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Concentrations of Urinary 8-Hydroxy-2′-deoxyguanosine and 8-isoprostane in Women Exposed to Woodsmoke in a Cookstove Intervention Study in San Marcos, Peru

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Abstract

Nearly half of the world's population is exposed to household air pollution (HAP) due to long hours spent in close proximity to unvented cooking fires. The effect of woodsmoke exposure on oxidative stress was examined by investigating the association between woodsmoke exposure and biomarkers of DNA oxidation (8-hydroxy-2′-deoxyguanosine [8-OHdG]) and lipid peroxidation (8-isoprostane) among control and intervention stove users. HAP exposure assessment was conducted within the framework of a community-randomized controlled trial of 51 communities in San Marcos Province, Cajamarca Region, Peru. The first morning urine voids after 48hr HAP exposure assessment from a subset of 45 control and 39 intervention stove users were analyzed for 8-OHdG and 8-isoprostane. General linear models and correlation analyses were performed. Urinary oxidative stress biomarkers ranged from 11.2 to 2270.0 μg/g creatinine (median: 132.6 μg/g creatinine) for 8-OHdG and from 0.1 to 4.5 μg/g creatinine (median: 0.8 μg/g creatinine) for 8-isoprostane among all study subjects (n=84). After controlling for the effects of traffic in the community and eating food exposed to fire among all subjects, cooking time was weakly, but positively associated with urinary 8-OHdG (r=0.29, p=0.01, n=80). Subjects' real-time personal CO exposures were negatively associated with 8-OHdG, particularly the maximum 30-second CO exposure during the sampling period (r=−0.32, p=0.001, n=73). 48hr time integrated personal $PM_{2.5}$ was negatively, but marginally associated with urinary 8-isoprostane (r=−0.21, p=0.09, n=69) after controlling for the effect of distance of homes to the road. Urinary 8-isoprostane levels

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Conflict of Interest statement
The authors declare no conflict of interest.

reported in the available literature are comparable to results found in the current study. However there were relatively high levels of urinary 8-OHdG compared to data in the available literature for 8-OHdG excretion. Results suggest a sustained systemic oxidative stress among these Peruvian women chronically exposed to wood smoke.

Keywords

8-OHdG; 8-isoprostane; cookstove; household air pollution; oxidative stress; Peru

1. Introduction

The use of solid fuels occurs mostly in the developing world where wood and crop residues are employed by households for cooking and heating (Smith and Mehta, 2003). These fuels are often used in unvented or poorly designed stoves which creates high levels of household air pollution (Rehfuess et al., 2006). Biomass combustion in the indoor environment is considered to be probably carcinogenic for humans (IARC, 2010). Although numerous pollutants including carbon monoxide, polycyclic aromatic hydrocarbons, formaldehyde and benzene are produced from biomass combustion, particulate matter (PM) is considered the best indicator of smoke exposure (Naeher et al., 2007; Perez-Padilla et al., 2010).

Mounting evidence points to the mutagenic, genotoxic and cytotoxic properties of biomass smoke, particularly for woodsmoke particulate matter (Danielsen et al., 2009). One toxicological mechanism by which PM has been shown to induce health effects, in *in-vitro* studies involving human cells or cell lines, is through the pathway of oxidative stress (Danielsen et al., 2009). Oxidative stress can cause damage to deoxyribonucleic acid (DNA) through the production of 8-hydroxylated guanine species such as 8-hydroxy-2′-deoxyguanosine (8-OHdG) (Arnett et al., 2005). Another toxicological effect of PM observed in *in-vitro* studies, specifically of woodsmoke PM, involves the generation of reactive oxygen species (ROS) such as perioxides that can react with the lipids of cell membrane. A common stable product of ROS-induced lipid peroxidation is 8-isoprostaglandin $F_{2\alpha}$ (8-isoprostane) (Danielsen et al., 2009). Hence, both 8-OHdG and 8-isoprostane are known markers of oxidative stress (Barregard et al., 2006; Loft et al., 1992).

8-Isoprostane is a prostaglandin (PG)-F2-like compound belonging to the F2 isoprostane class that is produced in vivo by the free radical-catalyzed peroxidation of arachidonic acid (Montuschi et al., 1999). On the other hand 8-OHdG is an oxidized nucleoside of DNA and it is considered as the most frequently detected and studied DNA lesion (Wu et al., 2004). 8-OHdG is excreted during the repair of DNA damage (Wu et al., 2004). Measurements of these compounds have been performed in biologic fluid, particularly in urine and can provide quantitative indices of oxidative stress (Montuschi et al., 1999). Aside experimental studies where the effect of woodsmoke on oxidative stress has been studied (Barregard et al., 2006; Barregard et al., 2008), the literature is scant on studies of biomass smoke exposure and resulting health effects, particularly regarding women in the developing world who cook with biomass fuels. Given the daily HAP exposure experienced by these women over a lifetime, there is the need to better understand the effect of woodsmoke exposure on oxidative stress in this vulnerable population.

In this study, we examined the concentrations of oxidative stress biomarkers of women exposed to woodsmoke. Study subjects from San Marcos, Cajarmarca Region, Peru, used wood as fuel for cooking and high PM levels have been measured among this population (Hartinger et al., 2013). Based on earlier studies among this population which did not reveal statistically significant differences in $PM_{2.5}$ and CO measurements (Commodore et al.,

2013; Hartinger et al., 2013), the primary focus of this study was to assess the association of urinary oxidative stress with biomass smoke exposure among control and intervention stove users as a combined population. Therefore this study examined the use of biomarkers in investigating oxidative stress, which plays a role in many diseases and in natural aging.

The aims of this study were (1) to determine whether increased exposure to biomass cookstove smoke was associated with increased urinary levels of 8-OHdG and 8-isoprostane among control and intervention subjects and (2) to investigate the factors that are associated with urinary 8-OHdG and 8-isoprostane concentrations among study subjects. We assessed woodsmoke exposure with personal and kitchen measurements of particulate matter and carbon monoxide. Woodsmoke exposure was also assessed with urinary hydroxylated polycyclic aromatic hydrocarbons (hydroxy-PAH), metabolites of PAHs generated through incomplete combustion (Li et al., 2012).

2. Material and Methods

2.1 Study Design and Study Homes

From June to August 2009, a cross sectional study was conducted within the framework of a community based randomized control trial (c-RCT) by the Instituto de Investigación Nutricional (IIN) and the Swiss Tropical and Public Health Institute (Commodore et al., 2013; Hartinger et al., 2013). The aim of the parent study was to evaluate an integrated home-based environmental intervention package (IHIP) against childhood diarrhoea and respiratory infections (Hartinger et al., 2011). The May–August period in the study region is characterized by dry conditions and cold nights with temperatures ranging from 7°C to 25°C (Hartinger et al., 2013). HAP exposure assessment occurred during this season, with no follow up during the rainy season. The altitude in the region ranges between 2200 and 3900 meters above sea level. Mean altitudes ± SD for intervention and control households are 2684 ± 284 and 2727 ± 438 meters above sea level respectively.

For this cross-sectional study, control and intervention households were from participating households in the parent c-RCT (n=250 and 253 for intervention and control homes respectively). The c-RCT involved 51 community clusters in which households used solid fuels for cooking in the Province of San Marcos, Cajamarca Region, Peru (Hartinger et al., 2011; Hartinger et al., 2012). The intervention was randomized at the community level, with the 51 community clusters allocated into control and intervention groups by using covariate-based constrained randomization (Hartinger et al., 2013). Field workers for the c-RCT visited all study homes during this 3 month period; however subjects' availability, willingness to participate, availability of air sampling equipment as well as time and budget constraints limited the total sample size of the present study.

Prior to the start of the c-RCT, a pilot study was conducted, where several potential stove designs were tested, and subjects were consulted on cooking habits and preferences to provide a user-friendly stove design which met their household and cooking needs (Hartinger et al., 2012). The final stove model, for the c-RCT was called the OPTIMA-improved stove (hereafter OPTIMA stove). Kitchen performance tests of the OPTIMA stoves revealed a 15% reduction in daily fuel and energy use and a 16% reduction in fuel and energy use per capita compared with the traditional open fire stoves, although there was no statistically significant differences in these reductions (Hartinger et al., 2011; Hartinger et al., 2012). The OPTIMA stove was built with red burnt bricks plastered with a mixture of mud, straw and donkey manure (Hartinger et al., 2012). It has three pot holes for cooking, a closed combustion chamber, metal chimney with a regulatory valve, a hood, and metal rods for support.

