

# **Urinary Biomarkers of Response in Relation to Biomarkers of Air Pollution Exposure in Schoolchildren from Stockton, California (SAPEP)**

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# Disclosure

The findings and conclusions of this study are those of the authors and do not necessarily represent the views or opinions of the University of California at Berkeley, Human Services Agency, the California Environmental Protection Agency, or OEHHA.

The authors declare no conflict of interest.

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# Project Collaborators



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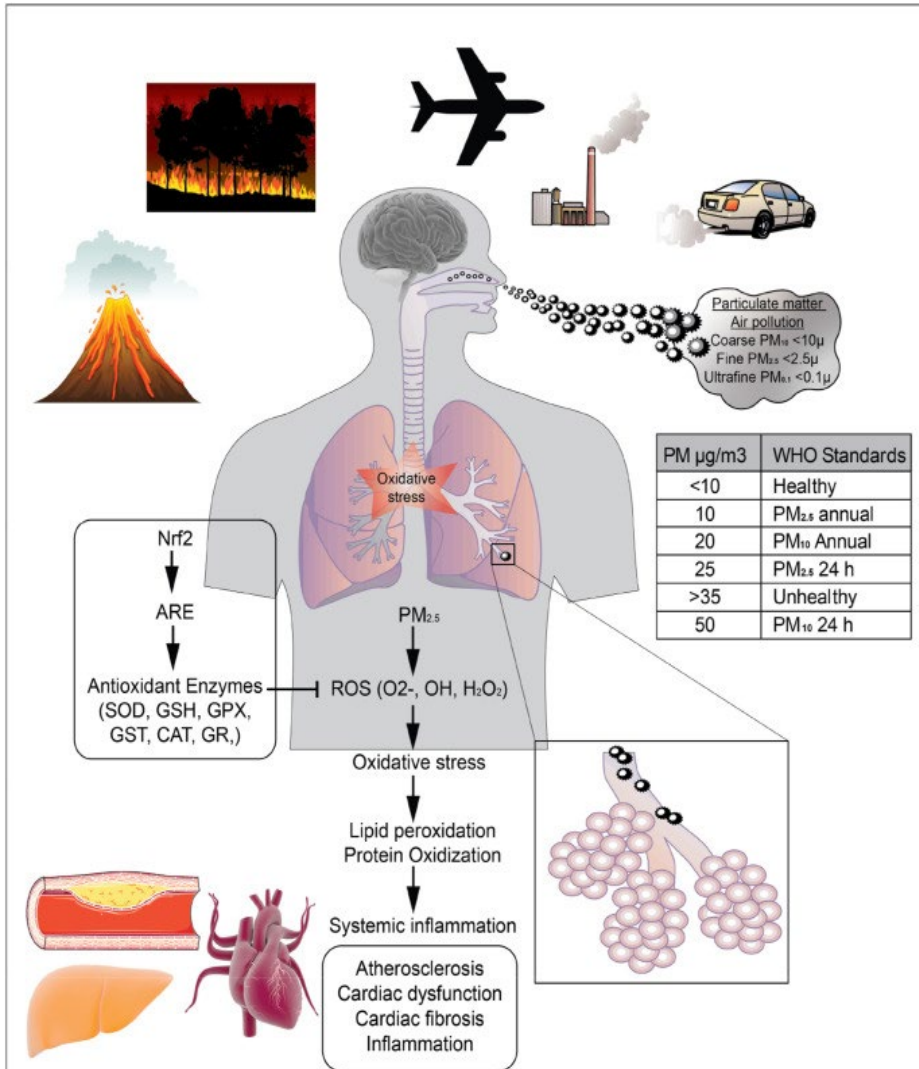
University of California  
San Francisco

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# Background-Air Pollution

- Air pollution is a growing concern worldwide:
  - ❖ Increased wild fires and extreme heat waves caused by climate change
  - ❖ Exposure to air pollution is associated with adverse health effects including asthma, respiratory and cardiovascular diseases, and lung cancer
  - ❖ Children, in particular, are among the most vulnerable populations



