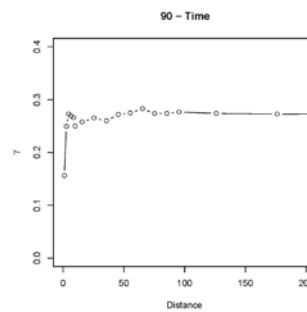
Temporal Semivariogram

Temporal Semivariogram (Cont.)

90 – Time
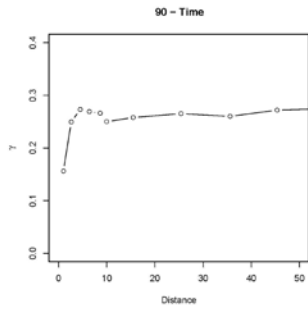
Temporal Semivariogram (Cont.)

model fit

Results from model fit:

	model	psill	range
1	Nug	0.07959511	0.000000
2	Sph	0.18841311	3.602777

Nugget effect= 0.07959511
Sill= 0.079595+0.188413=0.268
Range=3.603 (days)

7

8
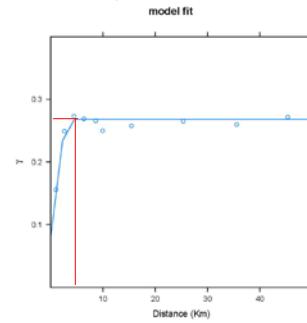
Spatial-Temporal Semi-variogram (contour)
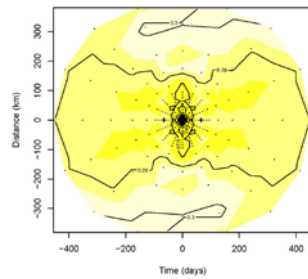
Spatial-Temporal Semi-variogram

Nugget effect
= 0.08
Sill=0.28
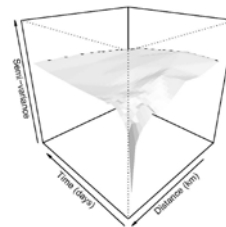
9

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Spatiotemporal variogram model:

- Coefficients

k1 = [Cs(0) + Ct(0) − Cst(0; 0)]/Cs(0)Ct(0);

k2 = [Cst(0; 0) − Ct(0)]/Cs(0);

k3 = [Cst(0; 0) − Cs(0)]/Ct(0):

- Product Sum Model:

Cst(hs; ht) = k1Cs(hs)Ct(ht) + k2Cs(hs) + k3Ct(ht)

γst(hs; ht) = [k2 + k1Ct(0)]γs(hs) + [k3 + k1Cs(0)]γt(ht) − k1γs(hs)γt(ht)

*Assume: Ks=1=(K2+K1Ct(0))
          Kt=1=(K3+K1Cs(0))

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Variograms

- <u>Semi-variogram (temporal):</u>
  - $\gamma\,(h;\theta)=C_0+C_s[(3/2)(h/a_s)-(1/2)(h/a_s)^3]$
    $=0.0796 +0.1884[(3/2)(h/3.603)-(1/2)(h/3.603)^3]$

- <u>Semi-variogram (Spatial):</u>
  - $\gamma\,(h;\theta)=C_0+C_s[(3/2)(h/a_s)-(1/2)(h/a_s)^3]$
    $= 0.0154+0.2327[(3/2)(h/31.979)-(1/2)(h/31.979)^3]$

- <u>Step2: Calculate Product Sum Model:</u>
  - $\gamma st(hs; ht) = [k2 + k1Ct(0)]\gamma s(hs) + [k3 + k1Cs(0)]\gamma t(ht) − k1\gamma s(hs)\gamma t(ht)$
    $= \gamma s(hs) + \gamma t(ht) − 3.5508\,\gamma s(hs)\gamma t(ht)$

12

A.3   SPACE-TIME VARIOGRAM-CO

Distribution of CO monitoring sites
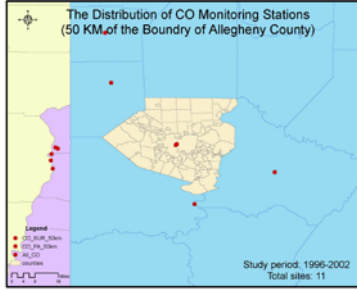
Semi-variogram: Spherical model