OPTIMA stoves were installed between October 2008 and January 2009 in 250 households (hereafter intervention households). There were no emissions tests or HAP exposure assessment prior to installation of the intervention stoves. The current study reports the only exposure assessment conducted for these stoves 6 to 8 months after installation (median 7.4 IQR = 6.6–8.1 month) (Hartinger et al., 2013). OPTIMA stoves were later stratified (after exposure assessment had occurred) into two categories based on their levels of functionality (FL). FL-I stoves were in good running conditions at the time of the assessment (plastered stove and no visible leaks when in use) and FL-II stoves were in need of repairs (re-plastering, filling small cracks, cleaning the chimney, chimney valve replacement). Field workers, during monthly visits, instructed OPTIMA stove users in the correct use of the stoves including cleaning and removal of ashes and wood residues. Although surveillance occurred in all study homes, stove repair and maintenance were not addressed during home visits until after air quality monitoring had occurred. Households with OPTIMA-improved stoves were re-visited 9 months (median 9.3 IQR= 9.0–9.7 month) after installation and repaired as needed by the original stove builders (Hartinger et al., 2013).

Control households in the c-RCT used a diversity of stove types (Hartinger et al., 2011). As such control households in this study had a wide range of stove types including (1) chimney stoves whose raw materials were provided by nongovernmental organizations (hereafter referred to as NGO), (2) chimney stoves built by the households themselves (hereafter referred to as self-improved by household), and (3) non-vented stoves with pot holes for cooking including the common three stone open fire stove (hereafter referred to as traditional). At the time of sampling, control households had stoves which had been in use between 4 months to over 10 years. Lastly, households were classified according to the primary stove in use and it is possible that some chimney stoves were used together with traditional stoves in some households, particularly for cooking animal feed or other meals which required substantial cooking times.

2.2 Exposure Monitoring

2.2.1 $PM_{2.5}$ and CO measurements—Forty eight-hr time integrated $PM_{2.5}$ and real time CO measurements were collected to assess the personal exposures of the household cook (usually the mother). Study subjects wore exposure monitoring equipment placed in vests, with sampling inlets in their breathing zones. Area 48-hr integrated $PM_{2.5}$ and real time CO measurements were also taken in the kitchen (Hartinger et al., 2013). Additionally, passive carbon monoxide (CO) diffusion tubes were used to obtain 48-hour time integrated CO measurements in study kitchens and personal CO exposures of the mother and a child under the age of five (Commodore et al., 2013). Questionnaires were administered on the second day of air sampling to obtain data on household air pollution, respiratory health-related symptoms, demographics, daily activities and commuting habits

2.3 Urine Sampling

After 48hr HAP exposure assessment had occurred, the first morning urine voids were provided by the subjects between 5 and 7 o'clock in the morning. This was to enable the investigation of the association between HAP measurements and urinary biomarkers. Samples were collected in sterile 100ml polypropylene containers by study subjects and stored in an insulated lunch bag with ice packs. Subjects were instructed not to touch the inner part of the polypropylene containers. The containers were then transported on ice packs to the study base and transferred into 50 ml polypropylene centrifuge tubes and frozen at −20°C until the end of the study. Samples were subsequently placed on dry ice and shipped to the United States. Upon arrival, the urine samples were still frozen and were stored at −80°C. The samples were later aliquoted at the National Center for Environmental Health (NCEH) laboratory at the Centers for Disease Control and Prevention (CDC) in

Atlanta, GA and shipped on dry ice to the Keck School of Medicine, University of Southern California, CA for 8-OHdG and 8-isoprostane analyses in May 2012. A total of 183 control and 155 intervention stove users provided urine samples. Results presented in this study are for oxidative stress analysis of urine samples from a subset of 45 control and 39 intervention stove users, for whom $PM_{2.5}$ and/or CO data were available.

In the current study, households were conveniently selected from participating households of the c-RCT: 50 control and 43 intervention households (Hartinger et al., 2013). Households from the parent study were eligible to participate, if they complied with the following criteria: (1) the stoves were located in an in-house kitchen environment (at least three full walls and a roof over the kitchen), (2) the households were within a half-hour walking distance from a road in order to transport the equipment and (3) the mother or caretaker agreed to wear the equipment to measure HAP and comply with the project instructions for the duration of the study (48hr) and agreed to sign the informed consent forms. Given the limited number of HAP measurement equipment, we stopped enrolment in each of the 51 communities after at least two households consented to participate. A total of 85 subjects provided urine samples (n=45 and 40 for control and intervention households respectively). Eight subjects did not provide urine samples (n=5 and 3 for control and intervention homes respectively). Of the 85 urine samples provided, only 84 had sufficient urine volume for analysis.

2.3.1 Exposure measurements: hydroxy-PAH analysis—Two mL urine aliquots were analyzed by the NCEH laboratory for polycyclic aromatic hydrocarbon metabolites, hydroxyl-substituted naphthalene, uorene, phenanthrene, and pyrene from urine samples of 155 intervention and 183 control stove users participating in the c-RCT (Li et al., 2012). A semi-automated liquid–liquid extraction and isotope dilution gas chromatography/high-resolution mass spectrometry (GC/HRMS) method were used (Li et al., 2006). We present hydroxylated polycyclic aromatic hydrocarbons (hydroxy-PAH) metabolite data for the 39 and 45 intervention and control subjects in the current oxidative stress study. The specific hydroxy-PAH metabolites include 1-naphthol (1-NAP), 2-naphthol (2-NAP), 2-hydroxy uorene (2-FLU), 3-hydroxy uorene (3-FLU), 9-hydroxy uorene (9-FLU), 1-hydroxyphenanthrene (1-PHE), 2-hydroxyphenanthrene (2-PHE), and 1-hydroxypyrene (1-PYR).

2.3.2 Oxidative stress measurement: Analysis of 8-OHdG—Urinary 8-OHdG concentrations were determined using the high performance liquid chromatography with electrochemical detection (HPLC-ECD) method (Pilger et al., 2002). In brief, a solution of 2 ml aliquot of the urine sample and 2 ml potassium dihydrogen phosphate buffer (KH_2PO_4) (0.1 M, pH 6) was applied to a solid phase extraction cartridge (Bond Elut-Certify, Varian) pre-conditioned with methanol, deionized (DI) water and KH_2PO_4 (0.1 M, pH 6). The cartridge was then washed with DI water and KH_2PO_4 (0.1 M, pH 6) and vacuum dried for 10 minutes. 8-OHdG was eluted by 2 ml solution of 30% methanol in DI water and 20 μl of eluted solution was injected into the HPLC (Alliance Waters 2695 with 2465 Electron-Chemical Detector). 8-OHdG was detected at a potential of +0.6 V at a range of 50 nA and a time constant of 1.0 sec. A linear calibration curve was obtained using aqueous solutions of 8-OHdG standard. The recovery rate of the analytical procedure was 99.6% with an analytic precision of 4.4% and a detection limit of 0.46 ng/ml.

2.3.3 Oxidative stress measurement: Analysis of 8-isoprostane—Urinary 8-iso-prostaglandin-$F_{2\alpha}$ (8-isoprostane) was analyzed using a liquid-chromatography tandem mass spectrometry (LC-MS/MS) technique modified from a previously published method (Liang et al., 2003). One milliliter of urine sample was spiked with 5 ng of 8-iso-$PGF_{2\alpha}$-D4, and diluted with 1 mL of water. After vortexing and centrifugation, the sample was purified

using solid phase extraction. A Bond Elut C18 cartridge was prewashed with 5 mL of methanol and 5 mL of water; then the sample was loaded and washed with 5 mL of water, 5 mL of methanol:water (5%:95%), and 1 mL of hexane, and then eluted with 2 mL of ethyl acetate. Following solvent evaporation, the sample extract was reconstituted with 100 μL of acetonitrile: water (15%:85%) and 20 μL was injected to LC-MS/MS for analysis. The LC-MS/MS was performed on TSQ Quantum Access MAX triple stage quadrupole mass spectrometer, coupled with an Accela 1250 pump and an Accela Open Autosampler.