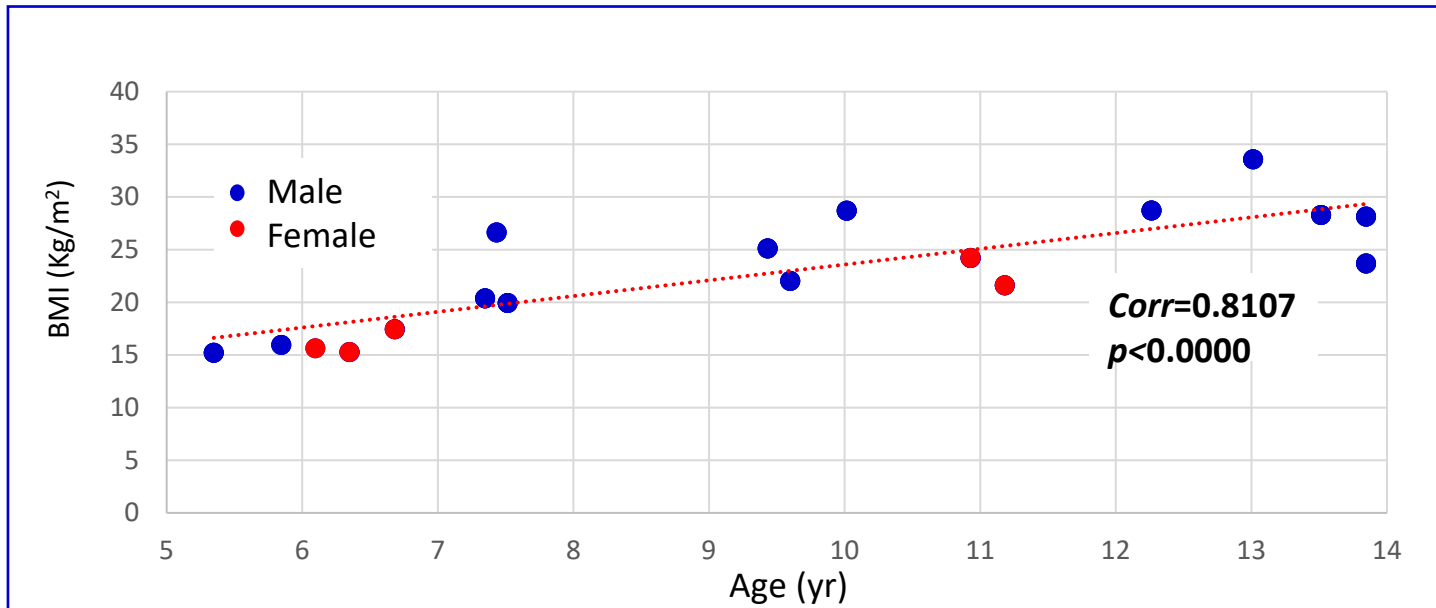
# Study Objectives and Design

- **To evaluate the distribution of four urinary biomarkers of response and their relationship with urinary biomarkers of exposures in schoolchildren**
  - ❖ **AB 617 school in Stockton, CA, Stockton Air Pollution Exposure Project (SAPEP)**
  - ❖ **Repeated measurements of biomarkers in schoolchildren (n=18) over 2 weeks in Dec. 2021**
  - ❖ **Biomarkers of response (repeated measurements = 67)—8-Isop, 8-OHdG, PGE2, and CC16**  
**Biomarkers of exposures (repeated measurements = 69)—metabolites of VOCs and PAHs**
  - ❖ **All biomarker measurements were adjusted for the specific gravity (SG) and creatinine (Cr), and log 2 transformed. SG adjusted values were used for data analysis.**

# Characteristics of the Study Participants

Total participants n = 18

- **Gender: Male = 13, Female = 5**
- **Age: 5-13 yrs**
- **BMI: Ranged from 15.2-33.6 Kg/m<sup>2</sup> with 39% obese/overweight**  
Ref: NHANES National Youth Fitness Survey (3yr-15yr, n = 1571, 2013):  
BMI range = 11.9 to 48.3 kg/m<sup>2</sup>
- **Ethnicity: Hispanic 11/18 = 61%**

