# Effects of Air Pollution on Biomarkers of Obesity and Oxidative Stress in Children from the San Joaquin Valley, CA

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## ABSTRACT

Air pollution is widely known to contribute to the development of chronic health conditions, such as respiratory and cardiac diseases, through mechanisms involving oxidative stress and inflammation. However, its effects on obesity, a prevalent health disorder defined by excess accumulation of body fat and oxidative stress, have yet to be extensively reviewed. Adipose tissue secretes adipokines, including adiponectin and leptin, which have inflammatory and hormonal functions contributing to increased levels of reactive oxygen species. Imbalance of these reactive oxygen species, or oxidative stress, can be measured via isoprostanes, providing insight into the relationship between pollutant exposure and obesity outcomes. This project aims to analyze the biomarkers of obesity and oxidative stress in order to determine the influence of air pollution in a cohort of children (N=210) living in the San Joaquin Valley, CA, an area with notoriously high levels of pollution. Urinary isoprostane and plasma leptin and adiponectin were quantified using enzyme-linked immunosorbent assays (ELISAs). Findings showed that leptin levels are positively and adiponectin levels are negatively associated with BMI ($p<0.001$). Isoprostane was found to be positively associated with daily PAH exposure ($p=0.03$) and with weekly PAH exposure ($p=0.04$). These results demonstrate that weight status is related to adipokines concentrations. Furthermore, air pollution exposure may influence oxidative stress and weight status, suggesting the need for further investigation of the role of air pollution in affecting biomarkers of obesity and oxidative stress and the development of obesity.

## KEYWORDS

adipokines, Children's Health and Air Pollution Study (CHAPS), isoprostane, ambient air pollution, oxidative stress, obesity

## INTRODUCTION

The health effects of ambient air pollution have been the target of much scientific inquiry. Worldwide, air pollution is estimated to be responsible for several millions of deaths per year, most of which are attributed to cardiovascular causes (Miller 2014). Short term exposure to airborne pollutants has increased hospital admissions and doctors visits for respiratory and cardiovascular illnesses in adults, as well as diagnoses of respiratory and pulmonary dysfunction in children (Katsouyanni et al. 1996, Chan-Yeung 2000, Padula et al. 2015). Such outcomes resonate especially in the San Joaquin Valley (SJV) of California, a region where air pollution concentrations are regularly above federal clean air standards and youth experience some of the highest asthma rates in the nation (Central California Asthma Collaborative n.d., US EPA n.d.). The social, economic and environmental factors classify the SJV as highly subject to environmental hazards, health risks, concentrated poverty, which together result in social vulnerability (Huang and London 2012).

Considering that many pollutants are potent oxidants (Lodovici and Bigagli 2011), potential mechanisms of the aforementioned health effects involve oxidative stress and inflammation. This is supported by studies reporting that ambient levels of particulate matter 2.5 (PM2.5) and benzopyrene (a polycyclic aromatic hydrocarbon (PAH)) were significant predictors of oxidative stress in a cohort of mothers and newborns (Ambroz et al. 2016). My study aims to examine the health implications of air pollution exposure with oxidative stress serving as a mechanistic link between the environmental pollutant and the health outcome.

Like air pollution, obesity poses a major public health issue, affecting approximately one in six children in the United States (Ogden et al. 2012). Obesity is characterized by an increase in body weight and excessive accumulation of adipose tissue due to an energy imbalance (Marseglia et al. 2014). Factors that influence this balance include genetics, dietary habits, geographical location, social behavior and socioeconomic status ("Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults: The Evidence Report - NHLBI, NIH" n.d.). Obesity is further characterized by a state of chronic inflammation (Marseglia et al. 2014). This inflammation can come about in two ways; first, obesity promotes the inflammation of adipose tissue through lipid peroxidation, which is modulated by adipokine hormones (Bouloumié et al. 1999, Nakanishi et al. 2005). Alternatively, initial weight gain

triggers an inflammatory response and prompts macrophages to infiltrate adipose tissue, directly contributing to and perpetuating the inflammatory state of fat (Weisberg et al. 2003, Johnson et al. 2012). Given the prevalence of obesity, there is a need to better understand its causes.

Inflammation resulting from oxidative stress, or the imbalance of free radical production and the body's ability to counter these molecules' harmful effect, may serve as a link between exposure to air pollutants and the development of obesity. Epidemiological evidence suggests that air pollution is a risk factor for childhood obesity, and that markers of ambient air pollution are associated with biomarkers of oxidative stress (Li et al. 2016, Wei et al. 2016). Obesity may be mediated by an inflammatory response triggered by pollutants (Xu et al. 2010, Møller et al. 2014). This study relies on the measurement of leptin, adiponectin and isoprostane; the former two are hormones produced by adipose tissue that can be used to characterize obesity status. Leptin is known to regulate satiety through communication with the hypothalamus, and increases in proportion to body fat content (Moran and Phillip 2003, Volberg 2013). Adiponectin serves in the regulation of blood glucose levels and combats free radical production from lipid peroxidation (Behre 2007). Generally, adiponectin decreases alongside body fat (Cnop et al. 2003, Volberg 2013). Isoprostane is a class of products formed by the free radical-catalyzed peroxidation of arachidonic acid (Montuschi et al. 2004). Isoprostane is one of the most reliable indicators of oxidative stress in vivo, and can serve as a proxy of total oxidative stress measurement (Roberts II and Morrow 2000, Montuschi et al. 2000). Studying these biomarkers can therefore provide valuable insight into the relationship between ambient pollution exposure and obesity development.

Air pollution has known influence on public health, particularly respiratory and cardiac health, and may have impacts on the development of obesity. As obesity is related to chronic inflammation and oxidative stress, isoprostane can serve as a useful biomarker and mediate a causal pathway between air pollution exposure and obesity development. This research addresses this little-studied intersection between ambient air pollution and obesity using biological samples from child participants in the Children's Health and Air Pollution Study (CHAPS) and the Children's Environmental Health Center at the University of California, Berkeley and Stanford University (PI-Hammond). This research seeks to: characterize the cohort by determining of the effect of age on isoprostane and identifying any sex differences for isoprostane; assess the relationship between isoprostane and weight status; evaluate the relationship between isoprostane

and air pollution exposure; and evaluate the relationship between weight status and air pollution exposure.