Chromatographic separation was achieved on a Phenomenex Luna 3μ C18 (50 × 2 mm) column, with 0.1% formic acid in water (solvent A) and acetonitrile (solvent B) as mobile phase. The flow rate was 100 μL/min, and the gradient elution was programmed as follows: hold at 85% A for 1 minute, decreasing to 20% A over 10 minutes, hold for 3 minutes then increasing to 85% at 13.5 minutes. The mass spectrometer was operated in the negative ESI mode. The capillary temperature and vaporizer temperature were 270°C and 320°C, respectively. The ion spray voltage was set at 3000 V. Nitrogen sheath and auxiliary gases were set at 40 and 20 arbitrary units, respectively. The ion pairs of m/z 353/193, m/z357/197 were used to monitor 8-iso-$PGF_{2\alpha}$ and 8-iso- $PGF_{2\alpha}$-D4, respectively. The calibration curve was constructed over a range of 50 pg/mL–5000 pg/mL. The recovery was 98.4% with analytic precision (%CV) of 3.3% and a detection limit of 50 pg/mL.

2.3.4 Correction of urinary 8-isoprostane and 8-OHdG by creatinine—The raw values (pg/ml) of urinary 8-isoprostane and (ng/ml) 8-OHdG were conventionally corrected by urinary creatinine (mg/dl) to count for the dilutedness of spot urine, allowing for between-person comparisons. Urinary creatinine was measured on a Roche Hitachi 912 Chemistry Analyzer (Hitachi Inc., Pleasanton, CA) using the Creatinine Plus Assay, as described in Roche's Creatinine Plus Product Application # 03631761003. All laboratory analysis documented in this manuscript were performed using validated methods with internal quality control procedures (i.e. laboratory personnel were appropriately trained to perform their roles and records were kept; all procedures were documented as standard operating procedures and were approved; routine internal audits and inspections were also conducted).

2.4 Statistical Analysis

Data were analyzed using SAS 9.3 (Cary, NC). All two-sided probability values (p values) less than 0.05 were considered statistically significant. Concentrations of oxidative stress markers, woodsmoke exposure measurements (CO and $PM_{2.5}$) and hydroxy-PAHs were log transformed for all analysis. The marginal association between log-transformed urinary oxidative stress biomarker concentrations and woodsmoke exposure measurements were examined using ordinary Pearson correlation coefficients. The following exposure measurements were used in the correlation analysis: 48hr time integrated personal $PM_{2.5}$, 48hr time integrated personal real time CO, highest 30 second CO measurement obtained during sampling, highest 15 minute average CO and highest 90 minute average CO. Subject's personal 48hr integrated personal CO data from passive diffusion tubes were available for 81/84 of study subjects, as such these CO measurements were also tested in the analysis. Then hydroxy-PAH metabolites used as biomarkers of exposure among this population (Li et al., 2012) were also used in the correlation analysis: 1-NAP, 2-NAP, 2-FLU, 3-FLU, 9-FLU, 1-PHE, 2-PHE, 1-PYR and the sum of all the hydroxy-PAHs.

In addition, data were analyzed with linear models (PROC GLM) to (1) examine associations between the oxidative stress biomarkers and woodsmoke exposure and (2) investigate factors that are associated with creatinine corrected urinary 8-OHdG and 8-isoprostane concentrations among study subjects. Predictor variables include the weight and

age of study subjects, cooking time, amount of time that mothers spent playing with children, volume of kitchen, stove type and wood type.

The time mothers spent in playing with their children during the day was also assessed to determine whether this affected their respective exposures. This variable was chosen as a proxy for how often the mother and child were together on any given day. Other variables included the distance of the subject's homes to the road, the frequency of traffic in subject's neighborhood and whether subject had eaten food exposed to fire (these include bread and foods exposed directly to fire).. Mother's weight, age, cooking time and the amount of time spent playing with children were centered on their respective means before inclusion in the models.

Predictors were included in the model individually to test for significant associations with each oxidative stress biomarker. Stepwise regression (PROC GLMSELECT) was then used for multiple effect selection with the significance levels of the F statistic for entering and removing effects set at 0.2 and 0.1 respectively. Second order interaction terms were also investigated but none were significant. Finally, the associations between the oxidative stress biomarkers and selected predictors were quantified with partial correlations. All partial correlations provided were controlled for potential confounders that were identified during the model selection process.

3. Results

3.1 Household characteristics

Household specific information is presented according to stove type (Table 1). Intervention households used OPTIMA FL-I (n=22) or OPTIMA FL-II (n=17) stoves and control households used NGO stoves (n=7), traditional stoves (n=29) or stoves that were self-improved by the household (n=9). Subjects' mean ages were similar among all study households (Table 1). Mean cooking time for study subjects was above 3 hrs (Table 1). *Eucalipto* (eucalyptus sp) was the most popular wood types used as cooking fuel by study subjects during the sampling period (Table 1).

Creatinine corrected urinary 8-OHdG and 8-isoprostane measurements for each study subject together with personal and kitchen $PM_{2.5}$ and CO measurements are provided in Table 2. Urinary 8-OHdG levels ranged from 11.2 to 2270.0 μg/g creatinine (median: 132.6 μg/g creatinine) (Table 2). Urinary 8-isoprostane levels ranged from 0.1 to 4.5 μg/g creatinine (median: 0.8 μg/g creatinine) (Table 2). There were no statistically significant differences in urinary oxidative stress biomarkers between control and intervention stove users, and no significant differences between the specific stove types. Mean (95% CI) urinary 8-OHdG and 8-isoprostane levels among intervention stove users were 132.9 (97.7, 180.8) μg/g creatinine and 0.8 (0.6, 1.1) μg/g creatinine respectively. Likewise, among control stove users, these levels were 139.3 (108.7, 178.4) μg/g creatinine and 0.7 (0.6, 0.9) μg/g creatinine respectively.

3.2 Association between hydroxy-PAH metabolites and oxidative stress biomarkers

Correlation analysis involving creatinine corrected hydroxy-PAH metabolites, which are used as biomarkers of exposure (Li et al., 2012) and (1) oxidative stress biomarkers, as well as (2) CO and $PM_{2.5}$ are presented (Table 3). When correlation analysis was performed between each specific hydroxy-PAH and the oxidative stress markers, 1-hyroxyphenanthrene (1-PHE) and 2-hyroxyphenanthrene (2-PHE) were marginally correlated with 8-isoprostane levels, p=0.099 and 0.06 respectively (Table 3). 8-isoprostane was positively correlated with the sum of the hydroxy-PAH metabolites although this association was not statistically significant (Figure 2).

3.3 Association between CO and $PM_{2.5}$, and oxidative stress biomarkers

Forty eight-hr time integrated personal $PM_{2.5}$ was negatively associated with 8-isoprostane (p=0.04) and 48hr personal CO was negatively associated with 8-OHdG (p=0.01) (Table 3 and Table 4). As seen in Table 4, the highest 30-second personal CO measured during the sampling period for each subject was significantly but negatively associated with both 8-OHdG (p=0.001) and 8-isoprostane (p=0.04) while 15 minute and 90 minute averages of real time CO were negatively associated with 8-isoprostane only (p=0.005 and 0.01). Results indicate a pattern of decreasing urinary oxidative stress biomarker levels with increasing woodsmoke exposure measurements. Scatter plots of $PM_{2.5}$ exposures against oxidative stress biomarkers allude to this and the trend suggest decreasing urinary oxidative stress biomarker levels with increasing $PM_{2.5}$ exposures (Figure 1).

3.4 Factors associated with oxidative stress biomarkers

Individual variables included in statistical models are presented in Table 4. All variables found to be associated with the oxidative stress markers were entered for the final model selection process using stepwise regression. Test statistics of the modeled effects of individual variables are listed (Table 4). Table 5 presents the final models for both oxidative stress markers.