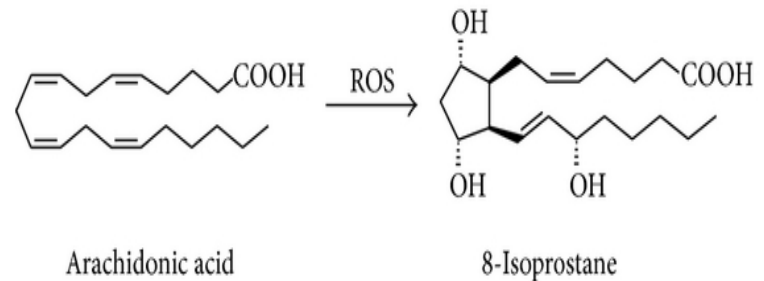


**BMI and Age: participant age and BMI are highly correlated**

# Biomarkers of Oxidative Stress

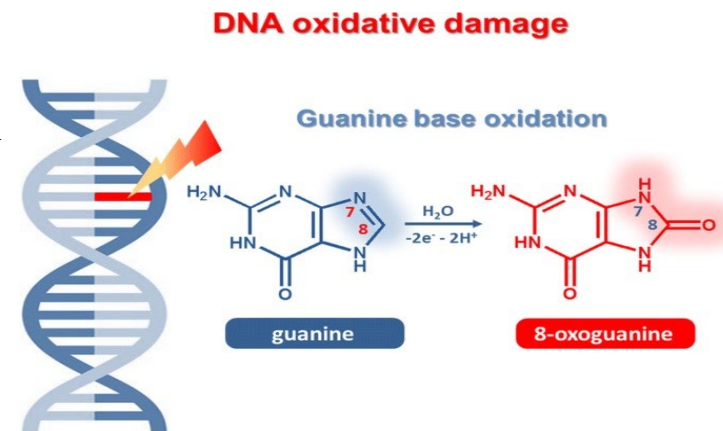
## Isoprostanes (8-Isop)

- Isoprostanes are products of free radical mediated **lipid peroxidation** (arachidonic acid)
- Increased 8-Isop levels have been associated with air pollution in:
  - CHAPS cohort children from Fresno (Mann et al, 2021)
  - Studies in adults (Wang. et al, 2021 and Lin et al, 2022)



## 8-hydroxy-2-deoxyguanosine (8-OHdG)

- 8-OHdG is produced by the **oxidative damage of DNA and other nucleic acids** by reactive oxygen and nitrogen species
- Hydroxylation of guanosine occurs in response to both normal metabolic processes as well exposures to environmental factors
- Increased levels of 8-OHdG are associated with air pollution exposure in:
  - Children (Zhu et al, 2023 and Svecova et al, 2009)
  - Adults (Maccarone et al, 2023 and Wang et al, 2021)



# Biomarkers of Response—Inflammation

## **Prostaglandin E2 (PGE2)**

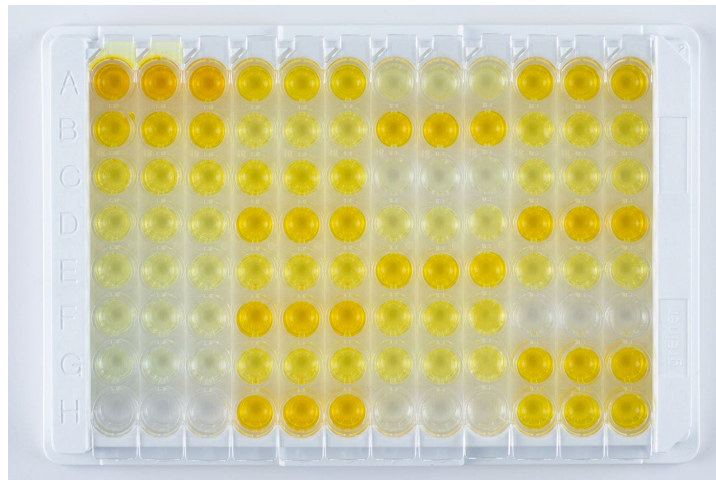
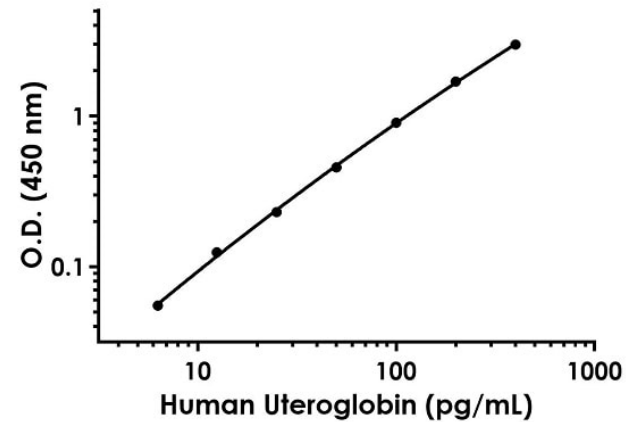
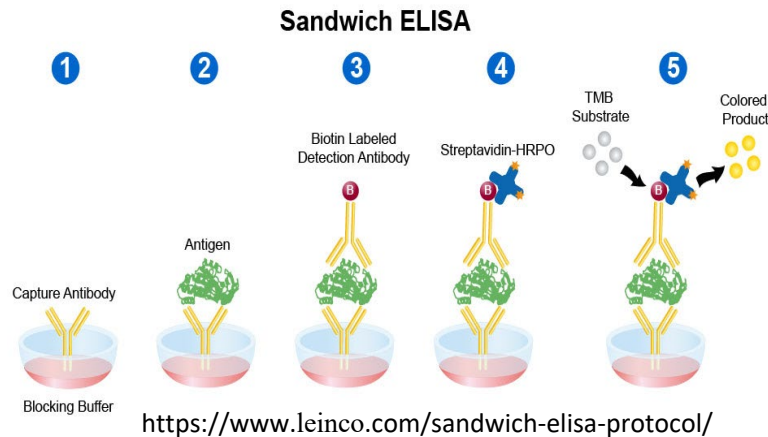
- PGE2 is one of the most abundant PGs produced in the body and involved in many biological functions, such as regulation of immune response, blood pressure, inflammation, pain, and fertility
  - Increased PGE2 may contribute to impaired airway function in children with severe asthma (Brugha et al, 2014)
  - Increased levels in former smokers with chronic obstructive pulmonary disease (COPD) (Tejwani et al, 2023)