## METHODS

### Study design and population

The Children's Health and Air Pollution Study (CHAPS) is a longitudinal study examining the effects of ambient air pollution on allergic disorders and metabolic syndrome. The objectives of the study are focused on immune- and metabolism-related health outcomes. In order to investigate the impact of air pollution on immune function and metabolism, CHAPS uses a "piece-wise natural history" design in their longitudinal study (Mann n.d.). All study subjects are recruited into one of three cohorts, with each representing a different age group: infants (20$^{th}$ week of pregnancy-2 years old), children (6-9 years old), and adolescents and young adults (12-23 years old). This project is focused on analyses in children, since is known that they are most susceptible to air pollutants (Committee on Environmental Health 2004).

Subjects for this project included 210 participants from the San Joaquin Valley, CA - an area known to have high air pollution and high social vulnerability (Huang and London 2012). All participants speak and are literate in English or Spanish, are currently non-smokers, and do not have a history of autoimmune disease, HIV, or cancer. Assessments from a single visit at UCSF Fresno Clinical Research Center were performed for this cohort. Urine, buccal cells, and 50 ml of blood were collected from each participant during the single visit. Anthropometry was collected, and a baseline interview and questionnaires were administered in order to obtain baseline historical, dietary, and residential information.

### Air pollution exposure data

Hourly, quality-assured, ambient air quality and meteorological data collected at the Fresno "Supersite" were obtained from California Air Resources Board (Watson et al. 2000). Daily pollutant exposures were assigned to participants based on measurements at the Supersite. We used the average 24-hour exposure for the day of the subjects' visit and the average exposure

values during the 1-week and 12 months prior to the visit for PM2.5 mass (micrograms per cubic meter), elemental carbon (EC; micrograms per cubic meter), and PAH456 (nanograms per cubic meter). Black carbon (BC) was determined from aethalometer (model AE42; Magee Scientific, Berkeley, CA) measurements of the optical absorption of PM2.5 ambient aerosol at 880 nm. EC concentrations were estimated from these BC measurements (EC = 1.19 × BC) (Chow et al. 1993). Pollutant data were subject to checks for quality assurance, including range checks, comparison of values at nearby monitoring sites, and consistency with historical temporal and/or diurnal patterns for each pollutant.

**Anthropometric data**

Individual height and weight for each participant were measured according to the NHANES Anthropometry Protocol. Height, without shoes, was measured in meters using a stadiometer with a fixed vertical backboard and adjustable headpiece. Weight was measured in kilograms using a Tanita scale. All measurements were measured in duplicate or triplicate and averaged. The averaged values were used to calculate body mass index (BMI) in weight (kg) / [height (m)]$^2$. Children were categorized into weight status categories (underweight, normal, overweight, and obese) according to their BMI percentile rankings using age-specific and gender-specific BMI cutoffs from the 2000 Center for Disease Control and Prevention child growth charts and NHANES growth curves.

**Adipokine data**

Whole blood samples from participants were collected and processed by field personnel. Plasma samples were aliquoted and stored at -80ºC until analysis at the Center for Environmental Research and Children's Health (CERCH). Adiponectin and leptin concentrations were measured using RayBio® Human Adiponection and RayBio® Human Leptin ELISA kits, respectively. For leptin analysis, plasma was diluted further than recommended in the standard protocol provided by RayBio® for a wider range of detection. Final dilutions for adiponectin and leptin were 1:30,000 and 1:70, respectively. The same internal controls were included on each

plate for quality assurance and reproducibility between each experimental run. All samples were run in duplicate to ensure accuracy of results. Analysis used averaged paired measurements.

**Isoprostane data**

Urine samples from all participants were collected and stored at -80ºC until analysis at the CERCH. 15-isoprostane$_{2t}$ concentrations were measured using Oxford Biomedical Research® Urinary Isoprostane ELISA kits. The same internal controls were included on each plate for quality assurance and reproducibility between each experimental run, and all samples were run in duplicate in order to assure accuracy of the results. Paired values were averaged for analysis. Creatinine was also measured using Oxford Biomed Creatinine Microplate assays. Isoprostane concentrations were normalized in order to adjust for urinary dilution by dividing isoprostane values (ng/mL) by creatinine values (mg/dL).

**Statistical analysis**

Prior to analyses, leptin, adiponectin and isoprostane values were log transformed in order to achieve a normal distribution because these values were right-skewed. BMI values are represented by the BMI percentile of each participant for comparability between ages and sexes. Simple linear regressions were used to characterize associations between age and biomarker concentration. T-tests were used to examine differences in biomarker concentration by sex. Differences in biomarker concentration by participant's weight status were examined through analyses of variance (ANOVAs). Linear regressions were performed to assess associations between biomarker concentration and BMI. Additional regressions were used to examine the relationship between concentrations of biomarkers and each pollutant of interest, as well as the associations between BMI and each pollutant of interest. Stata Version 12.1 was used to conduct statistical analyses. A statistical significance level of $p<0.05$ was used.

# RESULTS

## Cohort summary

The participants were children, ranging from 6 to 9 years of age, of which 110 were boys 55%) and 95 were female (45%). Participants were categorized by weight status in accordance with their BMI percentiles. Accordingly, 2% of participants were underweight, 54% were normal weight, and 18% were overweight and 26% were obese. Most participants were Hispanic (77%), African American (13%), or White (6%). These characteristics of the CHAPS cohort are summarized in Table 1. Biomarker concentrations for the cohort are summarized in Table 2.

