Environment International xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Full length article

Effect of a clean stove intervention on inflammatory biomarkers in pregnant women in Ibadan, Nigeria: A randomized controlled study

Christopher O. Olopade, MD [a,b,*], Elizabeth Frank [a], Emily Bartlett [c], Donee Alexander, PhD [a], Anindita Dutta, PhD [a,b], Tope Ibigbami, MSc [d], Damilola Adu, MSc [d], John Olamijulo, MPH [d], Ganiyu Arinola, PhD [e], Theodore Karrison, PhD [f], Oladosu Ojengbede, MD [g]

[a] Center for Global Health, University of Chicago, 5841 S. Maryland Avenue, MC 2021, Chicago, IL 60637, USA
[b] Department of Medicine, University of Chicago, 5841 S. Maryland Avenue, MC 6076, Chicago, IL 60637, USA
[c] Pritzker School of Medicine, University of Chicago, 924 East 57th Street, Suite 104, Chicago, IL 60637, USA
[d] Healthy Life for All Foundation, House 38, University College Hospital Campus, University of Ibadan, Oyo State, Ibadan, Nigeria
[e] College of Medicine, University College Hospital, Ibadan, Nigeria
[f] Department of Public Health Sciences, University of Chicago, 5841 South Maryland Ave, MC2000, Chicago, IL 60637, USA
[g] Department of Obstetrics and Gynecology, University College Hospital Campus, University of Ibadan, Oyo State, Ibadan, Nigeria

ARTICLE INFO

Article history:
Received 20 July 2016
Received in revised form 12 October 2016
Accepted 3 November 2016
Available online xxxx

Keywords:
Cleancook stove
Bio-ethanol
Cytokines
Systemic inflammation
Pregnant women
Nigeria

ABSTRACT

Background: Exposure to household air pollution (HAP) has been linked to systemic inflammation. We determined the impact of transition from traditional firewood/kerosene stove to bioethanol-burning stove on inflammatory biomarkers in pregnant Nigerian women.
Methods: Women ($n = 324$), cooking with kerosene/firewood, were recruited during their first trimester of pregnancy from June 2013–October 2015 and were randomly allocated to either control ($n = 162$) or intervention ($n = 162$) group using web-based randomization. Controls continued to use their own firewood/kerosene stove, while intervention participants received bioethanol CleanCook stoves. Serum concentrations of retinol-binding protein (RBP), malondialdehyde (MDA), tumor necrosis factor alpha (TNF)-α, interleukin (IL)-6, and IL-8 were measured by ELISA.
Results: After excluding 53 women (loss of follow-up, untimely biomarker assessments, incorrect dates of enrollment), data from 271 women were included in analysis. Mean (SD) change in RBP, MDA, TNF-α, IL-6, and IL-8 between baseline and third trimester was −2.16 (4.47), −19.6 (46.4), 3.72 (37.2), 0.51 (14.4), and 13.2 (197), respectively, in intervention and −2.25 (4.30), −24.6 (43.6), 7.17 (32.6), −1.79, (11.4), and 31.3 (296) in control groups. None of these changes differed significantly between the two treatment arms. However, changes from baseline in TNF-α levels were significantly different between intervention and control groups in subset of women ($n = 99$) using firewood before trial (−7.03 [32.9] vs. +12.4 [33.6]; 95% CI for group difference: −35.4 to −3.4, $p = 0.018$).
Conclusions: Decrease in TNF-α concentration from baseline to third trimesters in intervention group women could indicate reduced cardiovascular stress and prothrombotic effects from decreased HAP. Our findings suggest that ethanol-burning stoves may mitigate cardiovascular health risks.

1. Introduction

Nearly 3 billion people worldwide use biomass and coal for cooking and heating purposes (Organization WH, 2014). The use of poorly vented traditional and biomass stoves results in household air pollution (HAP) in many low- and middle-income countries (LMICs). HAP, the eighth leading contributor to overall global disease burden, is responsible for 2.9 million deaths annually and 85·6 million DALYs (Forouzanfar et al., 2015). Premature mortality related to air pollution in urban settings is projected to reach about 65% by 2050 (Lelieveld et al., 2015).

HAP threatens lives of the world's most vulnerable populations and contributes to environmental degradation (Brook et al., 2010). In some developing countries, particulate matter (PM) and carbon monoxide levels from incomplete combustion of biomass fuels for cooking are 20–100 times above World Health Organization (WHO) guideline limits and national standards (Burroughs Pena et al., 2015; Oluwole et al., 2013a). In Sub-Saharan Africa (SSA), biomass fuels are the primary

* Corresponding author at: The University of Chicago Medicine, 5841 S. Maryland Avenue, MC 6076, Chicago, IL 60637, USA.
E-mail address: solopade@bsd.uchicago.edu (C.O. Olopade).

http://dx.doi.org/10.1016/j.envint.2016.11.004
0160-4120/© 2016 Published by Elsevier Ltd.

source of energy used for cooking for 753 million people, which accounts for 80% of its population (IEA, 2015). In Nigeria, about 122 million people use biomass fuels for household energy needs (IEA, 2015). No other region in the world has such a large population relying on biomass fuels for cooking and household energy needs. Eighty percent of cardiovascular deaths worldwide occur in SSA and other LMICs (Organization WH, 2013). Nigeria is among 15 countries that account for most of the premature mortality linked to air pollution with 89 deaths per 100,000 (Lelieveld et al., 2015).

The greatest exposure burden to HAP in Nigeria is among women and children, as they typically assume the bulk of household cooking duties, and this can result in a multitude of detrimental health effects including respiratory, cardiovascular, and ocular damage, and increased susceptibility to infectious illnesses such as tuberculosis (Oluwole et al., 2013a; Pekkanen et al., 2002). However, these adverse health effects have not been studied in an integrated and scientifically rigorous manner.

Exposure to air pollution, especially $PM_{2.5}$, can trigger oxidative stress and systemic inflammation directly from pulmonary oxidative stress through inhalation of toxic pollutants (Brook et al., 2010). Hence, even though systemic inflammation is not generally associated with pregnancy in seemingly healthy women (Vitoratos et al., 2010), this process may be triggered by HAP exposure. Relative to the use of LPG stoves, regular cooking with biomass aggravates systemic inflammation and oxidative stress that may increase the risk of cardiovascular disease (CVD) (Dutta et al., 2012). Similar conditions in pregnant women could predispose them to increased CVD risk, a major cause of adverse pregnancy outcomes and maternal mortality (Moghbeli et al., 2008). Particulate matter has previously been found to induce interleukin-6 (IL-6), interleukin-8 (IL-8) and tumor necrosis factor (TNF)-alpha production (Delfino et al., 2009; Osornio-Vargas et al., 2003). Malondialdehyde (MDA), an indicator of oxidative stress, was also found to be elevated in rural populations exposed to HAP (Isik et al., 2005). HAP from burning of biomass fuel can be markedly reduced by clean cook stove interventions (Thomas et al., 2015), but residual levels of pollutants can remain high and can still pose significant health risks to household inhabitants (Oluwole et al., 2013a).

Clean cookstoves and fuels (i.e. high-efficiency and low emission) have been offered as a potential tool to reduce HAP exposure, improve health outcomes, and decrease greenhouse gas emissions and deforestation (Hanna et al., 2012). Though association between HAP and risk of developing CVD is well established, there are few studies that have investigated the effect of HAP intervention (viz., clean cook stoves) on attenuation of short and long-term CVD risks (Clark et al., 2013; McCracken et al., 2011). Prior studies have reported improved lung function (Heinzerling et al., 2016), decreased frequency of respiratory (e.g. cough, phlegm, wheeze, chest tightness), non-respiratory (e.g. eye discomfort, headache, backache) (Diaz et al., 2008) symptoms in intervention groups using improved cook stoves (Accinelli et al., 2014).

