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The Association of Urinary Polycyclic Aromatic Hydrocarbon Metabolites and C-Reactive Protein Levels in the HAPIN Trial

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Master of Science in Public Health

Environmental Health and Epidemiology

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Bachelor of Arts in Cellular Biology, Huntingdon College, 2018.

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Abstract

The Association of Urinary Polycyclic Aromatic Hydrocarbon Metabolites and C-Reactive Protein Levels in the HAPIN Trial

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Solid fuel sources are used by upwards of 3 billion people worldwide for cooking and heating homes, primarily in low- and middle-income countries. The resulting household air pollution accounted for an estimated 2.6 million deaths in 2016. The aim of the Household Air Pollution Intervention Network Trial is to conduct a large-scale, randomized control trial using a clean source of fuel (Liquid Petroleum Gas) for stoves as an intervention. PAHs are a group of chemicals that occur naturally in coal, crude oil, and gasoline, and are also byproducts of combustion and are formed when coal, oil, gas, wood, garbage, and tobacco are burned as a fuel source or heating source or when foods are grilled. Inhalation and ingestion of PAHs has been linked to blood and liver abnormalities, as well as inflammation of the respiratory system (CDC). Urinary PAHs were found to have a positive association with elevated baseline serum C-Reactive Protein levels, which are a biomarker for inflammation (Alshaarawy Et al). For the purpose of this study, we are only analyzing baseline data on the “other older adult woman” living in the household alongside the pregnant study participants. The analysis involved the creation of eight different multiple linear regression models with a PAH metabolite (PAHm) as the dependent variable and CRP as an independent predictor variable alongside demographic data, backwards elimination was performed until all variables were significant at p-value<0.05. Significant predictor variables included CRP, Specific Gravity, Charred Food, Stove Fuel, and IRC. All PAHm were significantly associated with Specific Gravity ($p<0.0001$), 2-Naphthol was associated with Charred Food and IRC ($p<0.05$), 1-Naphthol was associated with Stove Fuel and IRC, Fluorene and 1-Hydroxypyrene were both associated with Charred Food, Stove Fuel, and IRC ($p<0.05$), 1-Hydroxyphenanthrene and 2-Hydroxyphenanthrene were both associated with CRP, Charred Food, and Stove Fuel ($p<0.05$ for all, except charred was nominally significant at $p<0.1$ for OHPHE2), 4-Hydroxyphenanthrene was associated with Charred Food ($p<0.05$), and sumPAH was associated with Stove Fuel and IRC ($p<0.05$). The analysis highlights the need for further research after intervention to better understand the association of the predictor variables, particularly CRP.

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Purpose

The purpose of this study is to answer two research questions:

1. What does the urinary PAH metabolite distribution look like in adult women in the HAPIN Trial?
2. Are urinary PAH metabolites positively associated with C-Reactive Protein levels in a dose-response fashion in adult women in the HAPIN Trial?

Background

Household air pollution and health**:** Solid fuel sources are used by upwards of 3 billion people worldwide for cooking and heating homes, primarily in low- and middle-income countries (LMIC's). The resulting household air pollution (HAP) accounted for an estimated 2.6 million deaths per year in 2016, primarily woman and children. HAP exposure was listed as the number one environmental risk factor in the global burden of disease for this drastic loss of life. The aim of the Household Air Pollution Intervention Network Trial (HAPIN) is to conduct a large-scale, randomized control trial using a clean source of fuel (Liquid Petroleum Gas) for stoves as an intervention in 3200 study households across four LMICs. This study is designed to obtain rigorous evidence regarding health benefits across the lifespan from using cleaner burning sources. Each study site recruited 800 pregnant women <35 years of age and ≤ 9 weeks gestation. Half these households were then randomized into the LPG stove intervention arm with 18 months' supply of fuel, or the control arm which was to continue cooking with solid

biomass fuel sources. The control arm was compensated for participation in the study. The mother along with child will be followed until the child is 1 year of age. In households with another older non-pregnant adult woman, aged 40 to <80, she will also be followed for the 18-month follow-up period. In order to encourage intervention uptake behavioral change strategies were implemented. For the purpose of this specific study, samples from India were excluded. Data collection included extensive questionnaires and biospecimen collections (urine and dried blood spots (DBS)). For this study, we are specifically evaluating older adult women living in these households from Guatemala, Peru, and Rwanda.

