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Polycyclic Aromatic Hydrocarbons, Brachial Artery Distensibility and Blood Pressure among Children Residing near an Oil Refinery

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Abbreviations: Blood pressure (BP), Brachial artery distensibility (BAD), Body Mass Index (BMI), Cardiovascular disease (CVD), Centers for Disease Control and Prevention (CDC), dichloromethane (DCM), high performance liquid chromatography (HPLC), Particulate matter (PM), Particulate matter less than 2.5 microns in diameter ($PM_{2.5}$), Particulate matter less than 10 microns in diameter (PM_{10}), Polycyclic aromatic hydrocarbons (PAH), polyurethane foam (PUF)

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Abstract

Background: Polycyclic aromatic hydrocarbons (PAH) are produced by the burning and processing of fuel oils, and have been associated with oxidant stress, insulin resistance and hypertension in adults. Few studies have examined whether adolescents are susceptible to cardiovascular effects of PAHs.

Objective: To study associations of PAH exposure with blood pressure (BP) and brachial artery distensibility (BAD), an early marker of arterial wall stiffness, in young boys attending three schools in Jeddah, Saudi Arabia in varying proximity to an oil refinery.

Methods: Air samples collected from the three schools were analyzed for PAHs. PAH metabolites (total hydroxyphenanthrenes and 1-hydroxypyrene) were measured in urine samples from 184 adolescent males, in whom anthropometrics, heart rate, pulse pressure, brachial artery distensibility and blood pressure were measured. Descriptive, bivariate and multivariable analyses were performed to assess relationships of school location and urinary PAH metabolites with cardiovascular measures.

Results: Total suspended matter was significantly higher (444±143 μg/m3) at the school near the refinery compared to a school located near a ring road (395±65 μg/m3) and a school located away from vehicle traffic (232±137 μg/m3), as were PAHs. Systolic (0.47 SD units, $p=0.006$) and diastolic (0.53 SD units, $p<0.001$) BP Z-scores were highest at the school near the refinery, with a 4.36-fold increase in prehypertension ($p=0.001$), controlling for confounders. No differences in pulse pressure, BAD and heart rate were noted in relationship to school location. Urinary total hydroxyphenanthrenes and 1-hydroxypyrene were not associated with cardiovascular outcomes.

Conclusions: Proximity to an oil refinery in Saudi Arabia is associated with prehypertension and increases in PAH and particulate matter exposures. Further study including insulin resistance measurements, better control for confounding, and longitudinal measurement is indicated.

Introduction

Outdoor air pollution, a heterogeneous mix of pollutants largely produced through fossil fuel combustion and chemical reactions in the atmosphere (Brook et al., 2004), is well known to contribute to cardiovascular disease (Dockery et al., 1993). Motor vehicles and coal-fired power plants are major sources of these emissions, though oil refineries, aircraft, residential wood burning stoves and natural sources (lightning and wildfires) also are contributors (Bakker et al., 2000; Sun et al., 2010). Associations have been documented to be strongest in particular for two types of air pollution, particulate matter (PM) less than 10 microns in diameter (PM_{10}) and PM less than 2.5 microns in diameter ($PM_{2.5}$) (Bell et al., 2008; Dominici et al., 2006; Dominici et al., 2007). In 2010, 2.1% of the global burden of disease was due to PM-attributable cardiovascular disease (CVD, 53.2 million disability adjusted life years) (Lim et al., 2012).