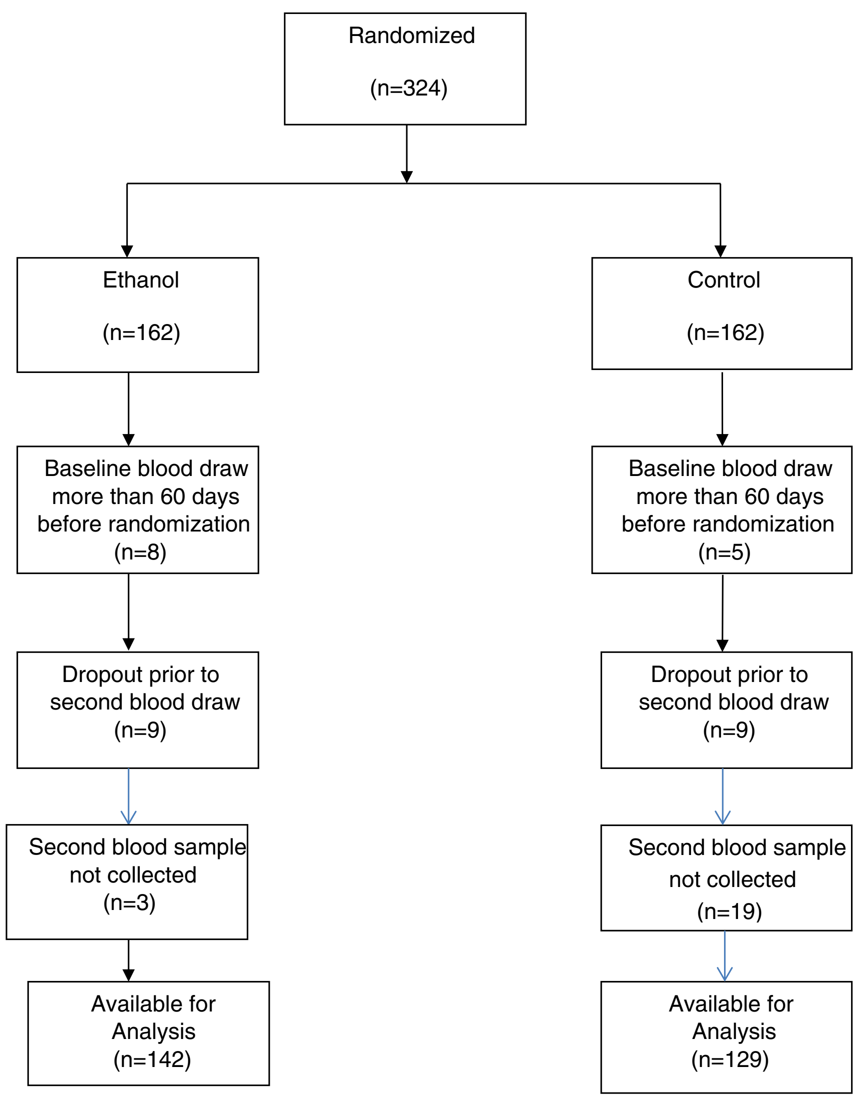


Fig. 1. Trial Profile.

We assessed the impact of transition from traditional wood or kerosene stove to a bioethanol-burning stove to determine if it could reduce inflammatory biomarkers and oxidative stress in pregnant women in Nigeria. To the best of our knowledge, this is the first study to investigate potential health benefits of cookstove intervention involving liquid fuels in pregnant women. We hypothesized that an ethanol cookstove intervention in a cohort of pregnant Nigerian women will produce a significant decrease in inflammatory biomarkers, which may reduce the risk of developing CVDs.

2. Methods

2.1. Study design

A randomized controlled trial (RCT) was conducted in Ibadan, Nigeria from June 2013–October 2015. Pregnant women were screened for eligibility and recruited at the time of their presentation at one of four participating primary health centers (PHCs): Agbongbon, Oranyan, Olorishaoko and Ijaye. The Institutional Review Board of the University of Chicago and the University of Ibadan approved the study protocol, which was registered at ClinicalTrials.gov: NCT02394574.

Primary outcome variables were pregnancy outcomes and are reported separately (manuscript in preparation). Exposure measurements, serum biomarkers assessment, blood pressure recordings, spirometry measurements, maternal symptoms, and fetal growth parameters were secondary.

2.2. Participants

Eligibility criteria for 324 women enrolled in this study included: i. apparently healthy women, ii. non-smokers, non-chewers of tobacco, iii. cooked regularly with firewood/kerosene, iv. <18 weeks gestational age as determined by self-reported first day of last known menstrual period (LMP) and confirmed by ultrasound biometry. Exclusion criteria included: i. smoker, ii. lived with smoker, iii. cooked for living, iv. HIV-positive, v. high-risk pregnancy (multiple gestations, uncontrolled maternal hypertension, maternal age > 35 for first delivery, three/more prior miscarriages, or a prior C-section). Written consent was obtained from participants at recruitment.

2.3. Randomization

Participants were randomized to ethanol or control cook stove group using randomization module in REDCap (Harris et al., 2009). Randomization was stratified by parity (≤4 vs. >4 children) and presence/absence of diabetes using method of permuted blocks (Matts and Lachin, 1988) and MT64 random number generator in STATA (College Station, TX). The study biostatistician (the only person with access to the lists) prepared the randomization lists in advance. A study personnel logged into REDCap to obtain assigned intervention, once a participant was deemed eligible and had consented to participate in the trial. Among 324 participants, 162 were randomly assigned to control arm and 162 to ethanol arm. Women assigned to control group continued to cook exclusively with the stove they were using at entry; i.e., firewood (n = 58) or kerosene (n = 104). Women randomized to ethanol group received CleanCook ethanol stove (CLEANCOOK Sweden AB), comprehensive training on operation of stove and supplies of ethanol every 2 weeks until 6 weeks after delivery. Additionally, field workers observed each woman refill, light and use the stove for the first time. Fifty-one of intervention group women had been firewood-users at time of entry into trial, while 111 were kerosene-users. Control group undertook an education awareness program on dangers of smoke exposure and were encouraged to reduce their exposure to smoke through behavioral and environmental modification.

2.4. Personal interviews

Information was collected on socio-demographics (age, education, habits, family, occupation of the participants, average family income, cooking hours per day, cooking-years, types of fuel used for cooking, home kitchen design, especially the presence or lack of windows), past medical history, dietary details, occupation of the spouse and exposure to environmental tobacco smoke (ETS). Data were collected through face-to-face interview with structured survey questionnaire in local language (Yoruba).

2.5. Serum IL-6, TNF-alpha, IL-8, RBP and MDA by ELISA

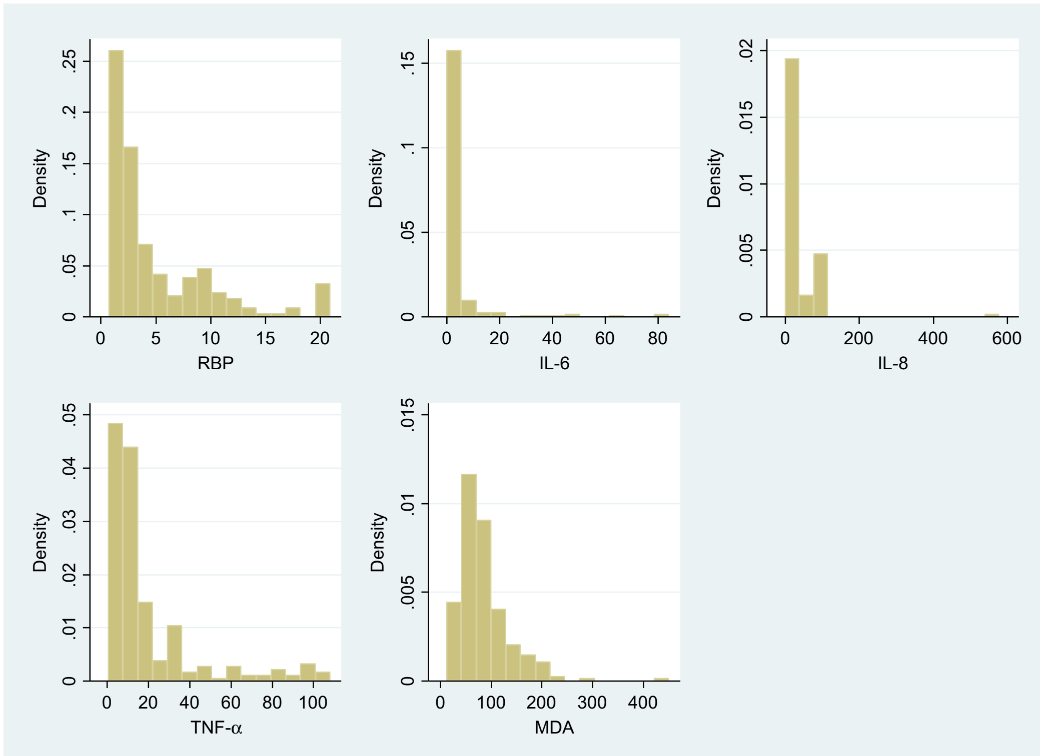
Blood was collected from antecubital vein of participants. Enzyme-linked immunosorbent assay (ELISA) was used for measurement of retinol-binding protein [RBP; by kit #E-80RBP (lot #15C1) of Immunology Consultants Laboratory, Inc., Portland, OR, USA], malondialdehyde [MDA; by kit #STA-832 (lot #0,613,008) of Cell Biolabs Inc., San Diego, CA, USA], interleukin-6 [IL-6; by kit #KHC0062 (lot #73992445B) of Life Technologies Corp, Frederick, MD, USA], interleukin-8 [IL-8; by kit #KHC0082 (lot #73916571B) of Life Technologies corp., Frederick,