Polycyclic Aromatic Hydrocarbons (PAHs)**:** PAHs are a group of chemicals that occur naturally in coal, crude oil, and gasoline. PAHs are also byproducts of combustion and are formed when coal, oil, gas, wood, garbage, and tobacco are burned as a fuel source or heating source or when foods are grilled (CDC). More than half of the global population uses these sources for cooking and heating indoors leading to high exposure (Li Et al). Exposure in industrialized countries is primarily from traffic pollution, while exposure in most third world countries is due to biomass fuel for cooking and heating (Choi, H). Exposure to PAHs can occur through dermal contact, ingestion of chargrilled and contaminated foods; however, the primary route of exposure is via inhalation of smoke and particulate matter from the burning of substances as described above. Typical environmental exposures to PAHs are much lower than occupational exposure or exposure from biomass-burning stoves. High exposure by dermal contact and inhalation have been linked to skin irritation, and blood and liver abnormalities. Inhalation of PAHs has been linked to inflammation of the respiratory system (CDC).

Hydroxylated PAH metabolites are often measured in urine samples due to ease of collection, their universal presence in urine, and difficulties measuring them in blood as the parent PAH or adducts. Urinary PAHs were found to have a positive association with elevated baseline serum C-Reactive Protein levels, which are a biomarker for inflammation (Alshaarawy Et al)

C-Reactive Protein (CRP)**:** CRP is an acute inflammatory protein that can increase one 1000-fold at the site of an infection or inflammation. CRP is primarily synthesized in the liver by hepatocytes but are also formed by many other cell types such as smooth muscle, macrophages, endothelial cells, lymphocytes, and adipocytes. Growing evidence suggest that CRP plays a major role in the inflammatory response to infection of the host and resulting pathways like “the complement pathway, apoptosis, phagocytosis, nitric oxide (NO) release, and the production of cytokines, particularly interleukin-6 and tumor necrosis factor-α (Sproston Et al).”

We are evaluating the association of hydroxylated PAH metabolites (PAHm) with CRP to determine if concentrated exposure to HAP increases inflammation, hence CRP levels, and if reducing exposure helps to reduce inflammation as measured by CRP.

Methods

PAH’s Extraction**:** Urine samples (n=202) from the adult older women in the HAPIN trial were analyzed to obtain PAHm levels. Samples were stored at -20^{o}C at International Research Centers (IRC) in the country in which the samples were collected until shipment on dry ice to the Biomarker Core Lab at Emory University. Once at the Biomarker Core samples were stored at -80^{o}C until analysis. Samples were taken out of

the freezer and placed in a warm water bath until thawed. 50 uL of isotopically labeled internal standard was added to a 1.5mL plastic microcentrifuge test tube. In addition, 250uL of β-glucuronidase/sulfatase enzyme solution was added to the tube along with 100uL of 12.5 mg/mL solution of L-Ascorbic acid then vortex mixed. 400uL of urine was added to the test tube and allowed to incubate overnight (17hrs) at 37^{o}C to liberate glucuronide bound PAHm. After incubation, 200uL of MeOH was added to the mixture in the tube to quench the enzyme then it was vortex mixed and centrifuged at 14,000vrpm for 10 minutes. The sample was then filtered using a 96-well sample filter onto a 96-well plate for analysis. The samples were analysed that day, not frozen, using online extraction with liquid chromatography-tandem mass spectrometry LC-MS/MS). 35 unknown samples were prepared and analysed per run. A full calibration curve, blanks and quality control samples were analysed concurrently with unknown samples. In addition, for further quality assurance, NIST Standard Reference Materials 3672 and 3673 were included in each run.