Subjects who resided in communities with frequent traffic (a car every few minutes) had geometric mean (95% CI) urinary 8-OHdG concentrations of 333.5 (199.0, 559.0) μg/g creatinine (n=12) compared to those who lived where cars passed by every hour: 148.3 (102.8, 214.1) μg/g creatinine (n=27) or seldomly (every few days): 133.6 (93.7, 190.5) μg/g creatinine (n=23) (Table 5). Also for 8-OHdG, subjects who reported consuming food exposed to fire had higher urinary levels of 250.4 (151.0, 415.2) μg/g creatinine (n=11) compared to those who had not consumed food exposed to fire: 140.7 (111.7, 177.0) μg/g creatinine (n=51). After controlling for the effects of traffic in the community and eating food exposed to fire, cooking time was significantly and positively associated with urinary 8-OHdG (p= 0.01, n=80) (Table 5). On the other hand, subjects' real time personal CO exposures were negatively associated with 8-OHdG (Table 5).

For urinary 8-isoprostane, subjects who lived ≤ 20 meters to the road had levels of 0.8 (0.6, 1.1) μg/g creatinine (n=25) compared to those who lived > 20 meters away with levels of 0.6 (0.5, 0.7) μg/g creatinine (n=38) (Table 5). After controlling for the effect of distance of road to subjects' homes, 1-hyroxyphenanthrene and 2-hyroxyphenanthrene were both positively associated with 8-isoprostane with a marginal statistical significance (p=0.05 and 0.06, respectively, n=84) (Table 5). In contrast, 48hr time integrated personal $PM_{2.5}$ was marginally but negatively associated with urinary 8-isoprostane after controlling for the effect of distance of homes to the road (p=0.09, n=69).

4. Discussion

It has been observed in *in-vitro* and human experimental studies that oxidative stress, in response to wood smoke, may play an important role in airway and alveolar epithelium injury (Barregard et al., 2006; Barregard et al., 2008; Danielsen et al., 2009; Danielsen et al., 2011). 8-Isoprostane is produced *in vivo* by the free radical-catalyzed peroxidation of arachidonic acid (Montuschi et al., 1999) while 8-OHdG is an oxidized nucleoside of DNA and the most frequently detected molecule of DNA lesion (Wu et al., 2004). In the present study, we investigated the effects of cookstove related biomass smoke exposure, subject characteristics and other factors on urinary concentrations of 8-OHdG and 8-isoprostane among women in rural communities in San Marcos, Cajamarca Region, Peru.

Urinary 8-isoprostane levels reported in the literature are comparable to results reported in the current study. Levels of this metabolite in normal individuals have been measured at 0.39 ± 0.18 μg/g creatinine (mean ± 2 SD) (Roberts and Morrow, 2000). A range of 0.2–1.5 μg/g creatinine using the LC-MS/MS technique has been documented for healthy individuals (Taylor et al., 2008). This biomarker has been shown to increase with increasing exposure regardless of the urinary analytical technique employed. Lai et al (2012), using an Enzyme-linked immunosorbent assay (ELISA), found that median concentrations (interquartile range) of urinary 8-isoprostane in 47 Taiwanese female highway toll station workers exposed to traffic exhausts were 3.69 (3.26 – 4.39) μg/g creatinine among exposed smokers (n=5) and 3.00 (2.63 – 4.17) μg/g creatinine among exposed nonsmokers (n=42).

The elimination half-life of isoprostanes in blood has been noted to be relatively short; less than 20 min (Gniwotta et al., 1997; Roberts II and Morrow, 2000) and this short duration is possible in urine as well. Once the exposure is removed, it is expected that levels of the biomarker will fall, particularly from nighttime until the following morning (Nuernberg et al., 2008). Also, 24hr urine samples may have been more informative since a single sample will provide only an index of isoprostane formation and only in chronic disease states is there expected to be a relatively steady rate of formation (Gniwotta et al., 1997). However, this is not the case among our study population as these women are mainly young women of child bearing age with no reported chronic diseases.

Elevated 8-OHdG levels have been detected in the urine samples of smokers and occupational workers (Chuang et al., 2003; Kim et al., 2004; Lodovici et al., 2000). It must be noted, however that the mean urinary 8-OHdG concentration recorded in the literature using HPLC-ECD have been between 3 – 10 μg/g creatinine (Bogdanov et al., 1999; Nakano et al., 2003; Pilger et al., 2002). When an ELISA assay, which has been reported to overestimate 8-OHdG levels by as much as 2 times (Shimoi et al., 2002; Song et al., 2009), was employed, typical concentrations ranged from 3– 20 μg/g creatinine (Chuang et al., 2003; Kim et al., 2004; Lai et al., 2005; Lee et al., 2010; Tamura et al., 2006). Wu et al. (2004), also employing ELISA, reported a mean urinary concentration of 8-OHdG for females as 43.9 ± 42.1 μg/g creatinine.

Given the lack of a similarly exposed comparison group from the developing world, we compare our findings to studies involving women exposed to air pollution in developed nations. Among 344 non smoking office workers in Taiwan, mean concentrations of urinary 8-OHdG ranged from 3.10 to 6.27 μg/g creatinine (Lu et al., 2007). Among a healthy Japanese population, women had mean (± SD) urinary 8-OHdG levels of 15.58 ± 5.49 μg/g creatinine (Kimura et al., 2006). Mean concentrations of urinary 8-OHdG were substantially higher among the 47 female highway toll station workers in Taiwan in the study by Lai et al (2005). Exposed non-smokers had mean urinary 8-OHdG of 13.6 μg/g creatinine while exposed smokers had 10.2 μg/g creatinine (Lai et al., 2005). Lai et al concluded that there was an increased amount of DNA damage in subjects who had worked under conditions of potential oxidative stress (e.g. traffic exhaust exposures). The continuous exposures experienced by subjects in our study throughout their lifetime suggests elevated levels of urinary 8-OHdG when compared to the above mentioned references from the developed world. There is an indication that this chronic inhalation of biomass smoke may lead to a sustained systemic oxidative stress status among our study subjects (Banerjee et al., 2012; Dutta et al., 2012).

Our results showed that study subjects were exposed to high levels of $PM_{2.5}$ and CO from cookstove related woodsmoke. We postulate that the presence of highly reactive electrophilic compounds in biomass smoke (Lewtas, 2007) and or the continual induction of intracellular ROS as a result of inhaling such exposures (Avakian et al., 2002) leads to high

urinary oxidative stress in our study population. Results also show that increasing biomass smoke exposure was not positively correlated with urinary levels of 8-OHdG and 8-isoprostane among study subjects. This was surprising since short term (4hr) exposure of healthy individuals to woodsmoke in experimental studies leads to increased airway inflammation and may affect lipid peroxidation (Barregard et al., 2008; Barregard et al., 2006). However, it must be noted that the mean $PM_{2.5}$ exposures experienced by the women in this study population were high. The mean 48hr $PM_{2.5}$ exposures [95% CI] among control and intervention subjects in this study population were 129 ug/m^3 [82, 176 ug/m^3] and 104 [64, 144 ug/m^3] respectively (Hartinger et al., 2013). These values are an order of magnitude higher compared to EPA's air quality standard of 35 ug/m^3 and WHO recommended air quality guideline of 25 ug/m^3 for a 24hr period. Additionally, the exposures were also continuous; and this allows little recovery time from one exposure period to the next.

In our study, 48hr mean CO was weakly but negatively associated with 8-OHdG and 48hr mean $PM_{2.5}$ was weakly but negatively associated with 8-isoprostane. These findings are surprising since PM from woodsmoke may increase in ammatory and oxidative stress response genes in cultured human cells (Danielsen et al., 2011). These findings may be due to negative correlations with ROS-inducing species in woodsmoke, or upregulation of mediator genes since PM from woodsmoke has been demonstrated to increase in ammatory and oxidative stress response genes in cultured human cells (Danielsen et al., 2011). Future studies need to focus on specific ROS species in woodsmoke to address this while noting the differences in experimental and natural setting studies. Other possible reasons may be as a result of decreased DNA damage or decreased DNA repair activity (Nuernberg et al., 2008). As has been hypothesized by others in the literature concerning workers exposed to metal fumes (Palmer et al., 2006; Wang et al., 2005) this finding may be due to a 'muted response' to inhaled exposure, in this case, HAP constituents. Future studies need to be designed to determine whether this response truly occurs, the mechanism by which it occurs, and most importantly, whether this response is damaging or represents an adaptive response (Nuernberg et al., 2008).