Table 1
Baseline demographic and clinical distributions by intervention arm.

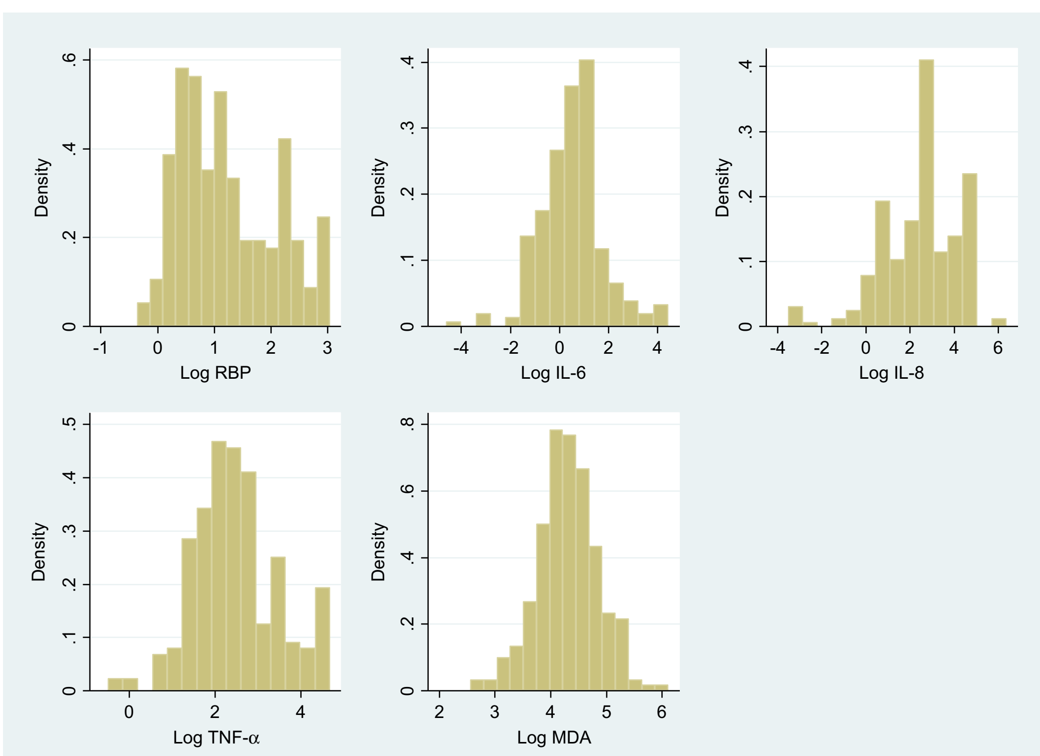
Variable	Baseline Kerosene Users		Baseline Firewood Users	
	Ethanol (KE) (n = 111)	Kerosene (KK) (n = 104)	Ethanol (FE) (n = 51)	Firewood (FF) (n = 58)
Clinic				
Agbongbon	67 (60.4%)	55 (52.9%)	9 (18.0%)	13 (22.8%)
Oranyan	37 (33.3%)	41 (39.4%)	5 (10.0%)	5 (8.8%)
Other	7 (6.3%)	8 (7.7%)	36 (72.0%)	39 (68.4%)
Missing			1	1
Diabetic				
Yes	2 (1.8%)	3 (2.9%)	0 (0.0%)	0 (0.0%)
No	109 (98.2%)	101 (97.1%)	50 (100%)	57 (100%)
Missing			1	1
Number of Children				
None	33 (29.7%)	26 (25.0%)	8 (16.0%)	16 (27.6%)
1–2	53 (47.8%)	53 (51.0%)	19 (38.0%)	18 (31.0%)
3–4	21 (18.9%)	23 (22.1%)	16 (32.0%)	22 (37.9%)
>4	4 (3.6%)	2 (1.9%)	7 (14.0%)	2 (3.4%)
Missing			1	
Marital Status				
Single	15 (13.5%)	3 (2.9%)	2 (4.0%)	4 (6.9%)
Married	96 (86.5%)	101 (97.1%)	47 (94.0%)	54 (93.1%)
Separated	0 (0.0%)	0 (0.0%)	1 (2.0%)	0 (0.0%)
Missing			1	
Mother's Age, yrs.				
Mean, SD	27.6, 5.6	28.2, 5.1	28.9, 7.0	27.4, 5.9
(range)	(15–42)	(17–39)	(15–44)	(14–42)
Missing	9	8	1	4
Mother's BMI				
Mean, SD	23.2, 4.2	24.7, 5.3	23.1, 4.2	24.7, 5.3
(range)	(14.2–35.2)	(17.1–45.0)	(17.6–36.2)	(17.2–43.9)
Missing	9	8	1	4
Education Level				
None	22 (19.8%)	26 (25.0%)	29 (58.0%)	32 (55.2%)
Primary School	15 (13.5%)	14 (13.5%)	1 (2.0%)	3 (5.2%)
Junior Secondary	4 (3.6%)	6 (5.8%)	5 (10.0%)	7 (12.1%)
Senior Secondary	54 (48.6%)	48 (46.2%)	14(28.0%)	12 (20.7%)
High School	9 (8.1%)	4 (3.8%)	1 (2.0%)	2 (3.4%)
Polytechnic	7 (6.3%)	5 (4.8%)	0 (0.0%)	1 (1.7%)
University	0 (0.0%)	1 (1.0%)	0 (0.0%)	1 (1.7%)
Missing			1	
Read/Write				
Yes	80 (72.1%)	67 (64.4%)	20 (40.0%)	25 (43.1%)
No	31 (27.9%)	37 (35.6%)	30 (60.0%)	33 (56.9%)
Missing			1	
Gestational Age at Entry, wks.				
Mean, SD	13.0, 2.9	13.1, 3.0	12.9, 3.2	13.1, 3.2
(range)	(7.5–18.0)	(7.1–18.0)	(6.7–17.9)	(7.4–17.9)
Missing		2	3	5

Please cite this article as: Olopade, C.O., et al., Effect of a clean stove intervention on inflammatory biomarkers in pregnant women in Ibadan, Nigeria: A randomized controlled stud..., Environ Int (2016), http://dx.doi.org/10.1016/j.envint.2016.11.004

MD, USA], and tumor necrosis factor-alpha [TNF-alpha; kit #KHC3012, lot# 73993297B) of Life Technologies Corp., Frederick, MD, USA] following manufacturers' protocol. Absorbance for all assays was measured at 450 nm in an ELISA reader (SpectraMax Plus 384 from Molecular Devices LLC, Sunnyvale, CA, USA). Samples were obtained at baseline and during third trimester of pregnancy.



A. Baseline distributions.



B. Baseline distributions—log scale.

Fig. 2. A. Baseline distributions. B. Baseline distributions—log scale.