CRP**:** DBS were collected onto standard Guthrie cards using a single drop of capillary blood per spot. They were dried at ambient temperature and were put into labeled zip top bags along with a desiccant pouch and humidity indicator card and stored at -20^{o}C at IRCs until shipment on dry ice to the Biomarker Core Lab at Emory University. Once at the Biomarker Core samples were stored at -80^{o}C until analysis. Using a 6-mm hole punch, one punch was taken from each DBS. The punch was submerged in 500 uL of phosphate buffered saline in 1.5 mL Eppendorf tubes. The sample was then vortexed and incubated overnight (16hrs) at 4^{o}C to enable the dissolution of all of the blood in the punch. In a new 1.5uL test tube, 190 uL of diluent 101 was added, then 10uL of the

eluted DBS sample. MSD plates are pre-coated with capture antibodies and exposed to a proprietary stabilizing treatment to ensure the integrity and stability of the immobilized antibodies. The MSD plate was washed 3 times with 150uL/ well with Wash Buffer. 25uL of sample was added to its assigned well and the plate was sealed and allowed to incubate at room temperature for 2 hours with a plate shaker set to 750. After incubation, 25uL of detection antibody were added to each assigned well and the plate was sealed and incubated at room temperature for 1 hour on plate shaker set at 750. The plate was then washed 3 times with Wash Buffer (150uL/well). 150uL of 1X Read Buffer T were added to each well, the plate was then analyzed on the MSD Plate Reader.

Data Analysis**:** A multiple linear regression was performed to describe urinary PAH levels in the HAPIN trial population, with demographic data as the independent variable and PAH as the dependent variable. A multiple linear regression controlling for Age, BMI, Education, SHS, Charred Food Ingestion, Stove Fuel, and IRC was performed to analyze the PAH/CRP dose response. With PAH being the dependent variable and CRP being the independent variable. Eight PAHm were measured and tested within the models: 2-Naphthol (_2N), 1-Naphthol (_1N), Fluorene (FLU), 1-Hydroxypyrene (OHP), 1-Hydroxyphenanthrene (OHPHE1), 2-Hydroxyphenanthrene (OHPHE2), 4-Hydroxyphenanthrene (OHPHE4), and sumPAH.

Results

Descriptive Statistics

CRP**:** There were 98 CRP samples analyzed from the OAW study population. The mean was 0.5667 (median 0.3334) and standard deviation of 1.0768, with a minimum value 0.0043 and a maximum of 9.3861. A relative standard deviation (RSD) was calculated for each CRP sample, the mean for the RSD was 3.9355, with a minimum of 0 and maximum of 14.6000.

Table 1							
	CRP_Mean	RSD_Mean	CRP_Min	CRP_Max	RSD_SD	RSD_Min	RSD_Max
Guatemala	0.5919	5.8917	0.0209	9.3861	4.3643	0.0000	14.6000
Rwanda	0.4976	6.8577	0.0043	2.4546	2.2998	2.1000	10.3000

Table 1. CRP distribution by Country.

PAH**:** There were 202 total samples analyzed for 7 different PAHs: _2N, _1N, FLU, OHPHE2, OHPHE1, OHPHE4, and OHP. Table 2 has the mean, SD, minimum and

Table 2					
	Variable	Mean	SD	Min	Max
Overall	_2N	11.6041	11.7301	0.5300	89.0500
	_1N	20.7845	112.7443	0.6300	1596.50
	FLU	3.1418	3.8966	0.2800	37.9300
	OHPHE2	1.7162	1.7012	0.2000	10.5700
	OHPHE1	1.3118	1.4438	0.1100	14.4100
	OHPHE4	0.2193	0.2296	0.0300	2.1900
	OHP	2.3412	2.3178	0.1800	15.3900
	sumPAH	269.3043	816.6721	16.6200	11374.57

maximum for each PAHm overall and by country.