## **Uteroglobin (CC16--Clara cell secretory protein)**

- Most circulating CC16 is produced by Clara cells that are predominant in lung bronchioles
- Increased plasma CC16 can result from disruption of the lung epithelial barrier and leakage into blood stream associated with environmental insults such as cigarette smoking and air pollution:
  - CHAPS children (Zhang et al, 2022)
  - Adults (Helen et al, 2013, Arjomandi et al, 2008)



# Biomarker were Assessed by Enzyme-Linked Immunosorbent Assays (ELISA)



**Each 96 well ELISA plate includes in duplicates:**

- **Calibration curve**
- **QC samples (lab volunteer)**
- **38 urine samples from study participants**

We evaluated several ELISA kits from different companies for each biomarker and selected the most reliable ones, respectively: from Oxford Biomedical Research (8-Isop), Abcam (8-OHdG and CC16) and R&D Systems (PGE2)

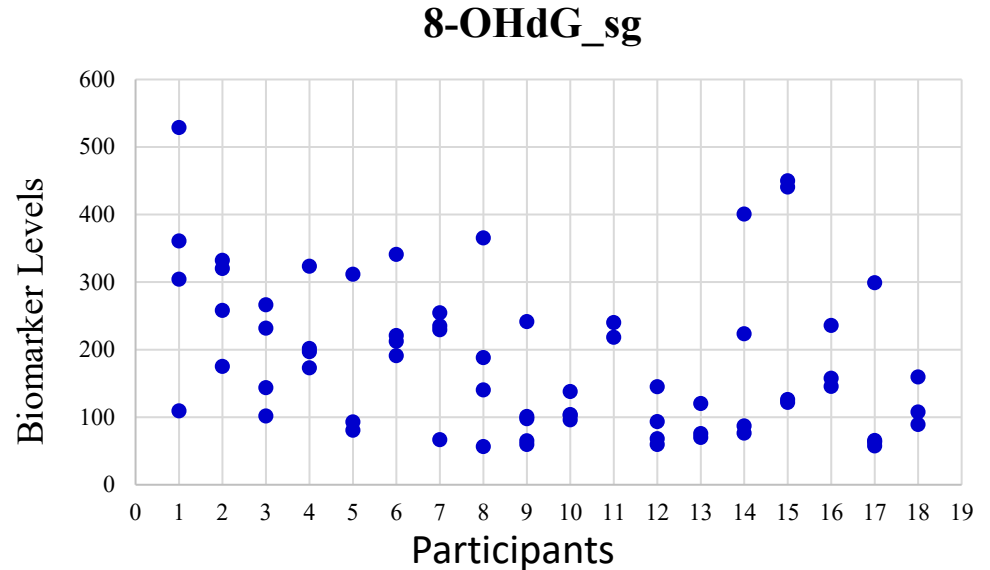
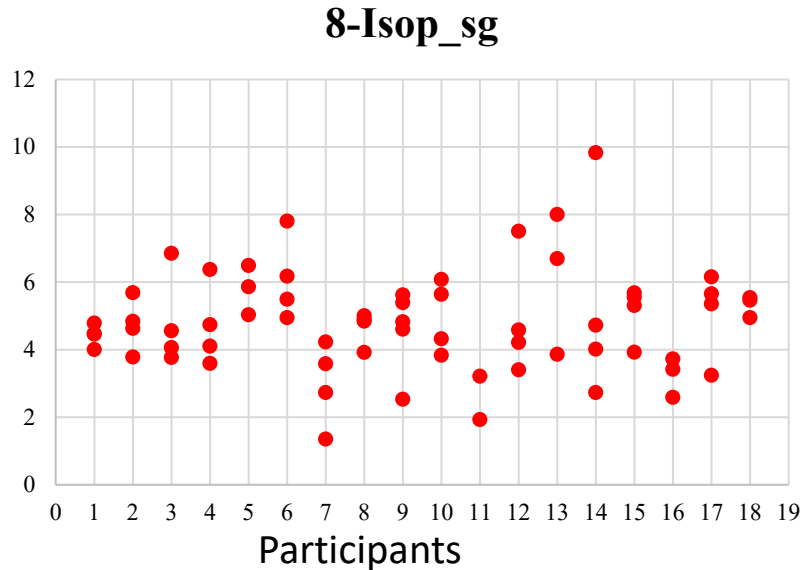
# Biomarkers of Response