Please cite this article as: Olopade, C.O., et al., Effect of a clean stove intervention on inflammatory biomarkers in pregnant women in Ibadan, Nigeria: A randomized controlled stud..., Environ Int (2016), http://dx.doi.org/10.1016/j.envint.2016.11.004

2.6. Monitoring stove use

All stoves in each household (including traditional firewood, kerosene and ethanol) were installed with a stove use monitoring system (SUMs). The SUMs is a small temperature data-logging sensor, first used by Ruiz et al. to monitors when stoves are is use (Ruiz-Mercado et al., 2013). The SUMs were placed 10 cm from the center of the kerosene cookstove burner and 14 cm directly in between the double burner of the CleanCook stove. These distances were determined pre-trial by defining an optimum length away from the stove burner that provided sufficient resolution of temperature fluctuation while not causing the SUMs to overheat and rupture. The temperature of the stoves were logged approximately every 10 min for the entire pregnancy period and the resulting data was compiled to determine when and how much the stove was used using an algorithm based on the amount of time the stove was above a threshold temperature. The SUMs recorded temperatures to the nearest 0.5 °C and were programmed to monitor either every three, ten or thirteen minutes based on the length of time between field visits. All data management and statistical analysis was conducted in RStudio version 0.98.507 (Northcross et al., 2016).

2.7. Personal monitoring of $PM_{2.5}$

Particulate matter with aerodynamic diameter < 2.5 μm ($PM_{2.5}$) was measured using RTI MicroPEM (PEM) for three consecutive days (72 h) during second and third trimesters. The exposure duration was based on a study from Guatemala that measured daily exposures for a year and showed that 72 h monitoring reflects 3 months exposure (Alnes, 2011). The MicroPEM is a new monitor developed and evaluated for use in settings with high concentrations of $PM_{2.5}$. Each woman in the study carried the MicroPEM in a small, culturally appropriate bag placed near the breathing zone. An internal accelerometer that was analyzed with the RTI software was used as a quality control check to ensure the monitor was worn. The accelerometer is sensitive enough to detect breathing movement even if the woman was sitting still. The following exposure indices adjusted for filter concentration; 72-h mean PM2.5 levels and the number of minutes spent with PM2.5 above 100 μg/m^3 during the 72-h period were recorded.

2.8. Statistical analysis

Out of 324 individuals, 215 were baseline kerosene-users randomized to ethanol (denoted KE: n = 111) or kerosene (KK: n = 104) and 109 were baseline firewood-users randomized to ethanol (FE: n = 51) or firewood (FF: n = 58). Three sets of analyses were, therefore, performed. First set compared Ethanol (E = KE + FE) vs. Control (C = KK + FF) groups. Second set compared ethanol vs. kerosene among subgroup of kerosene-users at baseline (i.e., KE vs. KK), and third ethanol vs. firewood among firewood-users at baseline (FE vs. FF). Biomarker levels were obtained at baseline and post-intervention (third trimester). Average interval between baseline and post-randomization time points was 145 days (range 54–187 days). Two-sample t-tests comparing changes from baseline between groups and analysis of covariance (ANCOVA), with post-randomization value as dependent variable and intervention group and pre-treatment biomarker level as predictor variables, were performed. Histograms of baseline biomarker levels indicated that the distributions for all five markers were positively skewed. Therefore, data were log-transformed prior to analysis. For ease of interpretation, however, mean changes are presented on original scale, while *p*-values correspond to tests based on log-transformed data. Scatterplots and Spearman rank correlation coefficients were generated to examine association between inflammatory biomarker levels and systolic and diastolic blood pressure.

Relationship between biomarker values obtained during third trimester with PM2.5 exposure levels measured during second trimester was examined. Multiple linear regression models were fit, which included exposure level as a continuous predictor and an indicator variable for whether exposure measurement was obtained during rainy or dry season, as pollution levels differed noticeably between the two seasons. As noted above, two summary measures derived for each participant during her three-day measurement period were used: average exposure level adjusted for filter concentration and number of minutes that the concentration of PM2.5 exceeded 100 μg/m^3. Both of these variables were highly skewed, and therefore, log and square root transformation were applied, respectively. Analyses were conducted using SAS, version 9.4 (SAS Institute Inc., Cary, NC) and Stata, version 14 (College Station, TX).

3. Results

Trial Profile is shown in Fig. 1. Participants whose baseline blood draw was obtained >60 days prior to randomization, those who dropped out of the study, and those in whom a post-intervention blood draw was not collected were excluded. More patients in control arm (N = 19) did not submit to post-intervention blood draw than in ethanol group (N = 3).

Table 1 shows baseline clinical and demographic variables by intervention arm, stratified according to baseline stove type. Overall, very few participants had diabetes and majority had 2 or fewer children at time of enrollment. Mean age was about 28 years (range 14–42 years) and mean BMI approximately 24 (range 14–44). Education levels varied from none to beyond high school; majority of participants were literate. Mean gestational age at entry was 13 weeks and ranged from 7 to

Table 2
Mean changes from baseline to third trimester (E vs. C).

		Ethanol (E)			Control (C)		
Variable	n	Mean Change	SE	n	Mean Change	SE	p-value[a]
RBP (mg/dl)	114	−2.15	0.41	101	−2.18	0.42	0.73
IL-6 (pg/ml)	116	0.46	1.30	100	−0.88	1.34	0.54
IL-8 (pg/ml)	112	16.2	18.2	99	26.8	28.6	0.41
TNF-α (pg/ml)	114	3.43	3.41	101	7.58	3.21	0.49
MDA (pmol/ml)	118	−19.7	4.2	101	−23.6	4.2	0.52

[a] p-value from two-sample *t*-test on log-transformed data; *p < 0.05.

Table 3A
Mean changes from baseline to third trimester (KE vs. KK subgroup comparison).

		Ethanol (KE)			Kerosene (KK)		
Variable	n	Mean Change	SE	n	Mean Change	SE	p-value[a]
RBP (mg/dl)	77	−3.11	0.57	65	-3.03	0.61	0.71
IL-6 (pg/ml)	78	1.42	1.74	66	-2.38	1.55	0.14
IL-8 (pg/ml)	75	35.5	26.0	64	55.4	43.8	0.95
TNF-α (pg/ml)	76	8.25	4.31	66	4.16	3.77	0.24
MDA (pmol/ml)	80	−20.3	5.8	66	-30.8	5.4	0.11

[a] p-value from two-sample *t*-test on log-transformed data.

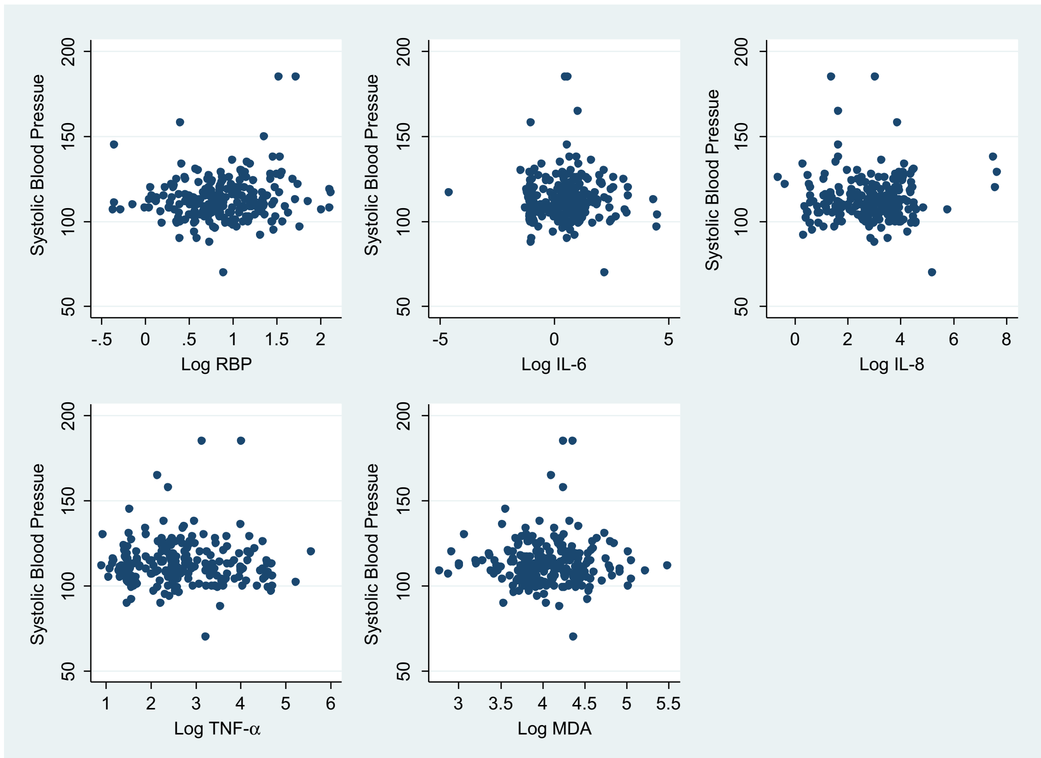
Table 3B
Mean changes from baseline to third trimester (FE vs. FF subgroup comparison).