**Table 1. Characteristics of CHAPS Children.**

| | Total (N=210) | | |
|---|---|---|---|
| | **Mean** | **SD** | **%** |
| Gender | | | |
| Boys, N=115 | | | 54.8 |
| Girls, N=95 | | | 45.2 |
| Age, yr | 7.43 | 0.62 | |
| Percent Body Fat, % | 27.16 | 11.81 | |
| BMI Percentile | | | |
| Underweight (<5$^{th}$ percentile), N=5 | | | 2.3 |
| Normal (5$^{th}$ to < 85$^{th}$ percentile), N=116 | | | 54.0 |
| Overweight (85$^{th}$ to < 95$^{th}$ percentile), N=38 | | | 17.7 |
| Obese (≥ 95$^{th}$ percentile), N=56 | | | 26.1 |
| Race/Ethnicity | | | |
| Hispanic, N=167 | | | 77.3 |
| African American, N=29 | | | 13.4 |
| White, N=12 | | | 5.6 |
| Asian or Pacific Islander, N=8 | | | 3.7 |

**Table 2. Biomarker Levels Summary**

| | Total (N=210) | | Boys (N=115) | | Girls (N=95) | |
|---|---|---|---|---|---|---|
| | **Mean** | **SD** | **Mean** | **SD** | **Mean** | **SD** |
| Leptin, ng/ml | 1.69 | 1.98 | 1.31 | 1.69 | 2.14 | 2.19 |
| Adiponectin, µg/ml | 25.49 | 22.12 | 25.85 | 23.42 | 25.07 | 20.56 |
| Isoprostane, ng/mg* | 6.06 | 5.77 | 5.18 | 3.33 | 7.13 | 7.64 |

*Adjusted by creatinine, mg/dL

**Biomarker inter-relationships**

The relationships between leptin, adiponectin and isoprostane were investigated to reveal that leptin and adiponectin have a significant inverse association, but no association was observed between adipokines and isoprostane. Details of this analysis are summarized in Table 3.

**Table 3. Inter-relationships between biomarkers.**

| | **$Log_{10}$(Adiponectin)** | | **$Log_e$(Isoprostane)** | |
|---|---|---|---|---|
| | **p-value** | **$R^2$** | **p-value** | **$R^2$** |
| $Log_{10}$(Leptin) | 0.04 | 0.02 | 0.53 | 0.002 |
| $Log_{10}$(Adiponectin) | - | - | 0.49 | 0.003 |

*Adjusted by creatinine, mg/dL

**Biomarkers and age**

There was no significant difference by age for either leptin, adiponectin, or isoprostane and age. Age was analyzed as a continuous variable. These relationships between biomarker concentrations and age are shown in Table 4.

**Table 4. Association between isoprostane and age.**

| **Variables** | **β** | **SE** | **95% Confidence Interval** | **p-value** |
|---|---|---|---|---|
| $Log_{10}$(Leptin) | 0.06 | 0.04 | (-0.01, 0.13) | 0.09 |
| $Log_{10}$(Adiponectin) | -0.04 | 0.05 | (-0.13. 0.05) | 0.36 |
| $Log_e$(Isoprostane*) | -0.02 | 0.08 | (-0.17, 0.14) | 0.82 |

*Adjusted by creatinine, mg/dL

**Biomarkers and sex differences**

A significant association between leptin concentrations and sex was observed ($p<0.001$). Girls had higher levels of leptin after log transformation. No significant differences were observed between adiponectin or isoprostane levels by gender (Table 5).

**Table 5. Biomarkers by sex of children.**

| | Boys (N=115) | | Girls (N=95) | | |
|---|---|---|---|---|---|
| **Variables** | **Mean** | **SD** | **Mean** | **SD** | **p-value** |
| $Log_{10}$(Leptin) | -0.003 | 0.26 | 0.18 | 0.33 | <0.001 |
| $Log_{10}$(Adiponectin) | 1.24 | 0.41 | 1.25 | 0.39 | 0.98 |
| $Log_e$(Isoprostane*) | 1.18 | 0.70 | 1.13 | 0.72 | 0.57 |

*Adjusted by creatinine, mg/dL

## Biomarkers and BMI

Significant associations between leptin and adiponectin concentration and BMI category were observed ($p<0.001$ for both), but this was not the case for isoprostane ($p=0.78$) (Table 6).

**Table 6. Distributions of leptin, adiponectin, and isoprostane by weight group.**

| | Underweight (N=5) | | Normal (N=116) | | Overweight (N=38) | | Obese (N=56) | | |
|---|---|---|---|---|---|---|---|---|---|
| **Variables** | **Mean** | **SD** | **Mean** | **SD** | **Mean** | **SD** | **Mean** | **SD** | **p-value** |
| Leptin, ng/ml | 0.60 | 0.08 | 0.89 | 0.32 | 1.26 | 0.70 | 3.79 | 3.00 | <0.001 |
| Adiponectin, µg/ml | 20.71 | 13.42 | 29.49 | 22.29 | 23.00 | 23.09 | 18.48 | 19.99 | <0.001 |
| Isoprostane, ng/mg* | 5.27 | 2.87 | 5.59 | 4.00 | 5.70 | 3.50 | 7.47 | 9.41 | 0.78 |

*Adjusted by creatinine, mg/dL

Normal weight children had average leptin values of 0.89 ng/mL, while overweight participants averaged 1.26 ng/mL, and obese 3.79 ng/mL. The positive relationship between leptin and BMI category is illustrated in Figure 1.