In an effort to identify sources and guide prevention, studies have rightly endeavored to disaggregate effects of the many chemicals found in air. Among chemical industries, petroleum refineries have been identified as large emitters of pollutants, especially PAHs (Polidori et al., 2010). In particular, polycyclic aromatic hydrocarbons (PAH) are known to be potent oxidant stressors (Araujo et al., 2008), which is important because oxidation of lipids contributes to inflammation. PAHs also reduce nitric oxide (Chuang et al., 2012), promoting vasoconstriction, platelet adhesion, and release of inflammatory cytokines (Harrison et al., 2003; Singh and Jialal, 2006). PAHs are also potent aryl hydrocarbon receptor agonists, inducting xenobiotic metabolizing enzymes that can augment generation of reactive oxygen species (Korashy and El-Kadi, 2006). Indeed, asphalt workers have been found to have increased fatal ischemic heart disease in association with urinary PAHs (Burstyn et al., 2005), and general population, cross-

sectional studies have associated urinary PAHs with CVD (Xu et al., 2010), peripheral arterial disease (Xu et al., 2013), and C-reactive protein (Everett et al., 2010), though another study failed to identify increases in other serum inflammatory markers commonly found in CVD (Clark Iii et al., 2012).

It is well known that children are at particular risk for health effects of air pollutants (Trasande and Thurston, 2005). Children tend to have higher PAH exposure to air, soil and dust than adults because of greater hand-to-mouth activity, time spent close to the ground, and inhalation rates per unit body weight (National Research Council, 1993). In the context of the rising obesity epidemic globally (de Onis et al., 2010), concerns have increased about the action of air pollutants and other environmental stressors on children, especially with increased adipose tissue substrate for oxidant stress. Adding to these concerns, epidemic increases in elevated blood pressure have been documented in the United States (Rosner et al., 2013). Indeed, a study of school-age children identified increases in oxidative stress biomarkers in association with urinary PAHs (Bae S et al., 2010).

Jeddah is the second largest city and the most significant commercial center in the Kingdom of Saudi Arabia. The growth of the city over the last thirty years has been rapid,(Saudi Network, 2008) without awareness of health consequences and regulations to limit environmental degradation. In Jeddah, stationary sources of air pollutants include an oil refinery, a desalinization plant, a power generation plant and several manufacturing industries. Many of the industrial facilities, for example, the Jeddah oil refinery, were originally built in nonresidential areas, but with subsequent urban development, the refinery is now surrounded by highly populated areas.(Al-Jahdali and Bin Bisher, 2008) The refinery has units for crude distillation,

desulphurization of gasoline, diesel and kerosene, vacuum distillation and for asphalt manufacture and produces 60,000 barrels of oil daily.(A Barrel Full) Indeed, a recent study identified elevated levels of $PM_{2.5}$ arising from heavy fuel oil near the refinery,(Khodeir et al., 2012) while another suggested genotoxicity of nearby emissions, with DNA damage directly correlating with organic particulate and PAHs concentrations in the air samples.(ElAssouli et al., 2007) Together, these findings suggest the potential for cardiovascular effects of air pollutants from the refinery for vulnerable subpopulations, including children.

In this manuscript, we describe airborne measurements of PM_{10} and PAH in schools in varying proximity to this large oil refinery, as well 1-hydroxypyrene and several monohydroxyphenanthrenes in the urine of children attending these schools. We also assessed associations of cardiovascular profiles in relationship to airborne and urinary measures of pollutants in these children.

1. Methods

2.1 Study population

Three schools were identified in varying proximity to the oil refinery and to vehicular traffic. All students enrolled in the three schools were male, and lived within 1km of the school. There are no mixed (male and female) schools in Saudi Arabia. The sampling sites in Jeddah city are shown in Figure 1. Site one (A, School 1) is located in Ghulail district, south of the city, close to the port and only 700m east of Jeddah oil refinery. The school belongs to a poor, highly

populated residential area. Site two (B, School 2) is located in Al-Muntazahat District, East of Jeddah, adjacent to the ring road (Al-Haramain road) and 9.5 km east of the refinery. The area has a high population and heavy traffic density. Site three (C, School 3) is located in Al-Murjan District, situated directly on Red Sea Creek (Sharm Obhur), 30.3 km to the north of the refinery and surrounded by resorts and a few residential areas. This school was selected because it was more likely to have "background" PM_{10} and PAH concentrations compared to schools near stationary and mobile sources of exposure, and is identified as the background school hereafter in the manuscript. Meterologic conditions (temperature, wind speed, pressure and relative humidity) were qualitatively similar across the three schools.