		Ethanol (FE)			Kerosene (FF)		
Variable	n	Mean Change	SE	n	Mean Change	SE	p-value[a]
RBP (mg/dl)	37	−0.16	0.29	36	−0.64	0.29	0.16
IL-6 (pg/ml)	38	−1.51	1.73	34	2.03	2.51	0.43
IL-8 (pg/ml)	37	−23.1	15.2	35	−25.7	6.1	0.090
TNF-α (pg/ml)	38	−6.20	5.24	35	14.03	5.89	0.011*
MDA (pmol/ml)	38	−18.4	5.0	35	−10.0	6.2	0.16

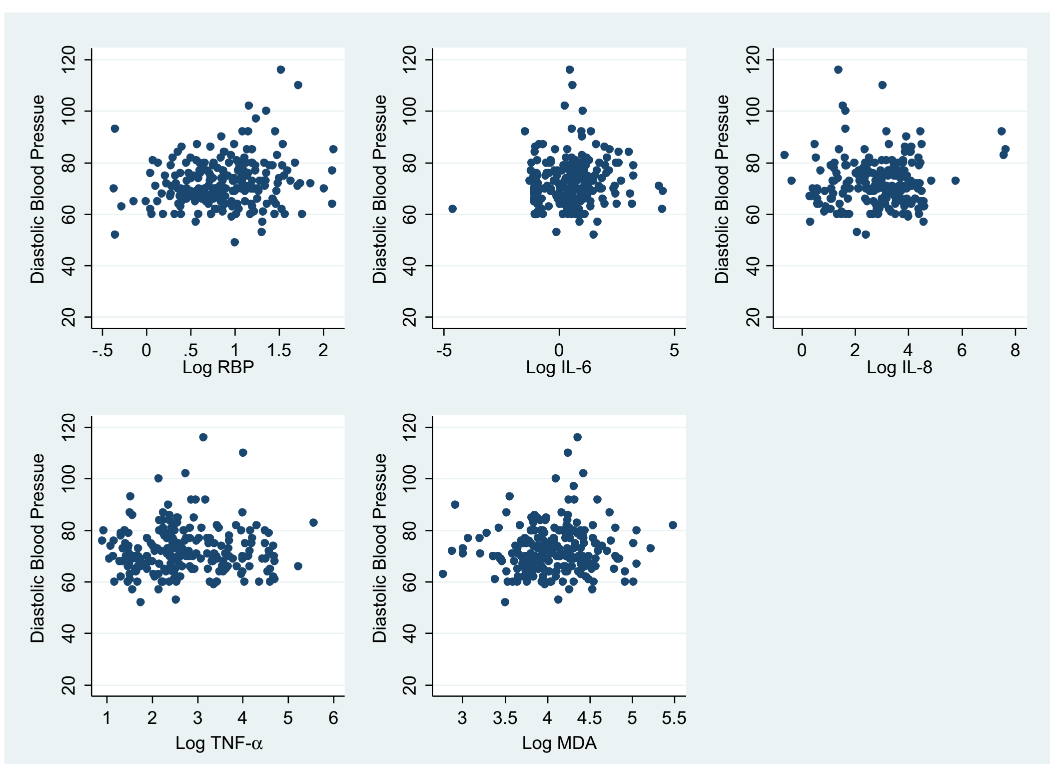
[a] p-value from two-sample *t*-test on log-transformed data.
* Stands for statistically significant difference (p < 0.05) when TNF alpha is compared between the ethanol and kerosene groups.

18 weeks. Randomization led to similar baseline distributions in ethanol and control arms; only (chance) imbalances were in marital status (among baseline kerosene-users) and small differences in BMI.

SUMs data showed that as many as 70% of the participants in the intervention arm adopted the CleanCook stove and gave away their kerosene stove(s) during the first 4 months of the study and used the



A. Systolic blood pressure vs. log biomarker levels.



B. Diastolic blood pressure vs. log biomarker levels.

Fig. 3. A. Systolic blood pressure vs. log biomarker levels. B. Diastolic blood pressure vs. log biomarker levels.

Please cite this article as: Olopade, C.O., et al., Effect of a clean stove intervention on inflammatory biomarkers in pregnant women in Ibadan, Nigeria: A randomized controlled stud..., Environ Int (2016), http://dx.doi.org/10.1016/j.envint.2016.11.004

Table 4
Spearman's rank correlation between blood pressure (measured at last visit prior to delivery) and blood levels of biomarkers (third trimester).

Parameters	SBP	DBP
	Rho value	Rho value
Serum TNF-α	−0.045	0.001
Serum IL-8	0.045	0.039
Serum IL-6	0.086	0.116
Serum RBP	0.093	0.135*
Serum MDA	−0.002	0.081

SBP, systolic blood pressure; DBP, diastolic blood pressure.
* $p < 0.05$.

CleanCook stove almost exclusively for the remainder of the study (Northcross et al., 2016).

Histograms of baseline serum biomarker levels are displayed in Fig. 2A and show considerable skewness. Log-transformed data (Fig. 2B) more closely approximate a normal distribution.

Tables 2 and 3A and 3B summarize mean changes in biomarkers from baseline to third trimester. Sample sizes reflect missing values due to failure of assay or removal of extreme outlying values. In E vs. C comparisons (Table 2), no statistically significant findings emerged. No statistically significant group differences in KE vs. KK subgroup comparisons (Table 3A) were noticed. In ANCOVA analyses, adjusting for baseline levels, mean post-randomization MDA was estimated to be −13.6% lower (95% CI: −24% to −2%) in KK group compared to KE group ($p = 0.025$). Comparing FE and FF groups, (Table 3B), statistically significant difference was found for TNF-α (*t*-test, ANCOVA). TNF-α in FE users decreased on average by −6.20 pg/ml (SE 5.24) whereas it increased in FF users by 14.03 pg/ml (SE 5.89) ($p = 0.011$; *t*-test). Similarly, mean post-randomization TNF-α was 68% higher (95% CI: 5% to 269%) in FF group compared to FE group ($p = 0.030$) in ANCOVA.

Relationships between third trimester serum biomarker levels and blood pressures recorded at last visit prior to delivery are presented in Fig. 3A and B. Spearman rank correlation coefficients are summarized in Table 4. There was little correlation between biomarkers and blood pressure. The only statistically significant association was between RBP and diastolic BP, but magnitude of the correlation was small ($r = 0.135$).

Fig. 4A and B shows scatterplots of biomarker levels as function of PM2.5 concentration and number of minutes above 100 μg/m^3 during 72-hour monitoring period. Measurements obtained during the rainy season are plotted in red and those from the dry season are shown in blue. The green and orange lines are the fitted values from the ANCOVAs for the rainy and dry seasons, respectively. Tables 5A and 5B provide corresponding estimates of slopes. There were statistically significant increases in IL-8 and TNF-α with increasing PM2.5 concentrations and with number of minutes above 100 μg/m^3. MDA decreased with increasing PM2.5 concentration (borderline significant) and with minutes above 100 μg/m^3. (See Table 5B.)