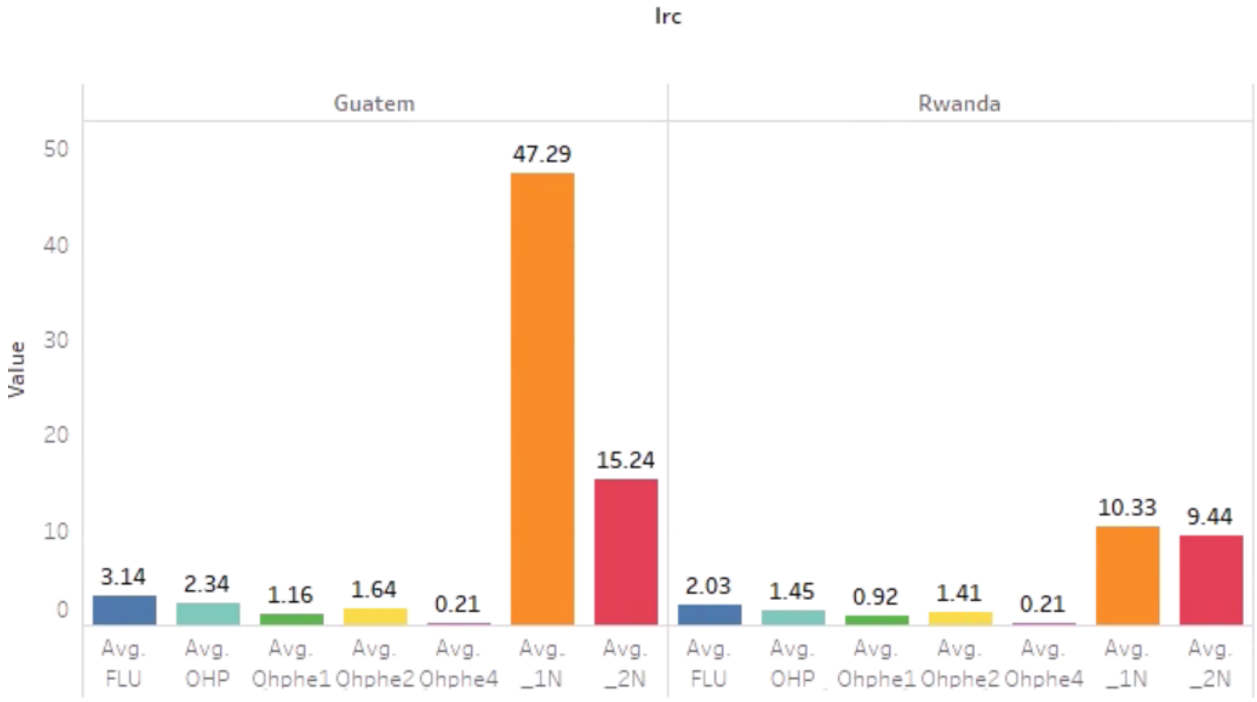

Figure 1. PAHm by Country.

Demographic Data**:** Study sites for this study were limited to Guatemala and Rwanda due to available CRP data. Guatemala accounted for 52 participants for this study's purpose, and Rwanda accounted for 24. The mean age of the women in the study population used for this research was 53.76 years old, minimum 40.50, maximum 74.30, and a standard deviation of 8.23 years. The mean BMI was 24.52, minimum 15.35, maximum 49.59, and a standard deviation of 4.87. Education was categorized into three categories with the following frequencies: no formal education (N= 32), Primary Schooling (N= 40), and some higher education completed (N= 3). Stove Fuel accounted for two sources with the following frequencies: Wood (N= 74), and charcoal (N= 2). Secondhand Smoke (shs) was measured in the study population by questionnaire with the following frequencies: Yes (N= 11), and No (N= 65). Charred food ingestion was measured in the study population by questionnaire with the following frequencies: Yes (N= 4), and No (N= 72).

Model Results: Eight general linear regression models were created, one for each PAHm and one for sumPAH. Each model used PAHm as the dependent variable and CRP, Sample Specific Gravity, Age, BMI, Charred Food, SHS, Stove Fuel Type, Education, and IRC as independent predictor variables. In order to fit the normality assumption, the following variables were logged: PAHm, CRP, Age, and BMI. Backwards elimination was then performed for each model until each predictor left was statistically significant at p-value < 0.05, or if necessary, nominally significant at p-value < 0.1.

Model	CRP	Spec_Grav	Charred	Stove_Fuel	IRC
sumPAH	No	Yes	No	Yes	Yes
_2N	No	Yes	Yes	No	Yes
_1N	No	Yes	No	Yes	Yes
FLU	No	Yes	Yes	Yes	Yes
OHPHE2	Yes	Yes	Yes	Yes	No
OHPHE1	Yes	Yes	Yes	Yes	No
OHPHE4	No	Yes	Yes	No	No
OHP	No	Yes	Yes	Yes	Yes

Table 3. PAHm Model and significant variables. Charred for OHPHE2 model was nominally significant at p-value<0.1.