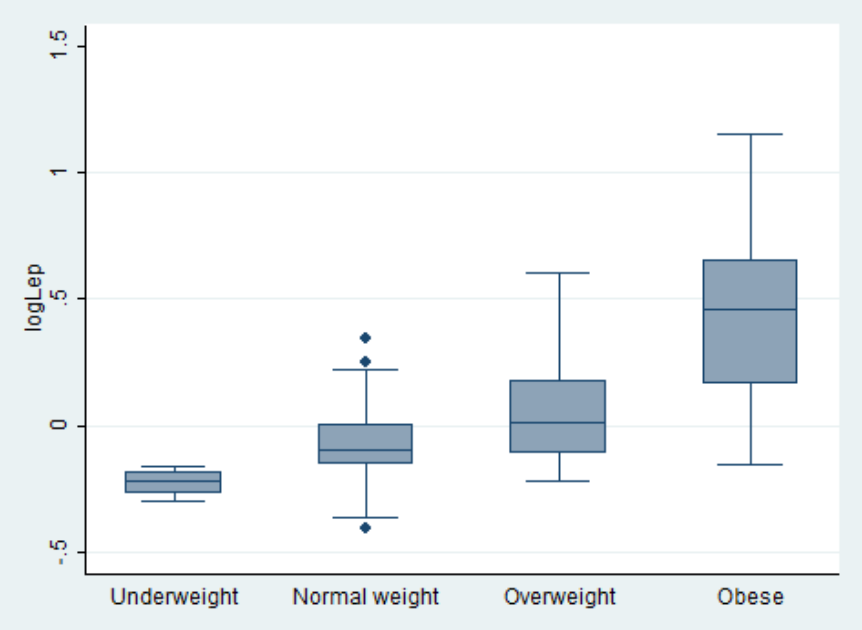


**Figure 1. Association between leptin and BMI category (underweight/normal/overweight/obese).**

Normal weight individuals had average adiponectin values of 29.49 ug/mL, overweight individuals had values averaging 23.00 ug/mL, and obese individuals had values averaging 18.48 ug/mL. This negative association between adiponectin and weight status is illustrated in Figure 2.

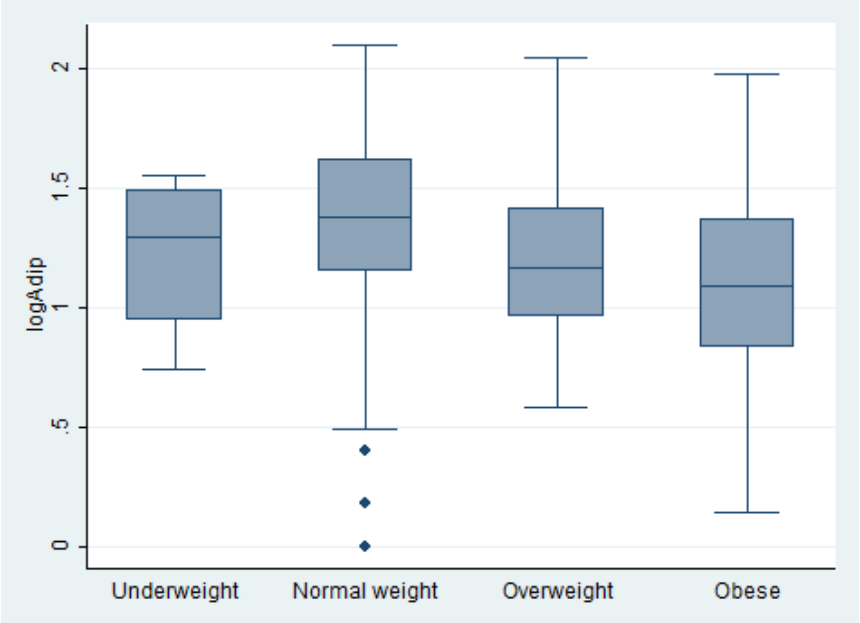


**Figure 2. Association between adiponectin and BMI category (underweight/normal/overweight/obese).**

BMI was also evaluated as a continuous variable. Findings of this analysis similarly revealed a positive association between leptin and BMI ($p<0.001$) and a negative association between adiponectin and BMI ($p=0.01$). No significant relationship between isoprostane and BMI was found. Results are shown in Table 7.

**Table 7. Associations between biomarkers and BMI.**

| | Total | | Boys | | Girls | |
|---|---|---|---|---|---|---|
| **Variables** | **β** | **p-value** | **β** | **p-value** | **β** | **p-value** |
| $Log_{10}$(Leptin) | 0.01 | <0.001* | 0.005 | <0.001* | 0.007 | <0.001* |
| $Log_{10}$(Adiponectin) | -0.003 | 0.01* | -0.002 | 0.25 | -0.004 | 0.02* |
| $Log_e$(Isoprostane) | 0.002 | 0.40 | -0.001 | 0.78 | 0.004 | 0.13 |

## Biomarkers and air pollution exposure

The relationship between leptin, adiponectin and isoprostane concentrations and air pollution exposure are summarized in Tables 8-10. Significant relationships were observed between isoprostane and daily PAH exposure, and isoprostane and weekly PAH exposure.

**Table 8. Association between biomarkers and polycyclic aromatic hydrocarbon (PAH) exposure.**

| **PAH Mean Day-of Exposure (ng/m3)** | **Total** | | **Boys** | | **Girls** | |
|---|---|---|---|---|---|---|
| **Variables** | **β** | **p-value** | **β** | **p-value** | **β** | **p-value** |
| $Log_{10}$(Leptin) | -0.58 | 0.57 | -0.90 | 0.56 | -1.05 | 0.50 |
| $Log_{10}$(Adiponectin) | 0.26 | 0.74 | 1.00 | 0.31 | -0.68 | 0.59 |
| $Log_e$(Isoprostane) | 0.95 | 0.03* | 0.74 | 0.23 | 1.11 | 0.08 |
| **PAH Mean Week Exposure (ng/m3)** | **Total** | | **Boys** | | **Girls** | |
| **Variables** | **β** | **p-value** | **β** | **p-value** | **β** | **p-value** |
| $Log_{10}$(Leptin) | -0.83 | 0.44 | -0.74 | 0.68 | -1.17 | 0.43 |
| $Log_{10}$(Adiponectin) | 1.05 | 0.20 | 1.90 | 0.09 | -0.03 | 0.98 |
| $Log_e$(Isoprostane) | 0.97 | 0.04* | 0.91 | 0.19 | 1.08 | 0.08 |
| **PAH Mean Year Exposure (ng/m3)** | **Total** | | **Boys** | | **Girls** | |
| **Variables** | **β** | **p-value** | **β** | **p-value** | **β** | **p-value** |
| $Log_{10}$(Leptin) | -0.06 | 0.54 | 0.05 | 0.74 | -0.02 | 0.26 |
| $Log_{10}$(Adiponectin) | 0.07 | 0.37 | 0.06 | 0.54 | 0.08 | 0.51 |
| $Log_e$(Isoprostane) | -0.07 | 0.11 | -0.11 | 0.07 | -0.03 | 0.65 |