4. Discussion

This is the first trial to propose use of bio-ethanol as an alternative cleaner fuel in a HAP intervention study in pregnant women. This RCT explored changes in systemic inflammation during traditional wood/kerosene stove to bioethanol-burning CleanCook stove transition in pregnant women. Personal exposure monitoring for $PM_{2.5}$ was performed on all participating women, thus, enabling more accurate description of the association between HAP exposure and measured biomarkers. Nigeria, as an implementing national partner of the Global Alliance for Clean Cookstove, had launched Clean Cookstove Alliance for Nigeria, which was aimed at reducing adverse health effects caused by exposure to smoke from HAP. This policy change and commitment by Government provided an opportunity to investigate the health impact of use of cleaner fuel for cooking.

Measuring levels of different inflammatory biomarkers allowed a determination of reduction in systemic inflammation due to transition from traditional to ethanol stoves. Overall, no statistically significant differences between those randomized to ethanol and those randomized to control group were observed. There was, however, a beneficial effect for TNF-α among subgroup of households that used firewood prior to enrollment in trial. This suggests that shifting to cleaner fuels can reduce systemic inflammation. The importance of systemic inflammation in pathogenesis of CVD is well recognized (Ryan et al., 2009; Knol et al., 2009; Touyz and Briones, 2011). In this study, mean post-randomization TNF-α was 68% higher in firewood-using control group compared to firewood-randomized intervention group ($p = 0.030$). TNF-α is an important inflammatory cytokine in the pathway leading to development of CVD. Similarly, IL-6 exhibited an increasing trend in FF users and decreased among the FE users but this change was not statistically significant. IL8 levels, though showed an increasing trend, increased at a lower rate among the KE users and the ethanol group (16.2 pg/ml) as a whole when compared with KK users (55.4 pg/ml) and the control group (26.8 pg/ml).

PM and ultrafine particles present in biomass smoke mediate oxidative stress and produce pro-inflammatory mediators that may lead to pulmonary as well as systemic inflammation (Donaldson et al., 2001; Pope et al., 2004). PMs not only stimulate production of TNF, which in turn increases production of interleukin (IL)-6 and IL-8, which promote thrombosis (Mutlu et al., 2007), but PMs are also involved in inducing liver to produce pro-inflammatory mediators and acute phase proteins like IL-6 and C-reactive protein (Delfino et al., 2009; Hertel et al., 2010). Ambient exposure to PM has been shown to accelerate coagulation through an IL-6 dependent pathway (Mutlu et al., 2007). Increased concentration of circulating pro-inflammatory cytokine TNF-α and IL-6, neutrophil chemo-attractant IL-8 and acute-phase protein CRP that might predispose them to developing CVDs has also been reported among biomass users (Dutta et al., 2012).

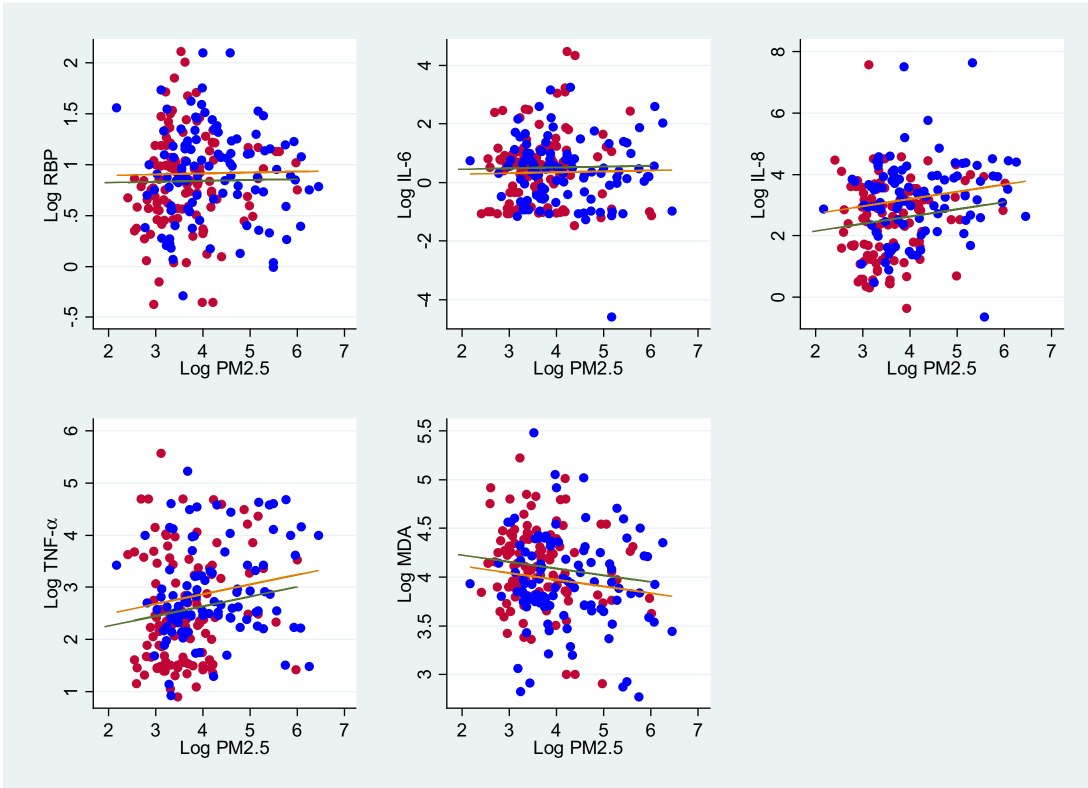
Malondialdehyde (MDA), an important markers of oxidative stress, which is generated in vivo via peroxidation of polyunsaturated fatty acids (Ho et al., 2013), is involved in promoting atherosclerosis by impairing the interaction between oxidized low-density lipoprotein and macrophages (Slatter et al., 2000). Women exposed to HAP from burning biomass fuels have significantly high levels of MDA (Isik et al., 2005). Decrease in MDA level related to transition from firewood to ethanol fuel was higher among FE users (−18.4 pmol/ml) compared to the FF users (−10.0 pmol/ml), but was not statistically significant.

Retinol-binding protein (RBP) is a nutritional biomarker with some anti-oxidant properties. RBP levels are generally lower among those chronically exposed to air pollution (Oluwole et al., 2013b). Low serum RBP concentration reflects anti-oxidant deficiency and allows induction or aggravation of systemic inflammation (Reifen, 2002). However, we did not observe any significant interval change in RBP levels as a result of the transition from traditional firewood/kerosene to the bioethanol stove.

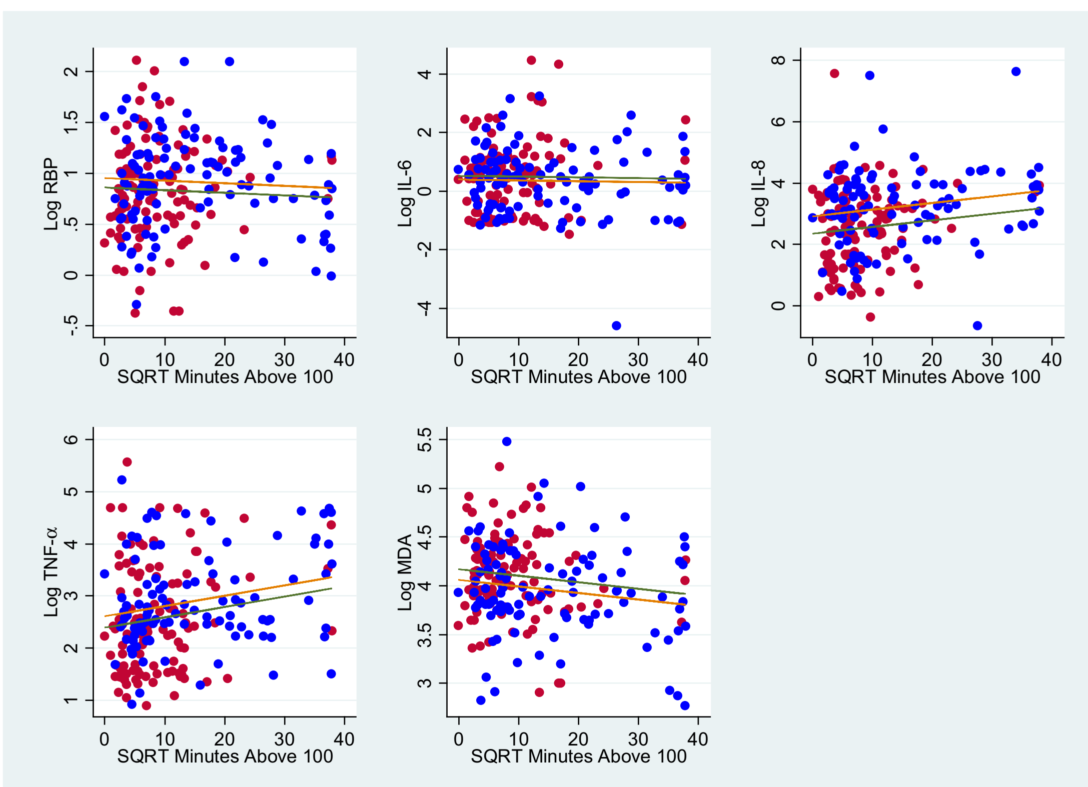
Overall, it may be concluded that there is some potential gain in health if women are able to switch to a better fuel and stove. Examination of serial blood pressure levels of pregnant women exposed to HAP (manuscript under journal review; Alexander et al.) showed statistically significant differences in diastolic blood pressure (DBP) profiles in pregnant women between intervention and control arms ($p = 0.0039$). Very little correlation was observed between inflammatory markers and blood pressure in this study, which is consistent with an earlier study that reported weak and non-significant association between hypertension and serum TNF-α (OR = 1.02, 95% CI: 0.76–1.18) (Dutta et al., 2012). Nonetheless, findings that blood pressure and TNF-α are reduced in intervention arm underscores the importance of transition to a much cleaner bioethanol-burning Cookstove.