Our results showed that hydroxylated phenanthrene (1-PHE and 2-PHE), used as a urinary biomarker of exposure (Li et al., 2012), was correlated with 8-isoprostane. Urinary 8-OHdG levels were not correlated with individual urinary hydroxy-PAHs or the summed total of the PAH metabolites. A study among children exposed to heavy traffic in Guangzhou, China did not observe a significant association between hydroxy-PAHs and 8-OHdG (Fan et al., 2012). Finally, our results showed no correlation between urinary 8-OHdG and 8-isoprostane. This is consistent with suggestions by others in the literature that there appears to be no correlation between oxidative DNA damage products and F_2-isoprostanes in healthy individuals (England et al., 2000; Sakano et al., 2009).

Cooking time was weakly but positively associated with urinary 8-OHdG concentrations. This finding is expected since longer cooking times implies longer exposures to HAP. Increasing cooking time increases the potential for oxidative DNA adduct formation due to continuous exposure to biomass smoke combustion products as well as the generation of heterocyclic aromatic amines in food which increases with increasing cooking time (Zaidi et al., 2012). Likewise eating food exposed to fire was also positively and significantly correlated with urinary 8-OHdG. Eating food exposed to fires also leads to the generation of reactive oxygen and nitrogen species which are precursors of oxidative stress (Tuohy et al., 2006).

Results from our study indicate that proximity of subjects' homes to the road side and the frequency of vehicular traffic lead to increased urinary oxidative stress in urine samples. Subjects who lived less than 20 meters to the road side and those whose communities had

frequent traffic had higher urinary 8-isoprostane and 8-OHdG levels respectively. Elevated levels of both markers have been recorded in the literature among subjects exposed to diesel and petroleum byproducts (Lai et al., 2005; Lee et al., 2010).

In the cross-sectional study of 47 Taiwanese female highway toll station workers exposed to traffic exhaust, there was a 9.32 mg/g creatinine increase of urinary 8-OHdG per 1000 cars/hour increase in average traffic density (Lai et al., 2005). The corresponding estimate for trucks and buses was of greater magnitude; 16.76 mg/g creatinine (Lai et al., 2005). This finding may help explain the elevated 8-OHdG levels in the current population, although traffic is not as heavy in the study region. It implies that aside biomass smoke combustion products from the household environment, subjects in this population were also exposed to traffic air pollution which represents an additional physiologic burden. Reductions in this type of vehicular air pollution is also necessary, as has been reported among healthy individuals in Beijing during the Beijing Olympic air pollution control period (Huang et al., 2012).

There were no statistically significant differences in urinary 8-OHdG and 8-isoprostane among control and intervention stoves users (see stove effect in Table 4). This finding is supported by earlier studies showing that exposure did not differ significantly among intervention and control stove users (Hartinger et al., 2013). Periodic maintenance over time, adequate stove design, proper and exclusive stove use (Commodore et al., 2013) may be important in lowering HAP exposure levels and resulting health effects among intervention stove users.

As the Global Alliance for Clean Cookstoves (GACC) aims to reduce HAP and the adverse health effects associated with it, these results add to the limited body of literature on the health effects experienced by populations exposed to HAP. The GACC, led by the United Nations Foundation, has the goal of 100 million households adopting clean and efficient cookstoves by the year 2020 (GACC, 2011). Certain issues need to be carefully addressed before these clean and efficient stoves are adopted globally. It is essential to determine the amount of HAP reduction necessary to improve health (Pope III et al., 2011; Smith et al., 2011; Smith and Peel, 2010), and develop new and rigorous means to evaluate the health bene ts of worldwide stove implementation programs (Martin et al., 2011). In the advent of national cookstove programs in Peru and other countries, field evaluation of new stove models is also an important step to understanding the impact of cookstove related woodsmoke exposure (Fitzgerald et al., 2012).

4.1 Strengths and Limitations

To our best knowledge, this is the first study looking at understanding systemic oxidative stress among women exposed to cookstove related biomass combustion in rural Peru. The use of two uncorrelated biomarkers of systemic oxidative stress represents a significant strength of this study, as 8-OHdG is a marker of DNA oxidative damage and 8-isoprostane is a marker of lipid peroxidation (mostly cell membrane damage). Additionally, study subjects were healthy, although chronically exposed to woodsmoke, and this may have minimized the effects of confounding from preclinical or overt disease regarding the concentrations of oxidative stress biomarkers.

The use of spot urine samples rather than 24hr urine samples did not allow us to examine the intra-individual variability in the biomarkers (Pilger et al., 2001; Pilger et al., 2002). Future studies can consider taking repeated simultaneous exposure (Dionisio et al., 2012; Smith and Peel, 2010) and urine samples (Gniwotta et al., 1997; Roberts II and Morrow, 2000) over extended periods of time to better characterize the exposure-response relationship.

Another limitation in our study was the lack of information on PAHs in soot residues (Lisouza et al., 2011) which had accumulated on the walls, ceiling and cooking utensils of study households regardless of whether a control or intervention stove was employed. Indeed, future studies can characterize these PAHs also in order to understand the association between HAP exposure and oxidative stress. In our study we also did not have data on alcohol drinking habits, antioxidant capacity, and the contribution of metals in drinking water on oxidative stress; future studies may need such information for further exposure characterization.

Households in our study were not sampled prior to and immediately after improved stove installation and this prevented evaluation of the effectiveness of the OPTIMA stoves at improving health soon after installation. Even more challenging, although the current measurements inform, the results suggest that the OPTIMA stove may not be effective over the long term at reducing HAP exposures and health effects.

5. Conclusion

We characterized urinary 8-OHdG and 8-isoprostane among intervention and control households in San Marcos, Cajamarca Region, Peru. Although both oxidative stress biomarkers did not differ significantly between control and intervention stove users, 8-OHdG levels appear to be elevated among our study subjects when compared to values of urinary 8-OHdG reported in healthy women in the scientific literature from the developed world. It is important to realize that reductions in wood smoke exposure may need to be more substantial in magnitude and sustained for longer term (e.g. years) to yield measurable health benefits. Future investigations into cumulative effects of daily exposure to HAP and evaluations of longer-term stove interventions are recommended.

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Highlights

- Urine samples from 84 biomass stove users were analyzed for8-OHdG and 8-isoprostane
- 8-OHdG levels ranged from 11.2 to 2270.0 μg/g creatinine for all subjects (n=84)
- 8-isoprostane levels ranged from 0.1 to 4.5 μg/g creatinine for all subjects (n=84)
- Results suggest there is sustained systemic oxidative stress among these women
- Future investigations into continuous HAP exposures are recommended

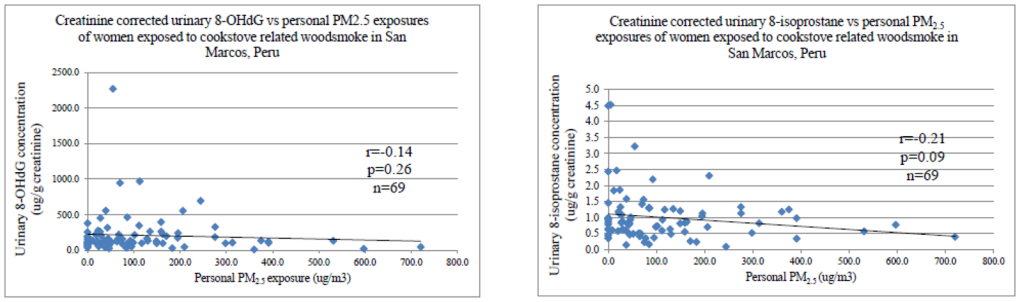
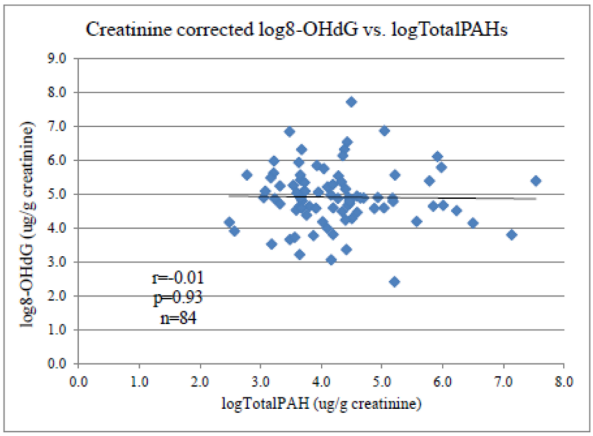


Figure 1.