Biomarkers (ng/mL SG)	Detection Rate	Min	Mean	Geometric Mean <sup>b</sup>	GM 95% CI <sup>b</sup>	Median	Max
8-Isop	100%	1.35	4.79	4.55	4.08-5.07	4.74	9.83
8-OHdG	100%	56.4	185	155	127-190	158	529
PGE2	100%	0.85	3.83	3.16	2.63-3.80	3.19	31.7
CC16	96%	0.01	10.3	2.15	0.74-6.22	2.96	91.8

<sup>b</sup> Geometric means and CI were calculated using a random effects model

- **8-Isop levels—slightly lower than in CHAPS children (n = 290, mean = 5.5 ng/mg Cr) (Mann, 2021)**
- **CC16 levels—higher than CHAPS children (n = 122, mean = 4.01 ng/mL ) from Fresno, CA (Zhang, 2022)**
- **8-OHdG and PGE2 no comparable data in children**

# Variations of Biomarker Levels in Repeated Samples



- **Study participants had between 2 and 4 repeated samples that show noticeable variation in urinary biomarker levels across time-points**
- **More detailed analysis of the variability (morning vs after school; 1<sup>st</sup> vs 2<sup>nd</sup> week) was not possible due to a relatively small number of participants**

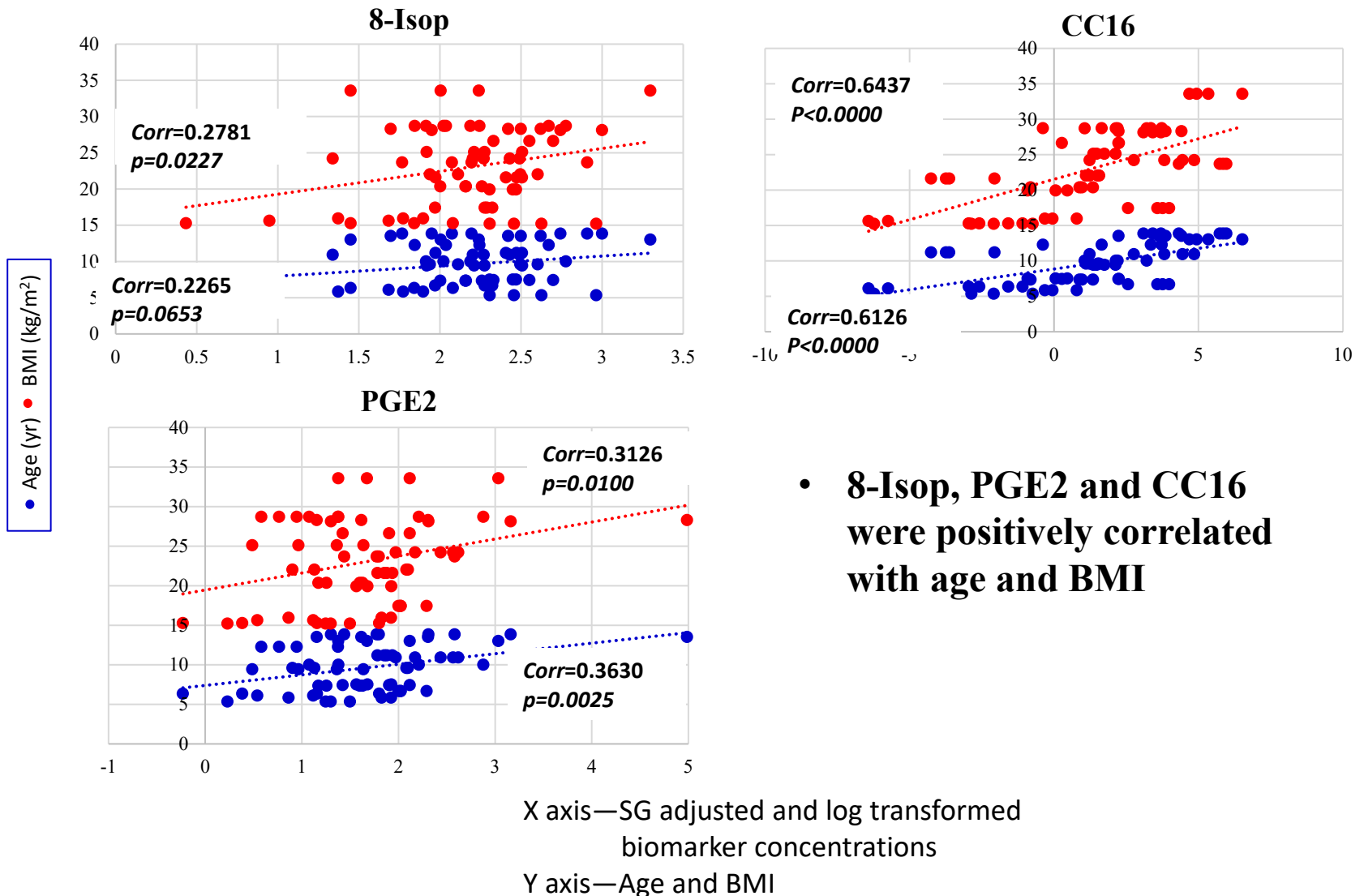
# Relationships Among Four Biomarkers of Response

Names	8-Isop		8-OHdG		PGE2	
	corr	p-value	corr	p-value	corr	p-value
8-OHdG	0.0327	0.7928				
PGE2	0.4641	<b>0.0001</b>	-0.3059	<b>0.0118</b>		
CC16	0.2394	<b>0.0510</b>	-0.2879	<b>0.0182</b>	0.4184	<b>0.0004</b>

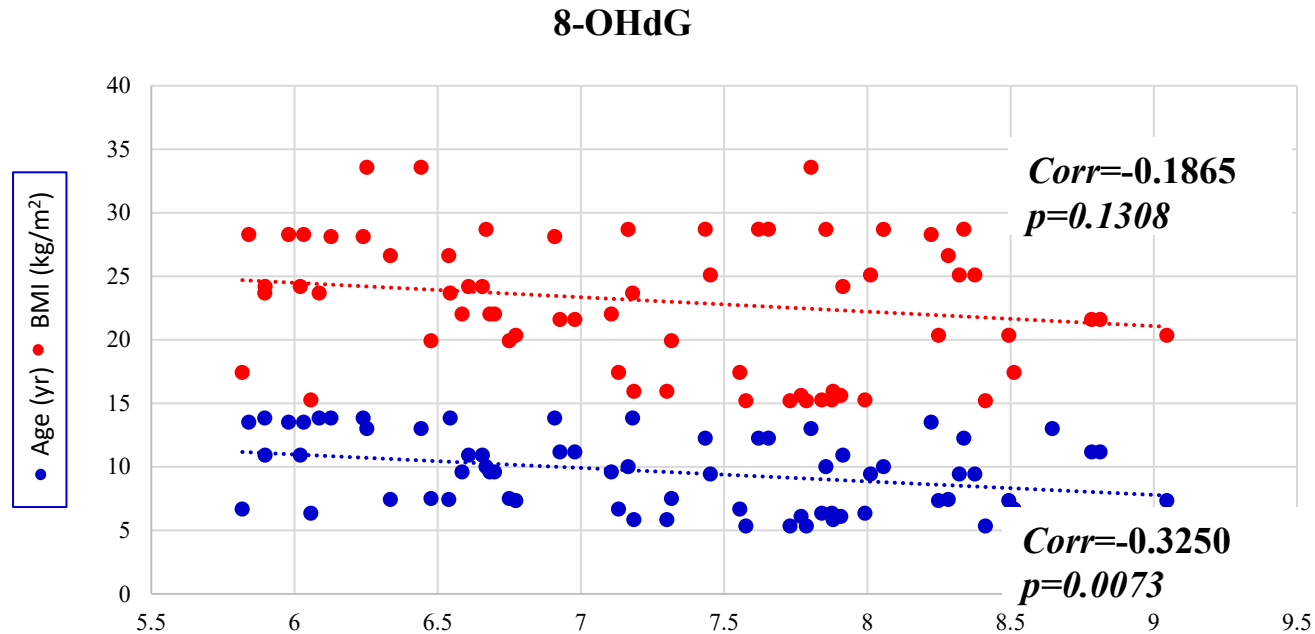
Log 2 transformed specific-gravity adjusted values were used for the Pearson correlations