Model 1: sumPAH

For every one unit increase in specific gravity, sumPAH increases 113.35 units on average (F-value= 44.86, p-value<0.001), controlling for stove fuel type and IRC. For every one unit increase by stove fuel type (charcoal), sumPAH decreases 0.77 units on average (F-value= 5.18, p-value<0.05), controlling for specific gravity and IRC. For every one unit increase by IRC, sumPAH increases 0.48 units on average (F-value= 7.78, p-value<0.05), controlling for specific gravity and stove fuel type. Model was significant at p-value<0.0001, F-value= 19.27, R^2= 0.45.

Model 2: _2N

For every one unit increase in specific gravity, _2N increases 110.07 units on average (F-value= 62.26, p-value<0.001), when controlling for charred food ingestion and IRC. For every one unit increase by charred, _2N increases 0.53 units on average (F-average= 4.47, p-value<0.05), controlling for specific gravity and IRC. For every one unit increase by IRC, _2N increased by 0.41 units on average (F-value= 8.91, p-value<0.05), controlling for specific gravity and Charred food ingestion. Model was significant at p-value<0.0001, F-value= 25.21, R^2= 0.51.

Model 3: _1N

For every one unit increase in specific gravity, _1N increases 111.75 units on average (F-value= 27.39, p-value<0.001), controlling for stove fuel type and IRC. For every one unit increase in stove fuel type (charcoal), _1N decreased 1.44 units on average (F-value= 7.98, p-value<0.05), controlling for specific gravity and IRC. For every one unit increase in by IRC, _1N increased 0.44 units on average (F-value= 4.09, p-value<0.05), controlling for specific gravity and stove fuel type. Model was significant at p-value<0.0001, F-value= 13.15, R^2= 0.35.

Model 4: FLU

For every one unit increase in specific gravity, FLU increased 104.85 units on average (F-value= 56.43, p-value<0.001), controlling for charred food ingestion, stove fuel type, and IRC. For every one unit increase by charred food intake, FLU increased 0.55 units on average (F-value= 4.35, p-value<0.05), controlling for specific gravity, stove fuel type, and IRC. For every one unit increase in stove fuel type (charcoal), FLU decreased

0.68 units on average (F-value= 6.01, p-value<0.05), controlling for specific gravity, charred food ingestion, and IRC. For every one unit increase by IRC, FLU increased 0.44 units on average (F-value= 9.27, P-value<0.05), controlling for specific gravity, charred food ingestion, and stove fuel type. Model was significant at P-value<0.0001, F-value= 19.02, R^2= 0.52.

Model 5: OHPHE2

For every one unit increase in CRP, OHPHE2 decreased 0.06 units on average (F-value= 4.08, P-value<0.05), controlling for specific gravity, charred food ingestion, and stove fuel type. For every one unit increase in specific gravity, OHPHE2 increased by 108.86 units on average (F-value= 59.32, P-value< 0.0001), controlling for CRP, Charred food ingestion, and stove fuel type. For every one unit increase in charred food intake, OHPHE2 increased 0.56 units on average (F-value= 3.35, P-value nominally significant at <0.1), controlling for CRP, Specific gravity, and stove fuel type. For every one unit increase by stove type (charcoal), OHPHE2 decreased 1.03 units (F-value= 6.43, P-value<0.05), controlling for CRP, Specific gravity, and charred food ingestion. Model was significant at P-value<0.0001, F-value= 18.30, R^2= 0.51.

Model 6: OHPHE1

For every one unit increase of CRP, OHPHE1 decreased 0.07 units on average (F-value= 5.36, P-value< 0.05), controlling for specific gravity, charred food ingestion, and stove fuel type. For every one unit increase of specific gravity, OHPHE1 increased 105.13 units on average (F-value= 64.73, P-value< 0.0001), controlling for CRP, charred food ingestion, and stove fuel type. For every one unit increase by charred food

ingestion, OHPHE1 increased 0.73 units on average (F-value= 6.84, P-value< 0.05), controlling for CRP, specific gravity, and stove fuel type. For every one unit increase by stove fuel type (charcoal), OHPHE1 decreased 0.90 units on average (F-value= 5.71, P-value< 0.05), controlling for CRP, specific gravity, charred food ingestion. Model was significant at P-value<0.0001, F-value= 20.66, and R^2= 0.54)

Model 7: OHPHE4

For every one unit increase in specific gravity, OHPHE4 increased 104.36 units (F-value= 56.54, P-value<0.0001), controlling for charred food ingestion. For every one unit increase by charred food ingestion, OHPHE4 increased 0.57 units (F-value= 4.25, P-value<0.05), controlling for specific gravity. Model was significant at P-value<0.0001, F-value= 30.39, R^2= 0.45.