**Table 9. Association between biomarkers and elemental carbon (EC) exposure.**

| EC Average Day-of Exposure (ug/m3) | Total | | Boys | | Girls | |
|---|---|---|---|---|---|---|
| **Variables** | **β** | **p-value** | **β** | **p-value** | **β** | **p-value** |
| $Log_{10}$(Leptin) | 0.10 | 0.89 | -0.10 | 0.38 | 0.05 | 0.72 |
| $Log_{10}$(Adiponectin) | -0.02 | 0.72 | 0.05 | 0.46 | -0.12 | 0.29 |
| $Log_e$(Isoprostane) | 0.05 | 0.18 | 0.05 | 0.31 | 0.04 | 0.46 |
| **EC Mean Week Exposure (ug/m3)** | **Total** | | **Boys** | | **Girls** | |
| **Variables** | **β** | **p-value** | **β** | **p-value** | **β** | **p-value** |
| $Log_{10}$(Leptin) | -0.10 | 0.22 | -0.08 | 0.54 | -0.11 | 0.31 |
| $Log_{10}$(Adiponectin) | 0.04 | 0.53 | 0.07 | 0.37 | -0.004 | 0.96 |
| $Log_e$(Isoprostane) | 0.06 | 0.08 | 0.09 | 0.07 | 0.04 | 0.35 |
| **EC Mean Year Exposure (ug/m3)** | **Total** | | **Boys** | | **Girls** | |
| **Variables** | **β** | **p-value** | **β** | **p-value** | **β** | **p-value** |
| $Log_{10}$(Leptin) | -0.01 | 0.79 | -0.02 | 0.55 | -0.001 | 0.97 |
| $Log_{10}$(Adiponectin) | 0.02 | 0.21 | 0.02 | 0.22 | 0.01 | 0.57 |
| $Log_e$(Isoprostane) | 0.003 | 0.73 | 0.001 | 0.94 | 0.01 | 0.64 |

**Table 10. Association between biomarkers and elemental carbon (EC) exposure.**

| PM2.5 Mean Day-of Exposure (ug/m3) | Total | | Boys | | Girls | |
|---|---|---|---|---|---|---|
| **Variables** | **β** | **p-value** | **β** | **p-value** | **β** | **p-value** |
| $Log_{10}$(Leptin) | 1.79 | 0.24 | 1.38 | 0.56 | 1.63 | 0.46 |
| $Log_{10}$(Adiponectin) | 0.50 | 0.67 | 1.28 | 0.40 | -0.51 | 0.77 |
| $Log_e$(Isoprostane) | 0.91 | 0.16 | 0.42 | 0.65 | 1.24 | 0.18 |
| **PM2.5 Mean Week Exposure (ug/m3)** | **Total** | | **Boys** | | **Girls** | |
| **Variables** | **β** | **p-value** | **β** | **p-value** | **β** | **p-value** |
| $Log_{10}$(Leptin) | -1.02 | 0.48 | -0.43 | 0.86 | -1.80 | 0.35 |
| $Log_{10}$(Adiponectin) | 1.13 | 0.31 | 1.93 | 0.21 | 0.09 | 0.95 |
| $Log_e$(Isoprostane) | 0.97 | 0.12 | 1.65 | 0.08 | 0.42 | 0.60 |
| **PM2.5 Mean Year Exposure (ug/m3)** | **Total** | | **Boys** | | **Girls** | |
| **Variables** | **β** | **p-value** | **β** | **p-value** | **β** | **p-value** |
| $Log_{10}$(Leptin) | -0.35 | 0.19 | -0.58 | 0.17 | -0.25 | 0.49 |
| $Log_{10}$(Adiponectin) | 0.10 | 0.61 | 0.26 | 0.33 | -0.10 | 0.75 |
| $Log_e$(Isoprostane) | 0.10 | 0.36 | 0.20 | 0.21 | 0.001 | 0.99 |

The relationship between biomarkers and each pollutant were also separately stratified for gender, but none were significant. Figure 3 shows the significant positive relationship

between isoprostane levels and daily polycyclic aromatic hydrocarbon exposure (p=0.03). Figure 4 illustrates similar positive associations between isoprostane and weekly PAH exposure (p=0.04).