At each of these three schools, all 10-14 year old children were approached about participation through their parents. Meetings at each school were held to explain the study objectives, sampling methods, and questionnaire guidelines. After obtaining signed and informed consent, questionnaires were administered to the parent, a urine sample was collected, and brachial artery distensibility and blood pressure were measured. On the eve of the sampling day, a guideline sheet, an individually labeled questionnaire, and a labeled sterile sampling tube were provided. They were instructed to get the first spot urine sample the next morning and bring back the samples together with the completed questionnaires to school for three consecutive days lagged one day from air sampling (Sunday to Tuesday). The exposure assessment questionnaire included information of child's home characteristics, medical history, demographic factors, semi-quantitative food frequency, food sources, habits, and time-activity profile. Special emphasis was given regarding the consumption of charbroiled food and cigarette smoking during the previous

seven days. Environmental assessments and urine sample collections were conducted simultaneously in all regions on the same days.

The study proposal was scrutinized and approved by King Abdulaziz University Research Ethics Committee (protocol number 700-12, approval date 4/1/2012). Dr. Trasande signed a self-certification that his participation did not represent human subjects research as defined by 45 CFR 46.102.

2.2 Air sample collection

Sampling was carried on the school's roof at a height of approximately 9 m from the ground. Atmospheric PAH samples were collected using a USEPA pesticide sampler (Environmental Tisch, Cleveland-Ohio, USA) In which the air was drawn through a TSP inlet followed by a quartz micro fiber filter (TE-QMA4 10.16 cm, Ohio, USA) to collect PAH compounds in the particulate phase and then through adsorbent polyurethane foam (PUF) to collect PAH compounds present in the gaseous phase. Fifteen daily 24–hr samples were collected from each school, with sampling beginning each day at 07:00 AM. The sampling flow rate was measured before and after sampling, and samples were collected during the period from February 23-April 23, 2013.

2.3 Body mass measurements

We measured weight and height and using calibrated stadiometers (Seca model 217; Hamburg, Germany) and scales (Beurer GmbH model PS07; Ulm, Germany). We derived Body Mass Index Z-scores from 2000 Centers for Disease Control and Prevention (CDC) norms, incorporating height, weight and gender; overweight and obese were categorized as BMI Z-score ≥1.036 and ≥1.64.(Ogden et al., 2002)

2.4 Cardiovascular measurements

The DynaPulse Pathway (PulseMetric, San Diego, CA) instrument derives brachial artery distensibility from arterial pressure signals obtained from a standard cuff sphygmomanometer (Brinton TJ, 1997). The pressure waveform is calibrated and incorporated into a physical model of the cardiovascular system, assuming a straight tube brachial artery and T-tube aortic system. DynaPulse has been previously validated with high correlation between compliance measurements obtained during cardiac catheterization and noninvasive brachial methods (r = 0.83).(Brinton TJ, 1997; Brinton TJ, 1998) Reproducibility studies using blind duplicates demonstrated good intraclass correlation coefficients for arterial compliance, from which distensibility is calculated (0.72) (Urbina et al., 2011; Urbina et al., 2010). Off-line analyses of brachial artery pressure curves are performed using an automated system.

This yielded measurements of brachial artery distensibility, systolic blood pressure, diastolic blood pressure, heart rate and pulse pressure. All measurements were averaged. Calculation of systolic and diastolic BP Z-scores utilized mixed-effects linear regression models described in The Fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in

Children and Adolescents. Height Z-scores, gender and age were input to compute expected systolic and diastolic BPs (derived from 1999-2000 NHANES data), and BP Z-scores were then calculated from the measured BPs using the formula $Zbp = (x - \mu)/\sigma$, where x is the measured BP, μ is the expected BP, and σ is derived from the same NHANES data.(National High Blood Pressure Education Program Working Group on High Blood Pressure in and Adolescents, 2004) Prehypertension was defined as SBP or DBP above the 90^{th} percentile for age, height and sex.