Urinary oxidative stress measures vs. $PM_{2.5}$ exposures of women exposed to woodsmoke in San Marcos, Peru. The association has been adjusted for distance of subject's home to the road.



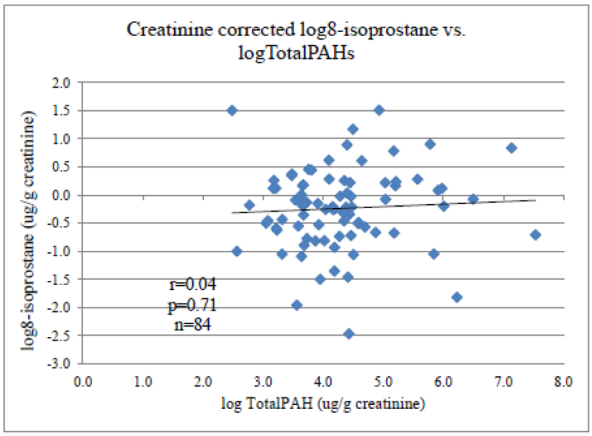


Figure 2.

Urinary oxidative stress measures vs. total urinary hydroxy-PAH exposures of women exposed to woodsmoke in San Marcos, Peru. The association has been adjusted for distance of subject's home to the road.

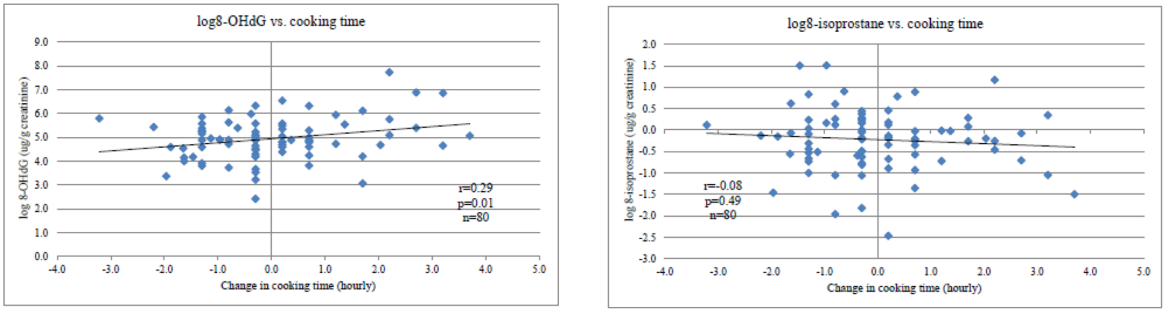


Figure 3.

Urinary oxidative stress measures vs. cooking time of women exposed to woodsmoke in San Marcos, Peru. The association has been adjusted for the amount of vehicular traffic in subjects' communities and eating food exposed to fire.

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Table 1

Mother's demographic information and household characteristics by stove type

Subject's stove type	Age (in years) Mean ± SD (n)	Cooking time (in hours) Mean ± SD (n)	Kitchen volume (in m^3) Mean ± SD (n)	Most common wood type used n (%)	Homes ≤ 20 meters from road n (%)	n (%) who have completed at least elementary school
Intervention	30.5 ± 9.3 (39)	3.2 ± 1.4 (39)	27.5 ± 11.0 (39)	Eucalyptus: 16 (45.7)	14 (37.8)	22 (56.4)
OPTIMA FL-I[a]	29.2 ± 7.9 (22)	3.1± 1.3 (22)	24.8 ± 9.8 (22)	Other: 9 (45.0)	7 (33.3)	13 (59.1)
OPTIMA FL-II[b]	32.1 ± 10.7 (17)	3.3 ± 1.6 (17)	30.9 ± 11.8 (17)	Eucalyptus: 6 (60.0)	7 (43.8)	9 (52.9)
Control	30.0 ± 6.7 (38)	3.3 ± 1.4 (43)	36.0 ± 26.4 (38)	Eucalyptus: 17 (39.5)	19 (44.2)	28 (73.7)
NGO[c]	35.4 ± 3.3 (5)	3.2 ± 1.1 (5)	29.0 ± 13.9 (4)	Other: 3 (42.9)	4 (80.0)	3 (60.0)
Self-improved by household	25.3 ± 3.8 (6)	3.3 ± 1.7 (9)	50.3 ± 40.1 (8)	Other: 5 (55.6)	4 (44.4)	4 (66.7)
Traditional	30.1 ± 7.0 (27)	3.3 ± 1.4 (29)	32.7 ± 21.8 (26)	Eucalyptus: 14 (48.3)	11 (37.9)	21 (77.8)

[a] Functionality level I refers to an OPTIMA stove in good conditions

[b] Functionality level II refers to an OPTIMA stove in need of minor repairs (eg re-plastering) or major repairs (eg chimney valve replacement)

[c] NGO: three main NGOs had improved stoves; JUNTOS-National cash transfer program. Part of the requirements is that families must build an improved stove with a chimney; SEMBRANDO & ADIAR are NGOs that work in nearby communities.

Total sample sizes for all stove types: OPTIMA FL-I=22, OPTIMA FL-II=17, NGO=7, self-improved by household=9 and traditional=29. Information on the total number of subjects who responded to questions on each of the demographic information and characteristics are presented in the table.

Description: This table presents the age and education levels of study subjects. Also provided are the amount of time spent cooking, volume of study kitchens, the most common wood type used for cooking, and the distance of the study households from roads.

Table 2

Creatinine corrected urinary oxidative stress measures together with personal and kitchen $PM_{2.5}$ and CO measurements

Sample type	Stove type	8-OHdG (ug/g creatinine)	8-isoprostane (ug/g creatinine)	48hr Kitchen $PM_{2.5}$ (ug/m^3)	48hr Personal $PM_{2.5}$ (ug/m^3)	48hr Kitchen Real Time CO (ppm)	48hr Personal Real Time CO (ppm)	48hr Personal Passive Tube CO (ppm)
Intervention	OPTIMA FL-I[a]	66.7	1.3	70.3	25.4	3.8	2.2	4.9
	OPTIMA FL-I	277.2	1.1	-	22.7	0.4	0.1	0.7
	OPTIMA FL-I	63.5	0.9	453.0	46.1	-	0.3	1.9
	OPTIMA FL-I	154.9	0.4	103.5	65.4	1.1	0.2	0.8
	OPTIMA FL-I	99.4	1.2	-	117.2	1.1	1.3	3.0
	OPTIMA FL-I	80.4	1.6	90.5	37.8	1.5	0.3	0.8
	OPTIMA FL-I	395.3	0.6	-	159.0	2.1	0.3	1.8
	OPTIMA FL-I	34.1	1.3	90.3	84.1	2.8	4.7	0.9
	OPTIMA FL-I	39.1	1.5	-	-	-	-	0.6
	OPTIMA FL-I	198.4	0.3	401.9	170.4	4.7	1.5	-
	OPTIMA FL-I	41.6	0.1	26.2	37.4	0.7	0.1	0.4
	OPTIMA FL-I	557.4	0.8	21.3	39.6	0.3	0.3	0.4
	OPTIMA FL-I	135.3	4.5	9.0	5.1	1.7	1.1	0.7
	OPTIMA FL-I	379.8	1.0	-	-	-	-	12.5
	OPTIMA FL-I	107.1	0.8	195.1	313.3	3.1	-	3.2
	OPTIMA FL-I	184.9	1.3	245.9	275.8	3.6	4.1	6.9
	OPTIMA FL-I	145.5	0.8	-	44.5	-	-	1.0
	OPTIMA FL-I	2270.0	3.2	56.3	55.0	0.9	0.1	0.6
	OPTIMA FL-I	29.0	0.2	674.7	183.2	22.0	4.2	7.5
	OPTIMA FL-I	130.4	0.5	-	40.9	0.4	0.4	0.4
	OPTIMA FL-I	111.9	0.3	35.2	-	2.7	1.0	1.0
	OPTIMA FL-I	65.2	4.5	-	-	3.6	1.2	2.2
	GM (95% CI)*	130.1 (82.5, 205.2)	0.9 (0.6, 1.3)	92.8 (44.9, 191.8)	62.5 (37.7, 103.6)	1.8 (1.0, 3.0)	0.6 (0.3, 1.2)	1.4 (0.9, 2.3)
	OPTIMA FL-II[b]	140.7	0.6	114.3	36.3	2.3	1.0	1.0
	OPTIMA FL-II	89.2	0.6	118.0	30.1	2.3	0.2	0.8
	OPTIMA FL-II	220.0	2.5	145.4	17.5	4.0	1.9	1.5