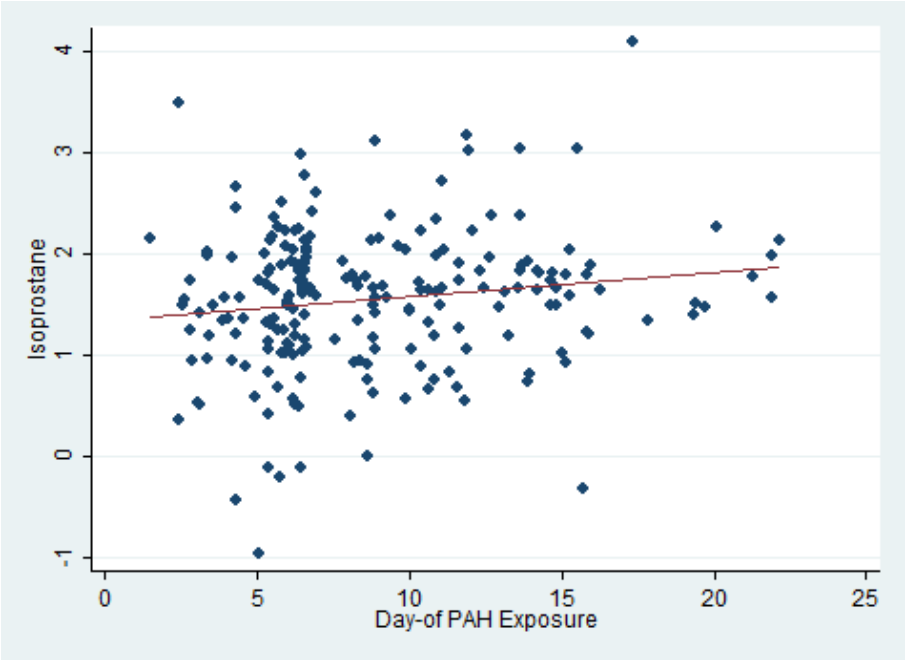


**Figure 3. Association between isoprostane and daily PAH exposure.**

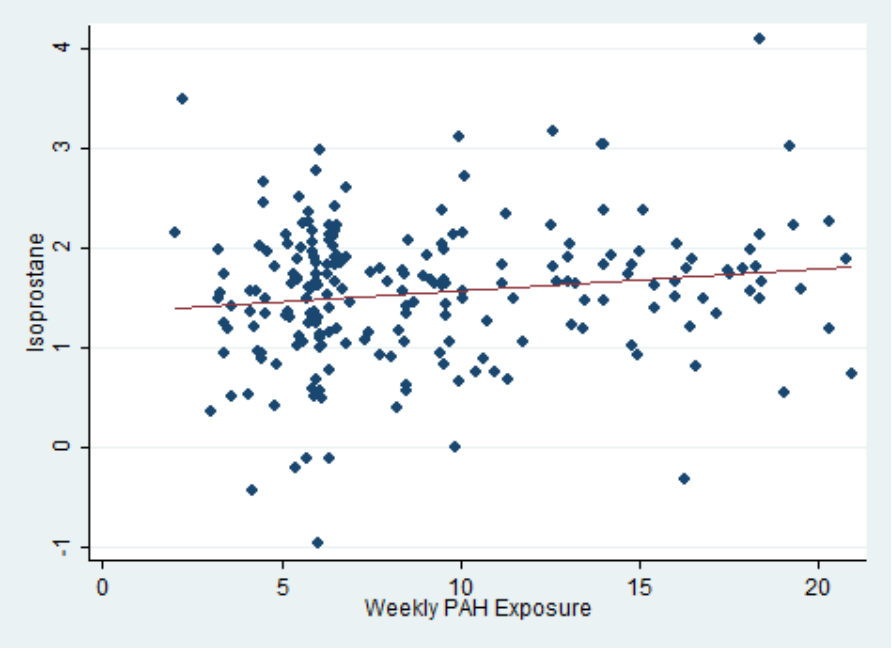


**Figure 4. Association between isoprostane and weekly PAH exposure.**

## Air pollution exposure and BMI

Exposure was analyzed as a continuous variable. Regression analysis of air pollution exposure and BMI (also treated as a continuous variable) found that there was an association between annual PM2.5 exposure and BMI (p=0.02). This is shown in Figure 5.

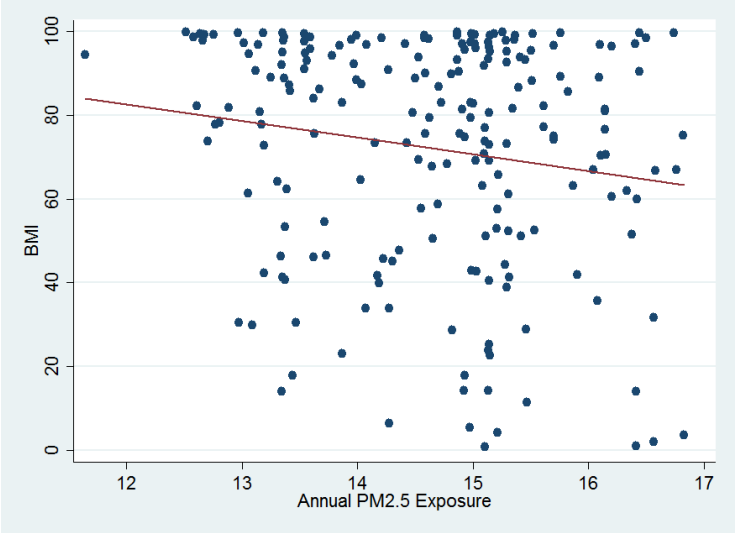


**Figure 5. Association between annual PM2.5 exposure and BMI.**

Analysis by gender revealed that BMI is associated with annual PM2.5 exposure in boys only (Figure 6). Results for measures of pollution exposure and BMI are presented in Table 11.