Model 8: OHP

For every one unit increase in specific gravity, OHP increased 92.36 units (F-value= 36.19, P-value<0.0001), controlling for charred food ingestion, stove fuel type, and IRC. For every one unit increase by charred food ingestion, OHP increased 0.73 units on average (F-value= 5.51, P-value<0.05), controlling for specific gravity, stove fuel type, and IRC. For every one unit increase by stove fuel type (charcoal), OHP decreased 0.95 units on average (F-value= 7.11, P-value<0.05), controlling for specific gravity, charred food ingestion, and IRC. For every one unit increase by IRC, OHP increased 0.37 units on average (F-value= 5.27, P-value<0.05), controlling for specific gravity, charred food ingestion, and stove fuel type. Model was significant at p-value<0.0001, F-value= 13.52, R^2= 0.43.

Discussion

With this cross-sectional study involving adult women who live in households with the pregnant women enrolled in the study, we successfully described the distribution of eight PAH metabolites in the adult women of the HAPIN trial, answering our first research question. Our second research question “Are urinary PAH metabolites positively associated with C-Reactive Protein levels in a dose-response fashion in adult women in the HAPIN Trial?” was successfully in finding a correlation, although minutely negative, with two of the PAH metabolites (OHPHE1 and OHPHE2).

CRP:

CRP was found to be significant at p-value<0.05 for two PAHm, OHPHE1 and OHPHE2.1-Hydroxyphenanthrene (OHPHE1) and 2-Hydroxyphenanthrene (OHPHE2) are both primarily associated with charcoal burning as home heating and stove fuel sources, and they primarily follow the inhalational exposure route (Avagyan Et al). With this knowledge, it is possible to conclude that CRP is a good indicator for inhalational exposure to PAHm. PAHm and CRP correlation is further discussed in the limitations and next steps section below.

Specific Gravity:

Specific Gravity was found to be significant at p-value<0.0001 for all PAHm and sumPAH. Specific gravity is the process of measuring the density of the solutes in a urine sample, and then comparing them to the density of water. The density of a urine samples can vary for multiple reasons including dehydration and kidney disfunction.

Specific gravity measurements allow for analyses to adjust for urine concentration and adjust the measurement of the analyte in question thereafter (Rugheed, G). With this, it is reasonable to conclude that the correlation and increase of PAHm with every one unit increase of Specific Gravity is expected, as this means the concentration of the urine sample has increased.

Charred Food Ingestion:

Charred food ingestion was found to be significant at p-value<0.05 for _2N, FLU, OHPHE1, OHPHE4, and OHP. Charred food ingestion was nominally significant at p-value<0.1 for OHPHE2. Due to the inability of Urine PAH tests to disassociate inhalational PAH exposure vs ingestion PAH exposure, and higher molecular weight PAHs often being excreted in higher concentrations through feces, charred food ingestion is modified by inhalational exposure. 2-Naphthol has been associated to inhalational exposure from wood burning stoves (U.S. National Library of Medicine), and 2-Naphthol is also found in charred foods and therefore can be accounted for through ingestion (Cotton, S.). In this study, 74 out of 76 participants used wood as their main stove fuel source, and only 4 out of 76 participants said that they ingest charred foods. So, it is likely that Charred Food Ingestion is significant for 2-Naphthol due to a confounding effect of stove fuel type and the charred food ingestion by the few participants. Fluorene is found in charcoal combustion as a byproduct, tobacco smoke, and fuel emissions (CDC, 2017). It is expected that fluorene was found in charred food due to confounding/contamination with fuel source and possible unknown exposure to secondhand smoke and fuel emissions. As discussed in the stove fuel section below, OHPHE 1, OHPHE2, and OHPHE4 are all associated with charcoal use as stove fuel,

and not with charred food, most likely they are associated to charred food ingestion due to confounding by charcoal use for cooking. 1-Hydroxpyrene is associated to both charcoal and charred food ingestion (Strickland Et al), with the low amount of study participants that use charcoal and consume charred foods, this correlation between charred food and OHP is consistent with previous findings.