Personal exposure monitoring of $PM_{2.5}$ for all participants provided the unique opportunity to assess individual HAP exposure and its relationship to measured biomarkers. Increases in IL-8 and TNF-α were

Please cite this article as: Olopade, C.O., et al., Effect of a clean stove intervention on inflammatory biomarkers in pregnant women in Ibadan, Nigeria: A randomized controlled stud..., Environ Int (2016), http://dx.doi.org/10.1016/j.envint.2016.11.004



A



B

Fig. 4. A. Serum biomarker levels (third trimester) vs. PM2.5 concentrations (second trimester readings). Red points/green line: rainy season, Blue points/orange line: dry season. B. Serum biomarker levels (third trimester) vs. minutes above 100 μg/m^3 (second trimester). Red points/green line: rainy season, Blue points/green line: dry season. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 5A
Regression of biomarkers on log PM2.5 concentration (estimated slopes).

Variable	Estimate	95% CI	p-value
Log RBP	0.0090	(−0.0651, 0.0832)	0.811
Log IL-6	0.0293	(−0.1659, 0.2245)	0.768
Log IL-8	0.2363	(0.0251, 0.4475)	0.028*
Log TNF-α	0.1863	(0.0278, 0.3449)	0.022*
Log MDA	−0.0686	(−0.1403, 0.0030)	0.060

* $p < 0.05$.

Table 5B
Regression of biomarkers on SQRT minutes above 100 (estimated slopes).

Variable	Estimate	95% CI	p-value
Log RBP	−0.0026	(−0.0092, 0.0040)	0.443
Log IL-6	−0.0027	(−0.0201, 0.0147)	0.758
Log IL-8	0.0216	(0.0029, 0.0403)	0.024*
Log TNF-α	0.0198	(0.0057, 0.0339)	0.006*
Log MDA	−0.0067	(−0.0131, −0.0003)	0.040*

* $p < 0.05$.

associated with increasing $PM_{2.5}$ concentrations. This means lowering HAP exposure through use of cleaner fuel and improved cook stove might lead to lower risk of CVD development. CleanCook stove used in this RCT surpassed WHO emission rate target for $PM_{2.5}$ and met ISO International Workshop Agreement (IWA) Tier 4 performance criteria for indoor emissions that are matched only by LPG gas stoves, solar, and biogas.

Relatively small sample size in baseline firewood subgroup was one limitation of this study. Follow-up for a longer time period post-randomization may have provided opportunity to investigate longer-term impacts on biomarker levels. Nevertheless, this study provides the basis for undertaking larger-sized RCTs in future.

5. Conclusions

Several million underprivileged women are chronically exposed to HAP in LMICs and it appears to be a menace to their health (Lim et al., 2012). Noubiap et al. (2015) made a compelling case to implement efficient strategies to educate populations on health issues associated with HAP health hazards and to build high quality data/evidence to facilitate efficient policy-making in SSA. Switching to ethanol-based stoves provides much-needed hope for a sustainable cooking alternative to unclean fuels in countries like Nigeria, where high-quality ethanol is already being produced locally for cooking. Additionally, the CleanCook stove used in this trial meets ISO performance criteria for indoor emissions that is comparable to LPG gas. In this context, the authors recommend measures to reduce HAP from unclean fuel/traditional stove use by introducing improved cook stoves that burn cleaner fuel. This study, which is important from public and global health perspectives, also supports and reiterates the need to follow WHO guidelines for indoor air quality and to craft and implement policy measures that will improve the health of vulnerable populations.

Role of the funding source

This work was supported by Global Alliance for Clean Cook (UNF-12-378) stoves through the United Nations Foundation (ClinTrials.gov NCT02394574). It had no role in study design, data collection, data analysis, data interpretation, or writing of the report. However, one of the authors Dr. D Alexander was a postdoctoral fellow in the senior author's program before being hired by the sponsoring agency towards the end of the study. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit.

Declaration of competing financial interests

We declare no competing financial interests.

Acknowledgement

Project supported by grant from Global Alliance for Clean Cook stoves. Project Gaia for assistance with securing gift of ethanol stoves from the National Biotechnology Development Agency, Abuja, Nigeria. Professor Adeyinka Falusi and Abayomi Odetunde for laboratory support in processing and storage of study samples.