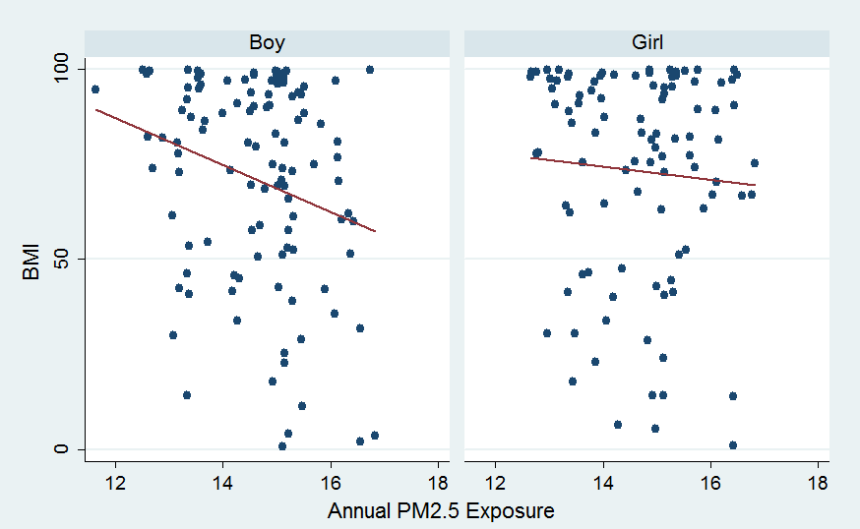


**Figure 6. Association between annual PM2.5 exposure and BMI by gender.**

**Table 11. Associations between air pollution exposure and BMI.**

| PAH (ng/m3) | Total | | Boys | | Girls | |
|---|---|---|---|---|---|---|
| Variables, Mean | β | p-value | β | p-value | β | p-value |
| Day-of exposure | 0.01 | 0.43 | 0.003 | 0.85 | 0.01 | 0.39 |
| Week | 0.01 | 0.43 | 0.01 | 0.58 | 0.01 | 0.56 |
| Year | 0.001 | 0.32 | 0.001 | 0.35 | 0.001 | 0.63 |
| EC (ug/m3) | Total | | Boys | | Girls | |
| Variables, Mean | β | p-value | β | p-value | β | p-value |
| Day-of exposure | 0.00004 | 0.99 | -0.001 | 0.17 | 0.001 | 0.28 |
| Week | -0.0001 | 0.88 | 0.00 | 0.88 | 0.00001 | 0.99 |
| Year | 0.0003 | 0.21 | 0.0001 | 0.73 | 0.0004 | 0.16 |
| PM2.5 (ug/m3) | Total | | Boys | | Girls | |
| Variables, Mean | β | p-value | β | p-value | β | p-value |
| Day-of exposure | 0.01 | 0.55 | -0.01 | 0.82 | 0.02 | 0.30 |
| Week | -0.01 | 0.54 | -0.01 | 0.69 | -0.01 | 0.66 |
| Year | -0.01 | 0.02* | -0.01 | 0.009* | -0.003 | 0.49 |

## DISCUSSION

Biomarkers of obesity and oxidative stress were analyzed to assess the relationship with exposure to air pollutants of interest, including polycyclic aromatic hydrocarbons, elemental carbon, and particulate matter, and associations with obesity, along with host factors such as age and sex. Leptin, adiponectin and isoprostane were not associated with age in this cohort of CHAPS children. Leptin levels were significantly higher in girls, and both leptin and adiponectin were found to have positive and negative relationships with BMI/BMI category, respectively. Isoprostane did not differ by sex, weight group status, or BMI. Associations between isoprostane and air pollution were observed specifically for both daily and weekly polycyclic aromatic hydrocarbon exposure. Moreover, investigation of air pollution exposures and BMI found that BMI and annual particulate matter 2.5 exposure were associated. This relationship was significant in boys only, and unexpectedly in the negative direction. These findings may be attributed to oxidative stress resulting from any number of stressors, as well as the process of lipid peroxidation caused by excess body fat, both of which warrant further exploration. It is important to note that these stressors may include ambient air pollutants of interest in this study

(PAH, EC and PM2.5), as well as other environmental exposures not investigated here. This suggested process is illustrated in Figure 7.

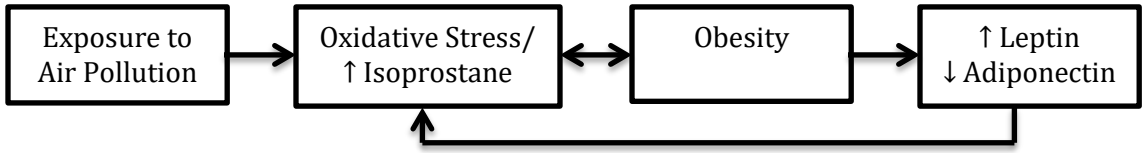


**Figure 7. Suggested mechanism of air pollution influencing biomarkers of obesity and oxidative stress.**

## Demographic associations

In this CHAPS cohort, age was not associated with any biomarkers measured, likely due to the children's similarity in age. Findings of no associations between leptin concentration and age were similar to results found by Zhong et al who also reported no significant associations (Zhong et al. 2005). However, there is conflicting research from other studies, which suggest that leptin levels increase across age groups in men and women (Isidori et al. 2000, Fulda et al. 2010). Besides the narrow age range of our study subjects, these differences in findings may be due to changes that occur in BMI and adiposity throughout life. Cnop et al concluded that adiponectin may also be related to age, finding a positive correlation between adiponectin and age (Cnop et al. 2003). Differences between our findings and those of Cnop et al may similarly be due to differences in body fat and the narrow age range of CHAPS children. With regards to oxidative stress, Cruz et al. reported an increase in isoprostane concentrations in adults as a result of accumulating metabolic substances that contributed to oxidative stress with age (Cruz et al. 2009). Our project was unable to reproduce similar results, likely due to the cohort's characteristics given that the subjects of this project ranged from 6 to 9 years old.

Differences in biomarker concentrations were only observed for leptin between CHAPS boys and girls, but not for adiponectin or isoprostane concentrations. The gender difference in leptin levels is well known, given that girls generally have more body fat. However, this difference was still significant when controlling for BMI, which is supported by the theory that male androgen levels play a role in reducing boys' leptin levels, hence resulting in girls having higher leptin (Wabitsch et al. 1997). Though no gender difference was observed for adiponectin concentrations in CHAPS children, studies do suggest that such differences may emerge during puberty as a consequence of serum androgen levels as well (Böttner et al. 2004). Findings on

isoprostanes and gender contribute to a body of conflicting literature. For example, in contrast to the Ide et al. report that females had lower levels of isoprostane and other oxidative stress markers in their plasma than males, Helmersson et al. reported significantly higher levels of the same biomarkers in females (Helmersson et al. 2002, Ide et al. 2002). Such variations could be the result of differences in sample handling techniques or biomarker measurement methods. The results of this project add to the equivocal prior findings on gender differences of oxidative stress biomarkers, suggesting a need for further future investigation into the role of gender in the presentation of oxidative stress.