Stove Fuel Type:

Stove Fuel Type, either wood or charcoal, was significant at p-value<0.05 for sumPAH, _1N, FLU, OHPHE2, OHPHE1, and OHP. 1-Naphthol is found in wood burning stoves, as well as 2-Naphthol, with 1-Naphthol at lesser concentrations than 2-Naphthol (Avagyan Et al). With 2-Naphthol being the main source of PAHm from wood as stove fuel, and our study population almost ubiquitously using stove fuel as mentioned above, the model would not find a significant difference in 2-Naphthol and Stove fuel type. Therefore 1-Naphthol, at lesser concentrations and varying by sample more, was significant, while 2-Naphthol was not. Fluorene is primarily found in charcoal combustion as a byproduct (CDC, 2017), therefore with the low amount of charcoal as stove fuel users, this finding is unlikely to be from effect modification from other sources. 1-Hydroxyphenanthrene (OHPHE1) and 2-Hydroxyphenanthrene (OHPHE2) are both primarily associated with charcoal burning as home heating and stove fuel sources, and they primarily follow the inhalational exposure route (Avagyan Et al). With this knowledge, and the few (N=2) households who use charcoal as a stove fuel source, it is reasonable to say that the correlation between 1-Hydroxyphenanthrene (OHPHE1) and 2-Hydroxyphenanthrene (OHPHE2) is valid. 1-Hydroxypyrene is associated with charcoal as a heating and stove fuel source, as well as with charred food ingestion. 1-

Hydroxypyrene is also commonly found in ambient air samples due to coal plants and pollution (Strickland Et al). With the amount of study participants who answered yes to eating charred food, and yes to using charcoal as a stove fuel source, the association between 1-Hydroxypyrene and Stove fuel type is valid. With the majority of PAHm's being produced by stove fuel type, it is reasonable to conclude that sumPAH is strongly associated with stove fuel type in this study.

IRC:

IRC, either Rwanda or Guatemala, was significant at p-value<0.5 for sumPAH, _2N, _1N, FLU, and OHP. SumPAH was relatively similar for both countries, but due to controlling for PAH source contributions and molecular weights there was a significant difference in PAH levels overall based on country (IRC). 2-Naphthol had a significant difference in levels based on country while controlling for other sources, with Guatemala on average having a _2N level of 15.24 and Rwanda having a _2N level of 9.44. Guatemala had a severely increased average level of 1-Naphthol leading to a significant difference between countries, Guatemala equaling 47.29, and Rwanda equaling 10.33. Guatemala had higher average levels of Fluorene as well, Guatemala equaling 3.14, and Rwanda equaling 2.03. For 1-Hydroxypyrene, Guatemala once again had higher levels than Rwanda, 2.34 and 1.45, respectively.

Limitations and Next Steps

Limitations to this study primarily involve that we are performing a cross-sectional analysis of a longitudinal study at baseline, which makes it hard to show correlation between CRP and PAHm as the exposures we are assessing are almost ubiquitous

across the study population. Wood was primarily used (N= 74) over charcoal (N=2) as a fuel source, and charred food were reported to be consumed by only 4 out of the 76 participants. These are some of the primary producers of PAHm and it is difficult to find statistically significant effects when the population is relatively evenly exposed. We believe as the intervention of LPG stoves is implemented, and more samples beyond the baseline visit are analyzed that the relationship between the exposures producing the PAHm, and CRP will become statistically significant and have a positive correlation.

Next steps for our research of PAHm, its' sources and exposure routes, and the populations effected most by this hazard are to continue to analyze the urine and dried blood spot samples from the HAPIN study participants beyond baseline visits and expand analysis to the pregnant women and their children who are involved in the study. Doing this will allow us to understand a broader range of affects including age, BMI, and the secondhand smoke component.

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