- If h=0 then $\gamma(h;\theta)=0$;
- If $0 < h \leq a_s$ then $\gamma(h;\theta)=C_0+C_s[(3/2)(h/a_s)-(1/2)(h/a_s)^3]$;
- If $h > a_s$ then $\gamma(h;\theta)=C_0+C_s$
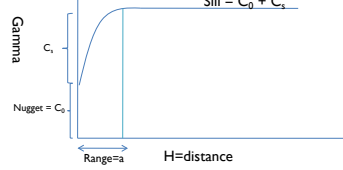
Spatial Semivariogram
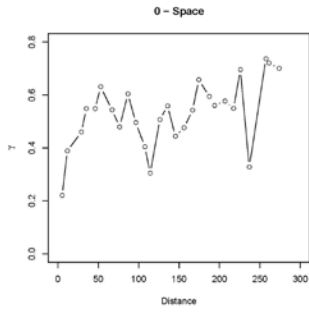
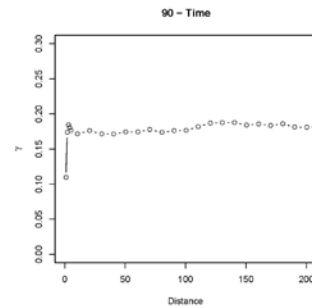## Spatial Semivariogram (Cont.)
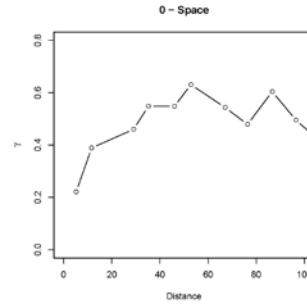
Spatial Semivariogram (Cont.)

Results from model fit:

model	psill	range
Nug	0.06272875	0.00000
Sph	0.46227815	22.50751

Nugget effect= 0.06272875
Sill= 0.06272875 + 0.46227815
  = 0.5250069
Range= 22.50751 (km)
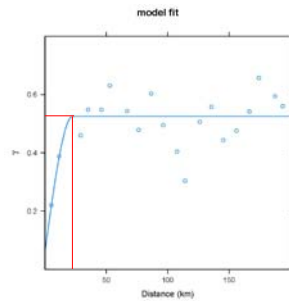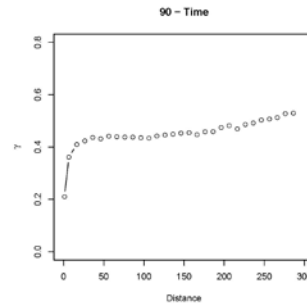
Temporal Semivariogram

Temporal Semivariogram (Cont.)

90 – Time — plot with Distance on x-axis and γ on y-axis
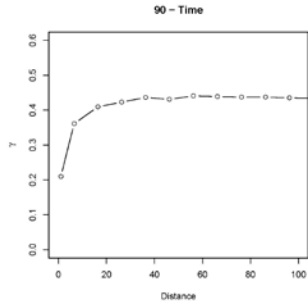
Temporal Semivariogram (Cont.)

Results from model fit:

model	psill	range
Nug	0.1787978	0.00000
Sph	0.2481167	12.06138

Nugget effect= 0.1787978
Sill= 0.1787978 + 0.2481167
   = 0.4269145
Range= 12.06138 (days)
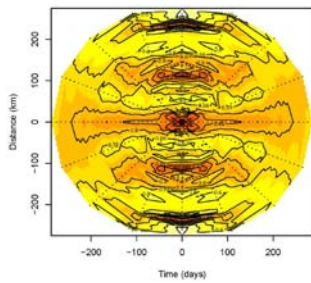
model fit — plot with Distance (km) on x-axis

Spatial-Temporal Semi-variogram (contour)

Spatial-Temporal Semi-variogram

Sill=0.6

Spatiotemporal variogram model:

- Coefficients

$k1 = [Cs(0) + Ct(0) − Cst(0; 0)]/Cs(0)Ct(0);$

$k2 = [Cst(0; 0) − Ct(0)]/Cs(0);$

$k3 = [Cst(0; 0) − Cs(0)]/Ct(0):$

*here Cs means sill

- Product Sum Model:

$Cst(hs; ht) = k1Cs(hs)Ct(ht) + k2Cs(hs) + k3Ct(ht)$

$γst(hs; ht) = [k2 + k1Ct(0)]γs(hs) + [k3 + k1Cs(0)]γt(ht) − k1γs(hs)γt(ht)$

*Assume: Ks=1=(K2+K1Ct(0))
         Kt=1=(K3+K1Cs(0))