Sample type	Stove type	8-OHdG (ug/g creatinine)	8-isoprostane (ug/g creatinine)	48hr Kitchen $PM_{2.5}$ (ug/m^3)	48hr Personal $PM_{2.5}$ (ug/m^3)	48hr Kitchen Real Time CO (ppm)	48hr Personal Real Time CO (ppm)	48hr Personal Passive Tube CO (ppm)
Intervention	OPTIMA FL-II	163.3	0.5	23.0	44.5	0.9	0.5	1.0
	OPTIMA FL-II	451.6	1.1	117.8	27.9	7.6	1.7	1.0
	OPTIMA FL-II	132.4	2.2	50.0	92.5	-	0.9	0.8
	OPTIMA FL-II	45.0	2.3	161.0	209.6	3.3	1.4	3.2
	OPTIMA FL-II	93.3	0.6	-	20.3	0.4	0.2	0.8
	OPTIMA FL-II	463.1	1.3	238.5	85.8	6.4	1.5	1.7
	OPTIMA FL-II	91.6	0.2	154.3	84.7	4.6	3.4	1.9
	OPTIMA FL-II	220.3	0.5	40.9	67.8	3.6	1.0	-
	OPTIMA FL-II	158.0	0.2	-	75.2	1.8	1.1	0.9
	OPTIMA FL-II	227.3	0.9	223.4	162.4	3.5	1.3	1.7
	OPTIMA FL-II	134.5	1.2	436.3	375.4	6.4	3.4	4.0
	OPTIMA FL-II	11.2	1.2	-	360.5	12.2	4.5	7.8
	OPTIMA FL-II	132.9	0.6	-	531.3	22.9	-	7.2
	OPTIMA FL-II	263.0	0.8	-	159.1	24.0	7.4	8.0
	GM (95% CI)*	136.6 (87.4, 213.5)	0.8 (0.5, 1.2)	116.5 (69.3, 195.9)	85.1 (49.8, 145.4)	4.0 (2.3, 7.2)	1.3 (0.8, 2.2)	1.9 (1.2, 2.9)
Control	NGO[c]	103.5	0.3	539.2	391.4	4.9	2.6	3.9
	NGO	43.8	0.4	-	-	6.6	3.6	4.0
	NGO	193.9	0.9	-	-	3.6	-	3.0
	NGO	263.1	0.8	74.9	27.0	24.0	3.0	2.1
	NGO	155.0	1.0	-	-	-	-	7.3
	NGO	133.9	0.6	43.5	8.1	-	0.4	1.0
	NGO	21.4	0.8	-	597.3	32.4	9.4	12.1
	GM (95% CI)*	100.4(44.3, 227.8)	0.7 (0.5, 0.9)	120.7 (4.5, 3239.7)	84.7 (3.1, 2309.5)	9.8 (2.9, 33.3)	2.6 (0.6, 10.5)	3.7 (1.7, 7.7)
	Self-improved by household	104.1	1.6	83.3	73.2	-	-	1.0
	Self-improved by household	72.6	0.3	547.7	77.7	29.9	0.8	2.1
	Self-improved by household	25.1	1.0	111.4	46.7	1.3	0.4	0.7
	Self-improved by household	946.1	1.4	298.3	70.3	3.1	0.1	0.7
	Self-improved by household	347.1	0.6	37.0	111.6	0.3	0.5	0.6
	Self-improved by household	132.8	1.2	540.1	21.2	5.3	0.6	7.2

Sample type	Stove type	8-OHdG (ug/g creatinine)	8-isoprostane (ug/g creatinine)	48hr Kitchen $PM_{2.5}$ (ug/m^3)	48hr Personal $PM_{2.5}$ (ug/m^3)	48hr Kitchen Real Time CO (ppm)	48hr Personal Real Time CO (ppm)	48hr Personal Passive Tube CO (ppm)
	Self-improved by household	54.5	1.9	17.4	24.0	8.8	0.4	1.6
	Self-improved by household	133.1	1.8	-	11.5	2.1	0.2	1.1
	Self-improved by household	969.1	0.9	100.5	112.8	1.1	0.5	1.7
	GM (95% CI)*	157.0 (60.1, 410.0)	1.1 (0.7, 1.6)	123.2 (43.9, 345.8)	48.1 (25.9, 89.3)	2.8 (0.8, 9.2)	0.3 (0.2, 0.7)	1.3 (0.7, 2.4)
	Traditional	97.9	0.5	366.6	298.4	8.2	5.0	6.8
	Traditional	315.8	0.8	225.5	43.0	14.1	0.5	3.0
	Traditional	45.4	0.4	-	720.7	23.4	8.4	7.6
	Traditional	262.8	1.3	107.4	135.0	1.8	0.5	4.1
	Traditional	693.4	0.1	257.1	244.6	3.7	2.9	3.8
	Traditional	189.8	0.6	-	-	-	-	13.2
	Traditional	69.3	2.4	-	-	-	-	12.6
	Traditional	104.7	0.4	179.4	-	0.2	0.9	1.0
	Traditional	107.7	0.7	150.9	98.8	2.8	0.8	1.5
	Traditional	50.1	0.4	54.9	95.2	0.5	0.8	0.8
	Traditional	241.3	1.1	54.6	195.6	1.0	0.3	0.4
	Traditional	86.8	0.6	146.7	-	0.1	0.2	0.6
	Traditional	65.9	0.4	-	-	-	-	7.0
	Traditional	112.9	0.5	-	52.0	0.6	0.5	0.9
	Traditional	126.5	0.8	-	149.1	2.3	0.7	1.7
	Traditional	120.2	1.0	360.7	391.3	4.6	3.6	3.8
	Traditional	99.5	0.8	-	-	8.0	2.0	3.0
	Traditional	120.6	0.5	53.2	63.6	1.4	0.4	0.5
	Traditional	173.6	1.0	717.1	195.0	16.1	0.7	-
	Traditional	162.8	0.6	207.0	128.5	3.7	-	2.0
	Traditional	209.3	0.9	82.1	29.3	1.1	0.1	0.4
	Traditional	555.3	0.7	-	206.2	2.5	2.2	0.8
	Traditional	214.6	0.7	152.7	101.8	2.6	2.2	3.1
	Traditional	98.3	0.9	930.9	162.8	17.0	2.0	3.1
	Traditional	254.8	1.0	-	-	-	0.3	0.6

Sample type	Stove type	8-OHdG (ug/g creatinine)	8-isoprostane (ug/g creatinine)	48hr Kitchen $PM_{2.5}$ (ug/m^3)	48hr Personal $PM_{2.5}$ (ug/m^3)	48hr Kitchen Real Time CO (ppm)	48hr Personal Real Time CO (ppm)	48hr Personal Passive Tube CO (ppm)
	Traditional	328.1	1.1	-	275.9	24.2	4.2	8.7
	Traditional	122.2	0.8	-	-	1.5	1.5	1.9
	Traditional	99.2	1.2	233.4	149.5	2.1	2.4	3.3
	Traditional	131.8	0.5	67.4	130.4	0.9	0.5	0.9
	GM (95% CI)*	145.2 (113.5, 185.8)	0.7 (0.5, 0.9)	170.1 (111.9, 258.7)	141.5 (100.5, 199.3)	2.6 (1.4, 4.6)	1.0 (0.7, 1.6)	2.1 (1.4, 3.2)

*GM (95% CI) refers to geometric mean (95% confidence interval)

[a]Functionality level I refers to an OPTIMA stove in good conditions

[b]Functionality level II refers to an OPTIMA stove in need of minor repairs (eg re-plastering) or major repairs (eg chimney valve replacement)

[c]NGO: three main NGOs had improved stoves; JUNTOS-National cash transfer program. Part of the requirements is that families must build an improved stove with a chimney; SEMBRANDO & ADIAR are NGOs that work in nearby communities.