- **8-Isop, PGE2 and CC16 were positively correlated with each other**
- **8-OHdG was negatively correlated with PGE2 and CC16**
- **8-Isop and 8-OHdG were not correlated**

# Biomarkers of Response by Age (Blue) and BMI (Red)



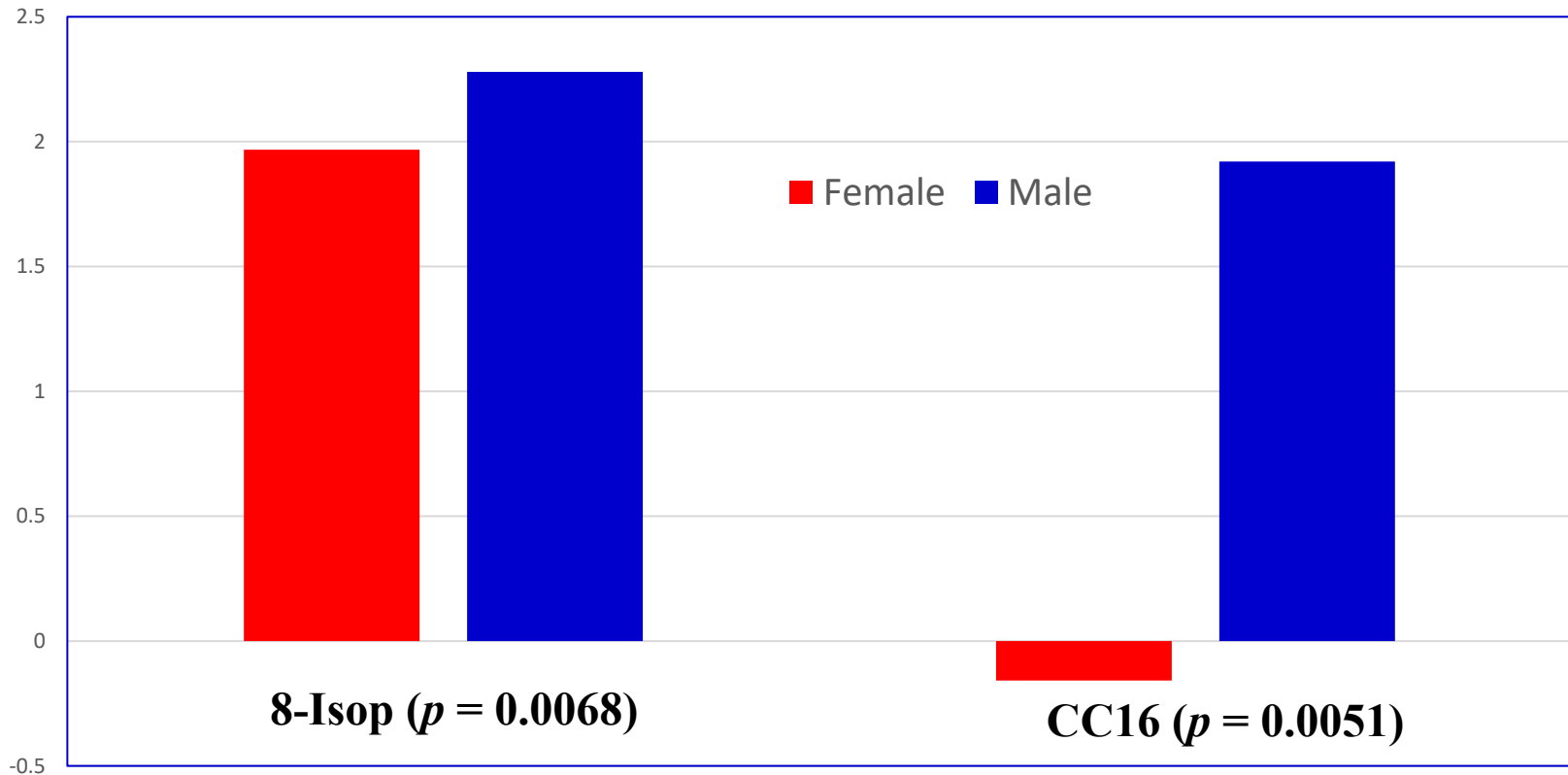
# 8-OHdG was Negatively Correlated with Age but Differences by BMI were not Statistically Significant