Variograms

- <u>Semi-variogram (temporal):</u>
  - $γ(h;θ)=C_0+C_s[(3/2)(h/a_s)-(1/2)(h/a_s)^3]$
    $=0.1787978 +0.2481167[(3/2)(h/12.06318)-(1/2)(h/12.06318)^3]$

- <u>Semi-variogram (Spatial):</u>
  - $γ(h;θ)=C_0+C_s[(3/2)(h/a_s)-(1/2)(h/a_s)^3]$
    $= 0.06272875+0.46227815[(3/2)(h/22.5075)-(1/2)(h/22.5075)^3]$

- <u>Step2: Calculate Product Sum Model:</u>
  - $γst(hs; ht) = [k2 + k1Ct(0)]γs(hs) + [k3 + k1Cs(0)]γt(ht) − k1γs(hs)γt(ht)$
 $= γs(hs) + γt(ht) − 1.570 γs(hs)γt(ht)$

Distribution of NO$_2$ monitoring sites

Semi-variogram: Spherical model

- If h=0 then γ (h;θ)=0;
- If 0< h $\leq$ a$_s$ then γ (h;θ)=C$_0$+C$_s$[(3/2)(h/a$_s$)-(1/2)(h/a$_s$)^3];
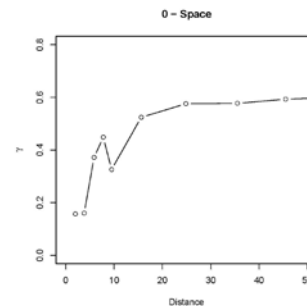- If h > a$_s$ then γ (h;θ)=C$_0$+C$_s$
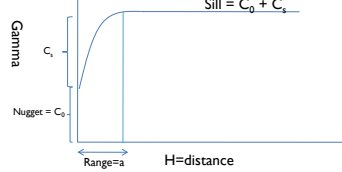
Spatial Semivariogram
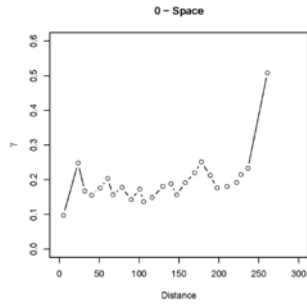
Spatial Semivariogram (Cont.)

Spatial Semivariogram (Cont.)

Results from model fit:

model	psill	range
Nug	0.06799291	0.0000
Sph	0.10314293	22.3977

Nugget effect= 0.06799291
Sill= 0.06799291 + 0.10314293
     = 0.17113581
Range= 22.3977 (km)

Temporal Semivariogram

Temporal Semivariogram (Cont.)

90 − Time

Temporal Semivariogram (Cont.)

model fit

Results from model fit:

model	psill	range
Nug	0.002035792	0.000000
Sph	0.175976225	2.304466

Nugget effect= 0.002035792
Sill= 0.002035792 + 0.175976225
= 0.178
Range= 2.304466 (days)

Spatial-Temporal Semi-variogram (contour)

Spatial-Temporal Semi-variogram

Sill=0.25
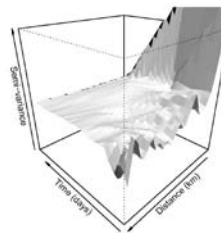
Spatiotemporal variogram model:

- Coefficients

$k1 = [Cs(0) + Ct(0) − Cst(0; 0)]/Cs(0)Ct(0);$

$k2 = [Cst(0; 0) − Ct(0)]/Cs(0);$

$k3 = [Cst(0; 0) − Cs(0)]/Ct(0):$

*here Cs means sill

- Product Sum Model:

$Cst(hs; ht) = k1Cs(hs)Ct(ht) + k2Cs(hs) + k3Ct(ht)$

$γst(hs; ht) = [k2 + k1Ct(0)]γs(hs) + [k3 + k1Cs(0)]γt(ht) − k1γs(hs)γt(ht)$

*Assume: Ks=1=(K2+K1Ct(0))
Kt=1=(K3+K1Cs(0))

Variograms

- <u>Semi-variogram (temporal):</u>
  - $γ(h;θ)=C_0+C_s[(3/2)(h/a_s)-(1/2)(h/a_s)^3]$
    $=0.0020 +0.175976[(3/2)(h/2.304)-(1/2)(h/2.304)^3]$

- <u>Semi-variogram (Spatial):</u>
  - $γ(h;θ)=C_0+C_s[(3/2)(h/a_s)-(1/2)(h/a_s)^3]$
    $= 0.06799291+0.10314293[(3/2)(h/22.3977)-(1/2)(h/22.3977)^3]$