2.5 Air analysis

Prior to sampling, the filters were preheated at 400 °C for 48 h in a box furnace, wrapped in clean preheated foil, placed in a cardboard box and sealed in an airtight metallic container. The PUF substrates were pre-cleaned prior to their use in the field by immersing in 100 mL of dichloromethane (DCM) and ultrasonicating at 20 °C for 30 min. The solvent was then drained and the PUF substrates were left to dry in a sealed metal container under a stream of nitrogen. The clean and dry PUF substrates were subsequently sealed in airtight plastic bags and stored in the freezer. Once exposed, the filter and PUF substrates were wrapped separately with clean preheated foil, enclosed in airtight plastic bags and stored at approximately -18 °C.
Samples were analyzed for 14 PAH using the methodology described previously.(Delgado-Saborit et al., 2013) Briefly, filter and PUF substrates were spiked with 1000 pg μL^{-1} deuterated internal standards for quantification. Filters were immersed in DCM and ultrasonicated for 15 min at 20 °C. The extract was subsequently dried and cleaned using a chromatography column filled with 0.5 g of anhydrous sodium sulfate (Puriss grade for high performance liquid chromatography, or HPLC). The extract was further concentrated to 50 µL under a gentle

N_2 flow. PUF substrates were immersed in 100 mL of DCM and ultrasonicated for 20 min at 20 °C. The sample was then concentrated to 10 mL using N_2 and subsequently dried and cleaned as outlined for the filters above.

Samples were analysed for PAH compounds using gas chromatography (6890, Agilent Technologies) equipped with a non-polar capillary column (Agilent HP-5MS, 30m, 0.25 mm ID, 0.25 µm film thickness – 5 % phenylpolysiloxane) in tandem with a mass spectrometer (5973N, Agilent Technologies).

2.6 Urine analysis

Determination of 1-, 2-, 3-, 4-hydroxyphenanthrene and 1-hydroxypyrene was carried out in urine samples pooled from multiple time points in each study participant using a method published by the German Research Foundation.(German Research Foundation) Urine samples of 6ml were buffered with 12ml 0.1M sodium acetate buffer, pH 5.0, and hydrolyzed with 80 µl glucuronidase/arylsulfatase for 16 h in a water bath at 37 ^{o}C. To separate the particulate matter, the solutions were centrifuged at 3000 r.p.m. for 15 min, and 12ml of the supernatant fraction was transferred into an autosampler tube. Then, 3 ml of this solution was injected into the high performance liquid chromatography (HPLC) system by an autosampler. The HPLC system consisted of a quaternary gradient pump, a single-channel pump, a column thermostat (L 7350), an autosampler, a fluorescence detector (L 7480), an automatic six-way valve and a PC for integration. All HPLC instruments were from Merck-Hitachi (Darmstadt, Germany). The

metabolites were enriched on a special precolumn consisting of copper phthalocyanine-modified silica gel, and were transferred on an RP-C 18 column (LiChrospher PAH 5µm 250-4 with precolumn) by a column switching program.