X axis—SG adjusted and log transformed biomarker concentrations

Y axis—Age and BMI

# Biomarker Levels in Girls (Red) vs Boys (Blue)



Y axis—SG adjusted and log transformed  
biomarker concentrations

- **8-Isop and CC16 levels were lower in girls compared to boys**

# Overview of Biomarkers of Response Findings

- **Most biomarkers of oxidative stress and inflammation were moderately correlated with each other**
- **All biomarkers show variability over two weeks of collection**
- **Differences by age and BMI were commonly observed**
- **Boys had higher levels than girls for 8-Isop and CC16**



## **The Next Question Was:**

**What is the relationship between biomarkers of exposure to air pollution (VOCs and PAHs) and four biomarkers of response (oxidative stress and inflammation) in schoolchildren?**

# Relationship Between Biomarkers of Response and Exposures

Urine Metabolites	Air Pollutants	8-Isop <sup>a</sup>		8-OHdG <sup>a</sup>		PGE2 <sup>a</sup>		CC16 <sup>a</sup>	
		corr	p-value	corr	p-value	corr	p-value	corr	p-value
<b>VOCs</b>									
CNEMA	Acrylonitrile	0.44233	<b>0.0002</b>	-0.024	0.8471	0.23621	<b>0.0543</b>	-0.03844	0.7575
3-HPMA	Acrolein	0.30006	<b>0.0136</b>	-0.075	0.5464	0.21242	0.0844	0.26147	<b>0.0326</b>
HPMMA	Crotonaldehyde	0.32608	<b>0.0071</b>	0.13146	0.2889	0.30662	<b>0.0116</b>	0.07059	0.5703
<b>PAHs</b>									
2-Naphthol	Naphthalene	0.43258	<b>0.0003</b>	-0.13962	0.2598	0.32625	<b>0.0071</b>	0.5091	<b>&lt;0.0001</b>
2-OH-fluorene	Fluorene	0.31968	<b>0.0084</b>	-0.07076	0.5693	0.21199	0.0850	0.35346	<b>0.0033</b>
1-OH-pyrene	Pyrene	0.09959	0.4227	0.31062	<b>0.0105</b>	-0.0026	0.9833	0.05444	0.6617

Log 2 transformed specific-gravity adjusted values were used for the Pearson correlations

**Metabolites with no significant relationship with biomarkers of response are not listed in the table (2-HPMA, 3-OH-fluorene, 1-OH-phenanthrene, 2-OH-phenanthrene and 3,4-OH-phenanthrene)**

# Relationship Between Biomarkers of Response and Exposures: Summary

- **8-Isop** was moderately correlated with 5 metabolites of 8 VOCs and PAHs (out of 11 measured)
- **CC16** and **PGE** showed positive correlations with 3 metabolites of VOCs or PAHs, but not the same ones
- **8-OHdG** showed positive correlation with 1-OH-pyrene

**Four different biomarkers provide complementary insights into biological response to air pollution exposure**

# Conclusions

- **To our knowledge, this is the first study to establish relationship between VOCs and PAHs with biomarkers of oxidative stress (8-IsoP and 8-OHdG) and inflammation (PGE2 and CC16) in children living in a community heavily impacted by air pollution**
- **A panel of four biomarkers of response presents a comprehensive picture of the relationship with air pollution as they show correlations with different urinary metabolites of VOCs and PAHs**
- **Biomarkers of oxidative stress and inflammation, as well as urinary biomarkers of exposure may be useful tools in biomonitoring air pollutants in children**

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