References

Accinelli, R.A., Llanos, O., Lopez, L.M., Pino, M.I., Bravo, Y.A., Salinas, V., et al., 2014. Adherence to reduced-polluting biomass fuel stoves improves respiratory and sleep symptoms in children. BMC Pediatr. 14, 12.
Alnes, L., 2011. How Many Days of Measurement Adequately Predicts Long-Term Indoor Concentration Means?
Brook, R.D., Rajagopalan, S., Pope, C.A.I., Brook, J.R., Bhatnagar, A., Diez-Roux, A.V., et al., 2010. Particulate matter air pollution and cardiovascular disease: an update to the scientific statement from the American Heart Association. Circulation 68, 224–230.
Burroughs Pena, M., Romero, K.M., Velazquez, E.J., Davila-Roman, V.G., Gilman, R.H., Wise, R.A., et al., 2015. Relationship between daily exposure to biomass fuel smoke and blood pressure in high-altitude Peru. Hypertension 65 (5), 1134–1140.
Clark, M.L., Bachand, A.M., Heiderscheidt, J.M., Yoder, S.A., Luna, B., Volckens, J., et al., 2013. Impact of a cleaner-burning cookstove intervention on blood pressure in Nicaraguan women. Indoor Air 23 (2), 105–114.
Delfino, R.J., Staimer, N., Tjoa, T., Gillen, D.L., Polidori, A., Arhami, M., et al., 2009. Air pollution exposures and circulating biomarkers of effect in a susceptible population: clues to potential causal component mixtures and mechanisms. Environ. Health Perspect. 117 (8), 1232–1238.
Diaz, E., Bruce, N., Pope, D., Diaz, A., Smith, K.R., Smith-Sivertsen, T., 2008. Self-rated health among Mayan women participating in a randomised intervention trial reducing indoor air pollution in Guatemala. BMC Int. Health Hum. Rights 8, 7.
Donaldson, K., Stone, V., Seaton, A., MacNee, W., 2001. Ambient particle inhalation and the cardiovascular system: potential mechanisms. Environ. Health Perspect. 109 (Suppl. 4), 523–527.
Dutta, A., Ray, M.R., Banerjee, A., 2012. Systemic inflammatory changes and increased oxidative stress in rural Indian women cooking with biomass fuels. Toxicol. Appl. Pharmacol. 261 (3), 255–262.
Forouzanfar, M.H., Afshin, A., Alexander, L.T., Anderson, H.R., Bhutta, Z.A., Biryukov, S., et al., 2015. Global, regional, and national comparative risk assessment of 79 behavioural, environmental and occupational, and metabolic risks or clusters of risks. 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet 388(10053), pp. 1659–1724.
Hanna, S., Britter, R., Argenta, E., Chang, J., 2012. The Jack rabbit chlorine release experiments: implications of dense gas removal from a depression and downwind concentrations. J. Hazard. Mater. 213–214, 406–412.
Harris, P.A., Taylor, R., Thielke, R., Payne, J., Gonzalez, N., Conde, J.G., 2009. Research electronic data capture (REDCap)–a metadata-driven methodology and workflow process for providing translational research informatics support. J. Biomed. Inform. 42 (2), 377–381.
Heinzerling, A.P., Guarnieri, M.J., Mann, J.K., Diaz, J.V., Thompson, L.M., Diaz, A., et al., 2016. Lung function in woodsmoke-exposed Guatemalan children following a chimney stove intervention. Thorax 71 (5), 421–428.
Hertel, S., Viehmann, A., Moebus, S., Mann, K., Brocker-Preuss, M., Mohlenkamp, S., et al., 2010. Influence of short-term exposure to ultrafine and fine particles on systemic inflammation. Eur. J. Epidemiol. 25 (8), 581–592.
Ho, E., Karimi Galougahi, K., Liu, C.-C., Bhindi, R., Figtree, G.A., 2013. Biological markers of oxidative stress: applications to cardiovascular research and practice. Redox Biol. 1 (1), 483–491.
IEA, 2015. World Energy Outlook 2015: Energy Access Database.
Isik, B., Isik, R.S., Akyildiz, L., Topcu, F., 2005. Does biomass exposure affect serum MDA levels in women? Inhal. Toxicol. 17 (12), 695–697.
Knol, A.B., de Hartog, J.J., Boogaard, H., Slottje, P., van der Sluijs, J.P., Lebret, E., et al., 2009. Expert elicitation on ultrafine particles: likelihood of health effects and causal pathways. Part. Fibre Toxicol. 6, 19.
Lelieveld, J., Evans, J.S., Fnais, M., Giannadaki, D., Pozzer, A., 2015. The contribution of outdoor air pollution sources to premature mortality on a global scale. Nature 525 (7569), 367–371.
Lim, S.S., Vos, T., Flaxman, A.D., Danaei, G., Shibuya, K., et al., 2012. A comparative risk assessment of burden of disease and injury attributable to 67 risk factor and risk factor clusters in 21 regions, 1990–2010: systematic analysis for the Global Burden of Disease Study 2010. Lancet 380, 2224–2260.
Matts, J.P., Lachin, J.M., 1988. Properties of permuted-block randomization in clinical trials. Control. Clin. Trials 9 (4), 327–344.
McCracken, J., Smith, K.R., Stone, P., Diaz, A., Arana, B., Schwartz, J., 2011. Intervention to lower household wood smoke exposure in Guatemala reduces ST-segment depression on electrocardiograms. Environ. Health Perspect. 119 (11), 1562–1568.
Moghbeli, N., Pare, E., Webb, G., 2008. Practical assessment of maternal cardiovascular risk in pregnancy. Congenit. Heart Dis. 3 (5), 308–316.

Please cite this article as: Olopade, C.O., et al., Effect of a clean stove intervention on inflammatory biomarkers in pregnant women in Ibadan, Nigeria: A randomized controlled stud..., Environ Int (2016), http://dx.doi.org/10.1016/j.envint.2016.11.004

Mutlu, G.M., Green, D., Bellmeyer, A., Baker, C.M., Burgess, Z., Rajamannan, N., et al., 2007. Ambient particulate matter accelerates coagulation via an IL-6-dependent pathway. J. Clin. Invest. 117 (10), 2952–2961.

Northcross, A., Shupper, M., Alexander, D.A., Olamijulo, J., Ibigbami, T., Ana, G., Ojengbede, O., Olopade, C.O., 2016. Replacing Kerosene Cookstoves with Bioethanol Cookstove Intervention in Nigeria: Measuring sustained usage. Energy Sustain. Dev. 35, 35–40.

Noubiap, J.J., Essouma, M., Bigna, J.J., 2015. Targeting household air pollution for curbing the cardiovascular disease burden: a health priority in Sub-Saharan Africa. J. Clin. Hypertens. (Greenwich) 17 (10), 825–829.

Oluwole, O., Ana, G.R., Arinola, G.O., Wiskel, T., Falusi, A.G., Huo, D., et al., 2013a. Effect of stove intervention on household air pollution and the respiratory health of women and children in rural Nigeria. Air Qual. Atmos. Health 6 (3), 553–561.

Oluwole, O., Arinola, G.O., Ana, G.R., Wiskel, T., Huo, D., Olopade, O.I., et al., 2013b. Relationship between household air pollution from biomass smoke exposure, and pulmonary dysfunction, oxidant-antioxidant imbalance and systemic inflammation in rural women and children in Nigeria. Glob. J. Health Sci. 5 (4), 28–38.

Organization WH, 2013. A Global Brief on Hypertension.

Organization WH, 2014. Fact Sheet no 292: Household Air Pollution and Health.

Osornio-Vargas, A.R., Bonner, J.C., Alfaro-Moreno, E., Martinez, L., Garcia-Cuellar, C., Ponce-de-Leon Rosales, S., et al., 2003. Proinflammatory and cytotoxic effects of Mexico City air pollution particulate matter in vitro are dependent on particle size and composition. Environ. Health Perspect. 111 (10), 1289–1293.

Pekkanen, J., Peters, A., Hoek, G., Tiittanen, P., Brunekreef, B., de Hartog, J., et al., 2002. Particulate air pollution and risk of ST-segment depression during repeated submaximal exercise tests among subjects with coronary heart disease: the exposure and risk assessment for fine and ultrafine particles in ambient air (ULTRA) study. Circulation 106 (8), 933–938.

Pope III, C.A., Burnett, R.T., Thurston, G.D., Thun, M.J., Calle, E.E., Krewski, D., et al., 2004. Cardiovascular mortality and long-term exposure to particulate air pollution: epidemiological evidence of general pathophysiological pathways of disease. Circulation 109 (1), 71–77.

Reifen, R., 2002. Vitamin A as an anti-inflammatory agent. Proc. Nutr. Soc. 61 (3), 397–400.

Ruiz-Mercado, I., Canuz, E., Walker, J.L., Smith, K.R., 2013. Quantitative metrics of stove adoption using stove use monitors (SUMs). Biomass Bioenergy 57, 136–148.

Ryan, S., Taylor, C.T., McNicholas, W.T., 2009. Systemic inflammation: a key factor in the pathogenesis of cardiovascular complications in obstructive sleep apnoea syndrome? Postgrad. Med. J. 85 (1010), 693–698.

Slatter, A.D., Bolton, H.C., Bailey, J.A., 2000. The importance of lipid-derived malondialdehyde in diabetes mellitus. Diabetologia 43 (5), 550–557.

Thomas, E., Wickramasinghe, K., Mendis, S., Roberts, N., Foster, C., 2015. Improved stove interventions to reduce household air pollution in low and middle income countries: a descriptive systematic review. BMC Public Health 15, 650.

Touyz, R.M., Briones, A.M., 2011. Reactive oxygen species and vascular biology: implications in human hypertension. Hypertens. Res. 34 (1), 5–14.

Vitoratos, N., Economou, E., Iavazzo, C., Panoulis, K., Creatsas, G., 2010. Maternal serum levels of TNF-alpha and IL-6 long after delivery in preeclamptic and normotensive pregnant women. Mediat. Inflamm. 2010.

Please cite this article as: Olopade, C.O., et al., Effect of a clean stove intervention on inflammatory biomarkers in pregnant women in Ibadan, Nigeria: A randomized controlled stud..., Environ Int (2016), http://dx.doi.org/10.1016/j.envint.2016.11.004