After separation, the analytes were quantified by a switchable wavelength fluorescence detector. For the detection of the phenanthrene metabolites, the excitation wavelength was 244nm and emission wavelength 370 nm. For the detection of 1-hydroxypyrene, the excitation wavelength of 236 nm and the emission wavelength of 386 nm were used. To quantify the co-eluting metabolites, the sum of 2- and 9 hydroxyphenanthrene was quantified by a calibration curve of 2-hydroxyphenanthrene. The limits of quantification were 16 ng/l urine for 1-hydroxyphenanthrene, 4 ng/l for 2/9- hydroxyphenanthrene, 5 ng/l for 3-hydroxyphenanthrene, 8 ng/l for 4-hydroxyphenanthrene and 12 ng/l for 1-hydroxypyrene. Creatinine in urine was determined photometrically as picrate according to the Jaffe method.(Taussky, 1954)

2.7 Statistical Analysis

Descriptive analyses were performed on the children who participated in the study. Based upon knowledge of socioeconomic strata in the city of Jeddah, income was categorized into <3000 Saudi Riyal (SR)/month (corresponding to <800 US dollars/month), 3000-9000SR/month and >9000SR/month. Residence of smokers in the home was categorized into a dichotomous variable as was burning incense (sometimes/rarely versus always) and use of charbroiled food (none during day before urine collection versus once or more during the day before sampling).

Means and standard deviations of particulate matter and total PAH as well as individual PAH compounds were computed, and compared by school location using an unpaired student's T-test.

Univariate analyses examined relationships of urinary 1-hydroxypyrene, total phenanthrenes, systolic and diastolic blood pressure, brachial artery distensibility, heart rate and pulse pressure with the above referenced sociodemographic and exposure variables. Multivariable analyses were then performed on these outcomes, using $p<0.10$ as a criterion for inclusion. For 1-hydroxypyrene and phenanthrene measurements, this resulted in exclusion of age, height and BMI Z-score in final multivariable models. Income category was included in the final multivariable model and subsequently excluded to assess potential effect modification on the school location-phenanthrene relationship. For cardiovascular measures, all sociodemographic and exposure covariates were included in final multivariable models in light of a strong literature suggesting socioeconomic relationships and tobacco smoke exposures with elevated blood pressure in children.(Johnson et al., 2009; Weitzman et al., 2005) Multivariable models examined both continuous systolic and blood pressure and Z-scores to assess whether use of American normative data were influential on the results.

2. Results

184 boys participated in the study (Table 1). The overall participation rate was 70.0% (63/88 from School 1, or 71.6%; 58/83 from School 2, or 69.8%; 63/92 from School 3, or 68.5%). All

participants were born in their current residence. The mean age was 12.1 years (SD 1.2); 22.6% were overweight or obese, and 51.1% met prehypertension criteria. Participants were near-equally distributed from the three schools. As expected, there were significant differences in family income by school location, with children from lower income families in the schools near the ring road and the refinery. Substantial second-hand smoke (37.2%) and incense exposure (88.9% sometimes or always) were reported, and nearly half had ingested charbroiled food in the day before the urine collection. Use of charbroiled food was less frequent (p=0.022) in the school away from the refinery without substantial nearby traffic (the "background" school), though BMI Z-scores were substantially higher (p=0.002) and elevated BMI categories were more frequent (p=0.004) in this school.

Substantial differences in PM_{10} and PAH were noted when the schools near the ring road and the oil refinery were compared to the school near the Red Sea (Table 2). PM_{10} and PAH did not differ substantially between the school near the ring road and the school near the oil refinery. Differences in total PAH of the schools near the ring road and the oil refinery with the "background" school were driven in magnitude by substantially higher phenanthrene concentrations (see Appendix), though nearly all PAHs measured were substantially higher in the other two schools compared with the school cited far from vehicular and point sources. Individual PAHs did not differ appreciably between the school near the ring road and the school near the oil refinery.

Univariate analyses revealed interesting individual-level differences in urinary biomarkers of PAH exposure (Table 3), in which proximity to the ring road (p=0.006) and the oil refinery

(p=0.016) were each associated with increases in 1-hydroxypyrene but not hydroxyphenanthrenes, whereas use of incense frequently was associated with substantially higher hydroxyphenanthrene concentrations (p=0.004) but not 1-hydroxypyrene. The highest category of family income was also associated with significantly lower urinary 1-hydroxypyrene (p=0.025). No socioeconomic, dietary, or household exposure factors were associated with blood pressure, though substantially higher diastolic blood pressure (4.71 mm Hg, 95% CI: 2.23, 7.18) was identified in the children near the refinery compared to the other groups. As expected, age, height and BMI Z-score were significantly associated with systolic and diastolic blood pressure.