Same factors may explain the isoprostane results by weight status group and BMI differences. This study found that there was no association between weight status category and isoprostane concentration, as well as between BMI and isoprostane. In contrast, Komakula et al. reported that elevated levels of 8-isoprostanes were associated with BMI. However, Il'yasova et al. reported lower $F_2$-isprostane levels among obese and diabetic subjects due to metabolic adaptation (Komakula et al. 2007, Il'yasova et al. 2012). Conflicting results may also be explained by the known association between obesity and inflammation, since leptin, a biomarker of obesity that increases with adiposity, contributes to the generation of oxidative stress (Fernández-Sánchez et al. 2011). This challenges the directionality of the relationship between oxidative stress and obesity, providing reason for obesity to cause the increase in oxidative stress. Further study is needed to clarify and understand the anthropometric determinants of isoprostane. However, previous studies have demonstrated that leptin and adiponectin concentrations are positively and negatively associated with BMI, respectively (Cnop et al. 2003, Moran and Phillip 2003). More specifically, Volberg et al illustrates that adipokine and BMI trends also are observed in children, reporting that leptin levels closely and positively correlate to child size in the CHAMACOS birth cohort, and adiponectin has an inverse association with body mass index (Volberg et al 2013). This project found similar results and reaffirmed that BMI is a significant determinant of leptin and adiponectin levels in children.

**Biomarkers and air pollution**

Limited data were available on the relationship between leptin and adiponectin levels and air pollution exposure. While the current study found no associations between adipokines and

any exposure metric, including daily, weekly and annual average exposure to polycyclic aromatic hydrocarbons, elemental carbon and particulate matter, future investigations with larger sample sizes are warranted. In contrast, previously conducted studies have demonstrated that isoprostane concentrations were positively associated with exposure to air pollutants. Specifically, Li et al. reported that particulate matter can exacerbate asthmatic response due to an effect on oxidative stress, as observed by increased isoprostane (Li et al. 2003). Investigation of the effects of polycyclic aromatic hydrocarbons similarly show that they elicit oxidative stress as a result of exposure (Araujo et al. 2008, Liu et al. 2009). The results of this project corroborate these findings that exposure to polycyclic aromatic hydrocarbons (measured both with a daily average and a weekly average) was positively related to isoprostane levels. Thus, this project confirmed that some air pollutants are determinants of isoprostane levels in CHAPS children. These findings imply that exposure to pollution is related to oxidative stress, and present support for inflammation and oxidative stress to be mechanistic pathways for the deleterious effects of accumulated exposure, such as well-known outcomes like respiratory and cardiac distress, and potentially others such as obesity.

**Air pollution and BMI**

A negative association between annual particulate matter exposure and BMI was observed, contradicting findings of Potera et al whose review of PM2.5 and inflammation found a positive influence of particulate matter exposure on total inflammation and subjects' weight (Potera et al 2014). Similarly to Potera, Sun et al found that exposure to fine particulate matter exaggerates adipose inflammation and visceral adiposity (Sun et al. 2009). The contradictory negative direction of the PM2.5 and BMI relationship observed in this study was strongly driven by the small subset of underweight children (N=5). These children may have underlying health conditions that are unaccounted for and thus affect the analysis. When these subjects were removed, there was no significant relationship between children's weight status and PM2.5 exposure. Given these results, there may still be an important relationship between air pollution in relation to the development of obesity, however it was not observed in this cohort.

## Limitations

Several limitations of the study design should be taken into account when interpreting the results. Participants may have health issues that influence study outcomes and are unaccounted for. Second, cross-sectional analyses were used; hence, they are not indicative of any causal relations. Third, the pollutants of interest for this study are by no means inclusive of all air pollution measures and may not be an adequate representation of exposures that may or may not contribute to oxidative stress. Moreover, the parameter of exposure assessment may not be the most accurate metric of the pollution burden of participants, since their proximity to the Fresno Supersite where data was obtained may vary for children by their residence. Future directions include utilizing other biomarkers of oxidative stress that may be more characteristic of accumulated pollution load. In addition, CHAPS contains other age-group cohorts, and this project might be expanded to those other cohorts or subjects may be followed over time in order to perform longitudinal analyses. Further, inclusion of additional metrics such as blood pressure and diabetic and asthmatic status may allow for the assessment of the associations between air pollution and diseases such as metabolic syndrome.

## Conclusions and broader implications

In conclusion, study results indicated that oxidative stress, as measured by isoprostane, was positively associated with polycyclic aromatic hydrocarbon exposure. This finding suggests that exposure to ambient air pollutants may play a role in disrupting metabolic balance and contribute to the development of obesity, implying a significant need to further investigate mechanistic pathways and preventative measures against such outcomes.

This study may encourage further understanding of factors contributing to chronic disease, in particular metabolic syndrome. As discussed before, populations such as those living in the San Joaquin Valley, CA are considered especially vulnerable to pollution due to the nature of their socioeconomic status and geographic location. This study may be beneficial in guiding policy and in environmental justice advocacy to protect people from harms resulting from their disproportionate burden of exposure to environmental contaminants.

## ACKNOWLEDGEMENTS

Thank you to my faculty mentor, Professor Nina Holland, for the guidance in completing this project. I gratefully acknowledge Children's Environmental Health Lab members, Maria Escudero and Michael Zhou, for their assistance in my work. Thank you to Professor Katharine Hammond (CHAPS Principal Investigator), Professor John Balmes, Dr. Mary Prunicki and Dr. Liza Lutzer for granting me access to CHAPS samples and exposure data. Special thanks to CHAPS staff and participants for making this work possible.

Thank you also to my thesis instructors, Dr. Patina Mendez and Dr. Kurt Spreyer, and especially to Abigail Cochran and the members of my peer reviewing group, Betty Huang, Ramanjot Kaur, and Cathy Ton for the extensive editing of drafts and the year-long support. This project was funded by the National Institute of Environmental Health Sciences and the Environmental Protection Agency. This project was funded by the National Institute of Environmental Health Sciences and the Environmental Protection Agency.

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