- <u>Step2: Calculate Product Sum Model:</u>
  - $γst(hs; ht) = [k2 + k1Ct(0)]γs(hs) + [k3 + k1Cs(0)]γt(ht) − k1γs(hs)γt(ht)$
    $= γs(hs) + γt(ht) − 3.254 γs(hs)γt(ht)$

A.5 SPACE-TIME VARIOGRAM-SO$_2$

Distribution of SO$_2$ monitoring sites
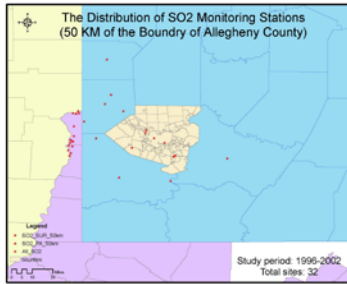
Semi-variogram: Spherical model

- If h=0 then γ (h;θ)=0;
- If 0< h ≤ a$_s$ then γ (h;θ)=C$_0$+C$_s$[(3/2)(h/a$_s$)-(1/2)(h/a$_s$)^3];
- If h > a$_s$ then γ (h;θ)=C$_0$+C$_s$
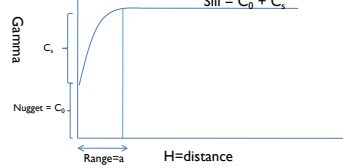
Spatial Semivariogram
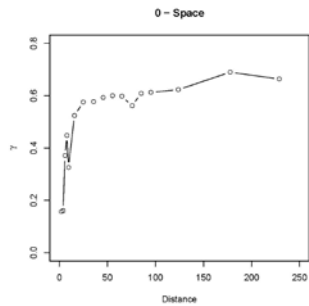
Spatial Semivariogram (Cont.)

Spatial Semivariogram (Cont.)

Results from model fit:
  model   psill   range
  Nug   0.07852457   0.00000
  Sph   0.51667000   20.15494

Nugget effect= 0.07852457
Sill= 0.07852457 + 0.51667000
   = 0.59519
Range= 20.15494 (km)

Temporal Semivariogram

Temporal Semivariogram (Cont.)

90 – Time
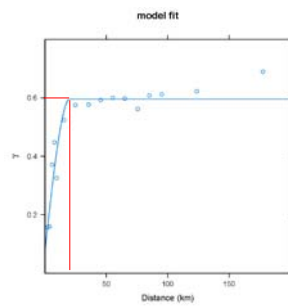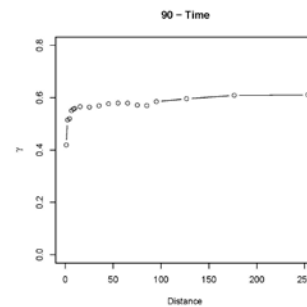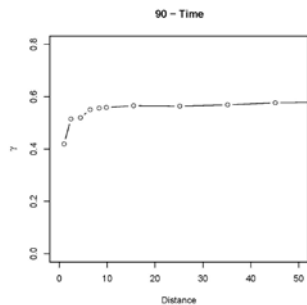
7

Temporal Semivariogram (Cont.)

model fit

Results from model fit:

model	psill	range
Nug	0.3367861	0.000000
Sph	0.2200411	3.935355

Nugget effect= 0.3367861
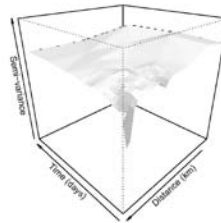Sill= 0.3367861 + 0.2200411
  = 0.556827
Range= 3.935355(days)

8
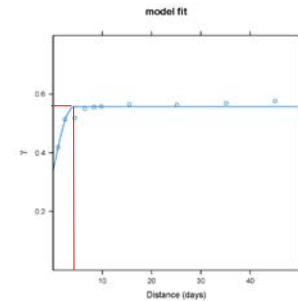
Spatial-Temporal Semi-variogram (contour)
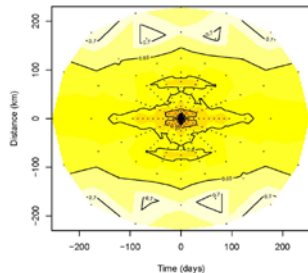
9

Spatial-Temporal Semi-variogram

Sill=0.65

10

Spatiotemporal variogram model:

- Coefficients

$k1 = [Cs(0) + Ct(0) - Cst(0; 0)]/Cs(0)Ct(0);$

$k2 = [Cst(0; 0) - Ct(0)]/Cs(0);$

$k3 = [Cst(0; 0) - Cs(0)]/Ct(0):$

*here Cs means sill

- Product Sum Model:

$Cst(hs; ht) = k1Cs(hs)Ct(ht) + k2Cs(hs) + k3Ct(ht)$

$\gamma st(hs; ht) = [k2 + k1Ct(0)]\gamma s(hs) + [k3 + k1Cs(0)]\gamma t(ht) - k1\gamma s(hs)\gamma t(ht)$

*Assume: Ks=1=(K2+K1Ct(0))
        Kt=1=(K3+K1Cs(0))

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Variograms

- Semi-variogram (temporal):
  - $\gamma (h;\theta)=C_0+C_s[(3/2)(h/a_s)-(1/2)(h/a_s)^3]$
    $=0.336786 +0.220041[(3/2)(h/3.935)-(1/2)(h/3.935)^3]$

- Semi-variogram (Spatial):
  - $\gamma (h;\theta)=C_0+C_s[(3/2)(h/a_s)-(1/2)(h/a_s)^3]$
    $= 0.0785+0.51667[(3/2)(h/20.1549)-(1/2)(h/20.1549)^3]$
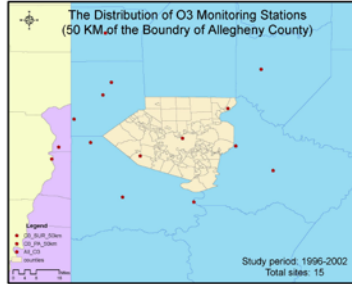
- Step2: Calculate Product Sum Model:
  - $\gamma st(hs; ht) = [k2 + k1Ct(0)]\gamma s(hs) + [k3 + k1Cs(0)]\gamma t(ht) - k1\gamma s(hs)\gamma t(ht)$
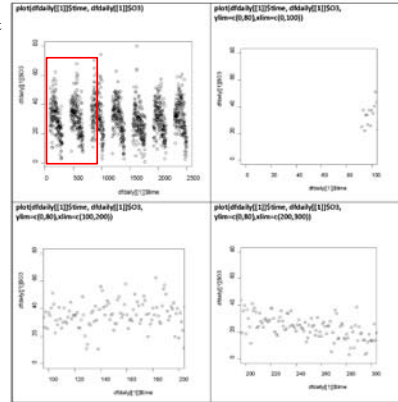 $= \gamma s(hs) + \gamma t(ht) - 1.514755 \gamma s(hs)\gamma t(ht)$

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Distribution of O$_3$ monitoring sites

O3 time series plot

Divided O$_3$ to separate time periods

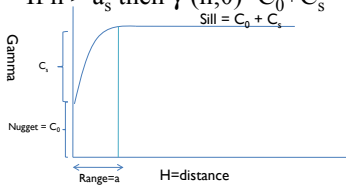| Time period	Number of measurement	Number of sites
91-305	6204	30
457-670	6822	34
822-1035	7075	34
1187-1400	7466	35
1553-1766	7661	36
1918-2131	7875	37
2283-2496	7442	35

Calculate overall variogram

- Calculate variogram for each subset and each direction by weight number of pairs
- Sum of gammas
- Average the distance
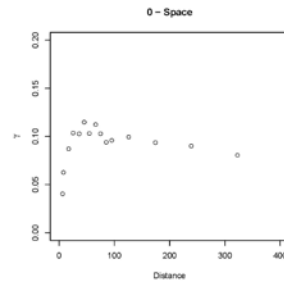- Calculate time- , space-, and time-space variograms in R

Semi-variogram: Spherical model

- If h=0 then γ (h;θ)=0;
- If 0< h $\leq$ a$_s$ then γ (h;θ)=C$_0$+C$_s$[(3/2)(h/a$_s$)-(1/2)(h/a$_s$)^3];
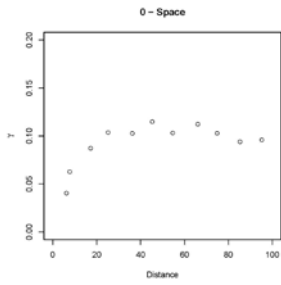- If h > a$_s$ then γ (h;θ)=C$_0$+C$_s$