Multivariable analyses identified substantially higher 1-hydroxypyrene concentrations in children attending the school near the ring road (Table 4), even after controlling for income category (107.3 ng/g creatinine, 95% CI: 6.4, 250.1). Children attending school near the oil refinery did have higher 1-hydroxypyrene concentrations compared to children attending the "background" school prior to controlling for income category (90.4 ng/g creatinine, 95% CI: 12.0, 1959) though this attenuated to nonsignificance on adding income category to the models (77.1 ng/g creatinine, 95% CI: -17.7, 212.7), suggesting residual confounding. Associations with total hydroxyphenanthrenes differed in that the only significant association was with incense use, in which children living in homes with heavy incense use had 124.3 ng/g creatinine higher total hydroxyphenanthrenes than other children (95% CI: 27.1, 203.5).

Multivariable regression models revealed strong associations of school location near the refinery with blood pressure outcomes, however modeled, and controlling either for urinary total hydroxyphenanthrenes or 1-hydroxypyrene (Table 5). Increments of 6.01 mm Hg systolic (95%

CI: 1.74, 10.27) and 6.44 mm Hg diastolic (95% CI: 3.08, 9.80) blood pressure, controlling for 1-hydroxypyrene, with similar associations when blood pressure Z-scores using US norms were applied or hydroxyphenanthrene was substituted for 1-hydroxypyrene. A 4.35-fold odds of prehypertension was identified (95% CI 1.42, 13.3) for attending school near the refinery, controlling for urinary 1-hydroxypyrene. Neither PAH biomarker was significantly associated with any blood pressure outcome, either in multivariable models with school location or without (data not shown). No differences in heart rate, pulse pressure, or brachial artery distensibility were significant.

3. Discussion

The main finding of this manuscript is that attendance of a school near a Saudi Arabian oil refinery is significantly associated with elevated blood pressure. Substantially elevated PAH biomarkers were identified with attending school in proximity to the refinery or high traffic density, though the association with refinery proximity was subject to residual confounding by family income. Coupled with biologically plausible mechanisms, they raise potential concern about vulnerability of children to the early development of cardiovascular disease with chronic air pollutant exposure.

As would be expected from a hypothesis-generating cross-sectional study, there are substantial limitations to interpretation. There are many well-known early life risks for cardiovascular disease (e.g., low birth weight(Barker, 1990; Barker and Osmond, 1986; Barker et al., 1992)) which we did not assess in the present study. Given the potential consequences of air pollutant

exposure for fetal growth, an alternative hypothesis is that exposure during pregnancy may have produced the associations described here, insofar as children experienced their gestations in the same areas as they attended school. We also did not collect data on caloric or salt intake which could have also contributed to elevations in blood pressure.(Yang et al., 2012) While a child spends forty hours or more per week at school, children could have lived in highly disparate areas with higher or lower PAH exposures that could have influenced cardiovascular parameters. Exposure imprecision is known to bias towards the null with categorical outcomes such as prehypertension,(Carroll, 1998; Fleiss and Shrout, 1977; Fuller, 1987) though the direction of this bias for the continuous outcomes is less clear. We did not exclude for preexisting medical conditions that could have contributed to the associations identified here, or other environmental chemical exposures, including phthalates and bisphenol A, which may independently contribute to insulin resistance, elevated blood pressure or signs of endothelial dysfunction including albuminuria.(Trasande et al., 2013a; Trasande et al., 2013b; Trasande et al., 2014; Trasande et al., 2013c) We were also unable to examine insulin resistance or oxidant stress biomarkers, which could both be mediators of the associations identified here. It is also important to recognize that the measurements of urinary biomarkers represent only a short snapshot of exposure, while the effects on cardiovascular outcomes are likely to be the result of chronic exposures which may not be well captured in the data for individual subjects, as even the pooled biomarker data from multiple time points reflect only exposures over the past 1-2 months.