Description: This table presents the concentrations of urinary 8-OHdG and 8-isoprostane obtained from study subjects. Where information is available, time integrated (48hr) personal exposures and kitchen concentrations of $PM_{2.5}$ and CO measurements are also provided.

Table 3

Correlation analysis between urinary oxidative stress measures, exposure measurements and hydroxylated PAHs.

		Creatinine corrected (ug/g creatinine)								
		log8- OHdG	**log8-isoprostane**	**log1-NAP**	**log2-NAP**	**log2- FLU**	**log3- FLU**	**log9-FLU**	**log1-PHE**	**log2-PHE**
log8-OHdG (ug/g creatinine)	r (p) n	1	0.08 (0.49) 84	−0.02 (0.87) 83	−0.05 (0.64) 84	−0.07 (0.55) 84	−0.02 (0.81) 84	−0.03 (0.81) 84	−0.01 (0.94) 84	−0.01 (0.91) 84
log8-isoprostane (ug/g creatinine)	r (p) n		1	0.03 (0.77) 83	0.11 (0.33) 84	0.14 (0.19) 84	0.10 (0.34) 84	0.10 (0.38) 84	0.18 (0.10) 84	0.20 (0.06) 84

		Creatinine corrected (ug/g creatinine)								
		log8-OHdG	**log8-isoprostane**	**log3-PHE**	**log1-PYR**	**logKPM[a] (ug/m3)**	**logMPM[b] (ug/m3)**	**logMRCO[c] ppm**	**logKRCO[d] ppm**	**logMTCO[e] ppm**
log8-OHdG (ug/g creatinine)	r (p) n	1	0.08 (0.49) 84	−0.02 (0.89) 84	−0.03 (0.77) 84	−0.07 (0.62) 55	−0.11 (0.36) 69	**−0.29 (0.01) 73**	−0.17 (0.14) 73	−0.19 (0.09) 81
log8- isoprostane (ug/g creatinine)	r (p) n		1	0.18 (0.11) 84	0.11 (0.31) 84	−0.17 (0.22) 55	−0.25 **(0.04)** 69	−0.08 (0.50) 73	0.04 (0.72) 73	0.01 (0.94) 81

[a] KPM refers to 48hr real time kitchen $PM_{2.5}$ concentrations

[b] MPM refers to 48hr personal $PM_{2.5}$ exposures

[c] MRCO refers to 48hr real time personal CO exposures

[d] KRCO refers to 48hr real time kitchen CO concentrations

[e] MTCO refers to 48hr time integrated personal CO exposures

1-NAP, 2-NAP, 2-FLU, 3-FLU, 9-FLU, 1-PHE, 2-PHE, 3-PHE and 1-PYR refer to 1-naphthol, 2-naphthol, 2-hydroxy uorene, 3-hydroxy uorene, 9-hydroxy uorene, 1-hydroxyphenanthrene, 2-hydroxyphenanthrene, and 1-hydroxypyrene respectively.

Statistically significant p values are in bold

Pearson's correlation coefficients are provided, with p values in parenthesis and sample sizes italized

Description: This table presents the results of correlation analyses between creatinine corrected oxidative stress biomarkers and hydroxylated PAHs, and household air pollution exposure measurements. All values were natural log transformed.

Table 4

Test statistics from individual general linear models for variables associated with urinary 8-OHdG and 8-isoprostane.

Parameter	Degrees of freedom[a]	8-OHdG		8-isoprostane	
		Overall F statistic	P value	Overall F statistic	P value
Continuous variables					
Cooking time	1, 78	5.55	**0.02**	0.62	0.43
48hr time integrated personal $PM_{2.5}$*	1, 67	0.87	0.36	4.54	**0.04**
30-second maximum personal CO*	1, 72	12.86	**0.001**	4.39	**0.04**
15-minute maximum personal CO*	1, 72	8.46	**0.005**	1.65	0.20
90-minute maximum personal CO*	1, 72	6.25	**0.01**	1.85	0.18
48hr time integrated personal CO*	1, 72	6.59	**0.01**	0.46	0.50
Categorical variables					
Age					
≤ 25 years (reference: ≥ 36 years)	2, 72	2.36	0.10	0.24	0.79
26 – 35 years			0.51		1.00
Distance of home to road					
≤ 20 meters (reference: > 20 meters)			**0.04**		0.57
Eating food exposed to fire	1, 78	0.85	0.36	4.56	**0.04**
No (reference: yes)	1, 78	3.27	0.07	0.19	0.66
Stove type	4, 79	0.31	0.87	1.00	0.41
OPTIMA FL-II[b] (reference: OPTIMA FL-I)			0.87		0.60
NGO[c]			0.51		0.31
Self-improved by household			0.60		0.54
Traditional			0.66		0.18
Traffic in the community[d]	2, 67	4.58	**0.01**	2.51	0.09
Frequently (reference: seldomly)			**0.004**		0.16
Hourly			0.34		**0.03**
Wood type	2, 75	0.16	0.86	1.39	0.26
Eucalyptus (reference: other wood types)			0.86		0.15
Hualango			0.70		0.97

[a] Degrees of freedom for model and error respectively

[b] Functionality levels I and II refer to OPTIMA stoves in need of minor repairs (eg re-plastering) or major repairs (eg chimney valve replacement)

[c] NGO: three main NGOs had improved stoves; JUNTOS-National cash transfer program. Part of the requirements is that families must build an improved stove with a chimney; SEMBRANDO & ADIAR are NGOs that work in nearby communities.

[d] Traffic in the community: frequently refers to a car every few minutes; Hourly refers to a car every hour; Seldomly refers a car every few days.

[*] Indicates negative regression coefficients for exposure measurements

Description: This table presents the results of general linear models where variables associated with urinary 8-OHdG and 8-isoprostane were tested. Only one predictor variable is included in each model, together with a specific oxidative stress biomarker as a response variable.

Table 5

Variables associated with urinary biomarkers of oxidative stress

Variables that are significantly associated with urinary 8-OHdG.		
After controlling for traffic[a] and eating food exposed to fire	**Correlation coefficent**	**P value**
Cooking time[b] (n=80)	0.29	0.01
48hr time integrated personal CO (n=73)	−0.26	0.03
90-minute maximum personal CO (n=73)	−0.23	0.06
15-minute maximum personal CO (n=73)	−0.25	0.04
30-second maximum personal CO (n=73)	−0.32	0.01
Variables that are significantly associated with urinary 8-isoprostane.		
After controlling for distance of home to road	Correlation coefficent	P value
1-hyroxyphenanthrene (n=84)	0.21	0.05
2-hyroxyphenanthrene (n=84)	0.21	0.06
48hr time integrated personal $PM_{2.5}$ (n=69)	−0.21	0.09

[a] Traffic in the community: frequently refers to a car every few minutes; Hourly refers to a car every hour; Seldomly refers a car every few days.

[b] Cooking time refers to the estimated cooking time of mothers in study region spent cooking (in hours). Each subject's time has been centered around the mean cooking time.

Description: This table shows significant variables associated with urinary oxidative stress biomarkers among study subjects, after controlling for factors such as traffic in the community and eating food exposed to fire (8-OHdG) and distance of study households to roads (8-isoprostane).