Spatial Semivariogram

Spatial Semivariogram (Cont.)
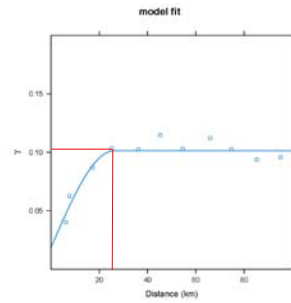
0 - Space

7

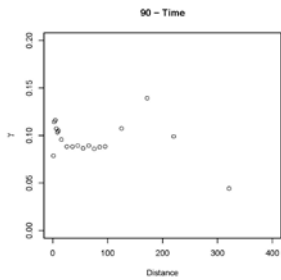## Spatial Semivariogram (Cont.)

model fit

Results from model fit:
  model   psill   range
1 Nug 0.01857383 0.00000
2 Sph 0.08276187 26.09189

Nugget effect= 0.01857383
Sill= 0.01857383 + 0.08276187
   = 0.101336
Range= 26.09189 (km)

8

Temporal Semivariogram

90 - Time

9

Temporal Semivariogram (Cont.)
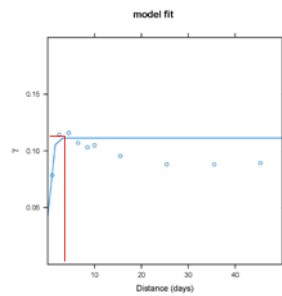
90 - Time
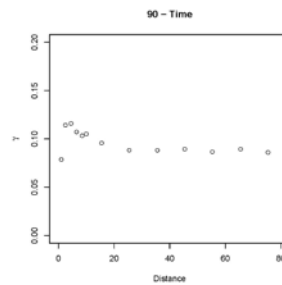
10

Temporal Semivariogram (Cont.)
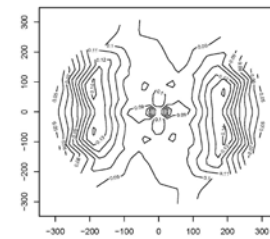
model fit

Results from model fit:
  model   psill   range
1 Nug 0.04131764 0.000000
2 Sph 0.07002842 2.147153

Nugget effect= 0.04131764
Sill= 0.04131764 + 0.07002842
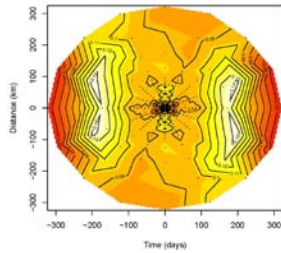   = 0.111346
Range= 2.147153 (days)

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Spatial-Temporal Semi-variogram (contour)

12

100

Spatial-Temporal Semi-variogram (contour)

Spatial-Temporal Semi-variogram

Sill=0.15
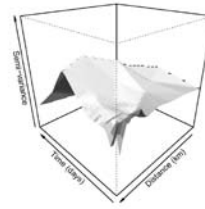
Spatiotemporal variogram model:

- Coefficients

$k1 = [Cs(0) + Ct(0) − Cst(0; 0)]/Cs(0)Ct(0);$

$k2 = [Cst(0; 0) − Ct(0)]/Cs(0);$

$k3 = [Cst(0; 0) − Cs(0)]/Ct(0):$

*here Cs means sill

- Product Sum Model:

$Cst(hs; ht) = k1Cs(hs)Ct(ht) + k2Cs(hs) + k3Ct(ht)$

$\gamma st(hs; ht) = [k2 + k1Ct(0)]\gamma s(hs) + [k3 + k1Cs(0)]\gamma t(ht) − k1\gamma s(hs)\gamma t(ht)$

*Assume: Ks=1=(K2+K1Ct(0))
    Kt=1=(K3+K1Cs(0))

Variograms

- <u>Semi-variogram (temporal):</u>
  - $\gamma(h;\theta)=C_0+C_s[(3/2)(h/a_s)-(1/2)(h/a_s)^3]$
    $=0.0413 +0.07[(3/2)(h/2.147)-(1/2)(h/2.147)^3]$

- <u>Semi-variogram (Spatial):</u>
  - $\gamma(h;\theta)=C_0+C_s[(3/2)(h/a_s)-(1/2)(h/a_s)^3]$
    $= 0.01857 +0.0828[(3/2)(h/26.09)-(1/2)(h/26.09)^3]$

- <u>Step2: Calculate Product Sum Model:</u>
  - $\gamma st(hs; ht) = [k2 + k1Ct(0)]\gamma s(hs) + [k3 + k1Cs(0)]\gamma t(ht) − k1\gamma s(hs)\gamma t(ht)$
    $= \gamma s(hs) + \gamma t(ht) − 5.56\ \gamma s(hs)\gamma t(ht)$

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