The well-documented disparities in environmental exposures by socioeconomic status posed difficulty in our study.(Freudenberg et al., 2011; Woodruff et al., 2003) We unfortunately could not identify a school located in a high socioeconomic status area near the refinery, and are aware

that unmeasured dietary or environmental factors that coexist with low socioeconomic status could also have confounded the relationships of school location with PAH biomarkers, and school location with elevated blood pressure. While these caveats are appropriate, the study also has many strengths, including careful measurement of cardiovascular profiles in a way that minimizes "white coat hypertension,"(Sorof et al., 2001; Verdecchia et al., 1992) assessment of airborne as well as biological markers of PAH exposure, and assessment of multiple potential confounders (e.g., tobacco smoke exposure, incense burning) in three relatively homogeneous samples permitting careful comparison.

Ours is the first study to our knowledge to relate environmental measures and biomarkers to measures of peripheral arterial wall stiffness in children. Though associations with brachial artery distensibility were nonsignificant, it should be noted that the population in this study is younger than those previous studies and may have experienced too short a period of exposure to see an effect on distensibility.(Urbina et al., 2011; Urbina et al., 2010) The mean BMI in our study population is also much lower than in past studies, and the lower BMI in the school near the refinery compared with the background school could also have confounded our interpretation. The effect size may have been too small to detect at this young an age.

Air pollution is a complex mixture, and the absent association of PAHs with blood pressure in the study population raises interesting questions. Another plausible group of mediators of the PM effect on cardiovascular disease are heavy metals, especially nickel and vanadium, which are prominent, along with PAHs, in oil burning and refining.(Bakker et al., 2000) Nickel and vanadium are also known to be potent oxidant stressors.(Chen et al., 1998; Chen et al., 2003;

Krejsa et al., 1997; Manzo et al., 1992; Stohs and Bagchi, 1995; Valko et al., 2005) Inhalation studies in mice have induced cardiovascular disease, and analyses of large longitudinal cohorts have also identified associations of high levels of these heavy metals in PM with increased adverse cardiovascular events.(Dominici F et al., 2007 ; Lippmann M et al., 2006) We did not measure heavy metals in our study population, and especially in a highly exposed subpopulation such as children attending school near an oil refinery heavy metals or other unmeasured air pollutants could explain the association of blood pressure with proximity to the refinery.

The absent association of PAH biomarkers or proximity to the refinery or traffic with brachial artery distensibility in the presence of the association with blood pressure bears some mention. There is no gold standard for assessing arterial stiffness, with each method based on different physiologic assumptions.(Woodman et al., 2005) While brachial artery distensibility is a robust measure of peripheral aortic stiffness, central aortic stiffness may have been more sensitive to detect the microvascular changes that would be induced by PAH or other air pollutant exposures. Pulse wave velocity in particular may therefore have been more useful. Given the alternative explanation that heavy metals in the PM may contribute uniquely to cardiovascular risk in our study population, it should be noted that heavy metals such as methylmercury have been documented to contribute to autonomic instability.(Grandjean et al., 2004; Murata et al., 2006) While we did not measure heart rate variability in the present study, future work in such populations should consider the possibility of autonomic cardiovascular effects rather than oxidant stress-mediated endothelial dysfunction as the potential mechanism.

In conclusion, proximity to an oil refinery in Saudi Arabia is associated with prehypertension and increases in PAH and PM_{10} exposures. Further study including insulin resistance measurements, better control for confounding, and longitudinal measurement is indicated.

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Figure 1. Study locations (red dots) in the context of locations of the refinery, city center and King Abdulaziz International Airport (KAIA, green dots).