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Journal

International Journal of Environmental Health Research, 32(3)

ISSN

0960-3123

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Publication Date

2022-03-04

DOI

10.1080/09603123.2020.1785402

Peer reviewed

Published in final edited form as:

Int J Environ Health Res. 2022 March ; 32(3): 565–578. doi:10.1080/09603123.2020.1785402.

Acute Differences in Blood Lipids and Inflammatory Biomarkers Following Controlled Exposures to Cookstove Air Pollution in the STOVES Study

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DISCLOSURE OF INTEREST:

The authors have no competing interests to declare.

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Abstract

Household air pollution is a leading risk factor for morbidity and premature mortality. Numerous cookstoves have been developed to reduce household air pollution, but it is unclear whether such cookstoves meaningfully improve health. In a controlled exposure study with a crossover design, we assessed the effect of pollution emitted from multiple cookstoves on acute differences in blood lipids and inflammatory biomarkers. Participants (n=48) were assigned to treatment sequences of exposure to air pollution emitted from five cookstoves and a filtered-air control. Blood lipids and inflammatory biomarkers were measured before and 0, 3, and 24 hours after treatments. Many of the measured outcomes had inconsistent results. However, compared to control, intercellular adhesion molecule-1 was higher 3 hours after all treatments, and C-reactive protein and serum amyloid-A were higher 24 hours after the highest treatment. Our results suggest that short-term exposure to cookstove air pollution can increase inflammatory biomarkers within 24 hours.

Keywords

air pollution; biomass burning; lipoproteins; inflammation

INTRODUCTION

Nearly 3 billion people burn solid fuels to meet their household cooking needs (Bonjour et al. 2013). Exposure to fine particulate matter air pollution ($PM_{2.5}$; particles less than 2.5 micrometers in aerodynamic diameter) from the use of solid cooking fuels resulted in an estimated 1.6 million premature deaths in 2017; approximately 40% of these premature deaths were a result of cardiovascular outcomes such as ischemic heart disease and stroke (Stanaway et al. 2018). Many interventions have been attempted to lower this disease burden using various types of cleaner-burning cookstoves. While some interventions succeed in reducing levels of household air pollution, whether these reductions lead to improved health outcomes remains unclear (Bruce et al. 2015; Quansah et al. 2017).

Evidence from previous literature suggests that exposure to household air pollution is associated with adverse cardiovascular outcomes related to blood pressure, endothelial function, and heart rate variability (McCracken et al. 2012; Fatmi and Coggon 2016).

Biomarkers, such as blood lipids and markers of inflammation, are important risk factors for development of cardiovascular disease (Bai and Sun 2016; Wu et al. 2018), yet they are difficult to assess in the context of household air pollution due to logistical and cost constraints of conducting field studies (Young et al. 2019). Laboratory-based studies can help overcome some logistical difficulties and complement field studies by allowing researchers to measure complex health outcomes in a controlled environment. Although previous studies have assessed the impact of wood smoke exposures on inflammatory biomarkers in controlled-exposure settings, these studies have been limited by small sample sizes ($n \leq 20$) and have reported few meaningful changes in inflammatory markers (Barregard et al. 2006; Forchhammer et al. 2012; Ghio et al. 2012; Riddervold et al. 2012; Stockfelt et al. 2013; Bonlokke et al. 2014). As a result, the overall body of evidence assessing household air pollution and cardiovascular-related biomarkers is limited and inconsistent.

Studying the impact of household air pollution on cardiovascular-related biomarkers will enhance our understanding of how these exposures influence the progression of cardiovascular disease. Blood lipids and inflammatory biomarkers are closely related determinants of vascular function and injury that can lead to advanced cardiovascular disease and mortality (Gonzalez and Selwyn 2003; Bai and Sun 2016). Atherosclerosis, a major cause of cardiovascular disease, is an inflammatory process that begins with endothelial dysfunction and accumulation of low-density lipoprotein (LDL) in the extracellular matrix of the intima (Bai and Sun 2016). LDL can be oxidized and stimulate the release of adhesion molecules, which facilitate the uptake of leukocytes to the site of vascular injury (Bai and Sun 2016). Macrophages then take up the oxidized LDL, leading to foam cell formation and subsequent fibrous plaques (Bai and Sun 2016). Triglyceride-rich lipoproteins contribute to this process by accumulating in the plasma and initiating a pro-atherogenic inflammatory cascade (Talayero and Sacks 2011). In contrast, high-density lipoprotein (HDL) is strongly protective against atherosclerosis by binding to and removing excess cholesterol from cells and extracellular tissues (Bai and Sun 2016).

The present study attempts to fill in gaps in the literature of how household air pollution impacts blood lipids and inflammatory biomarkers while also improving on previous studies by using a larger sample size of participants and a more robust study design. We assessed acute differences in blood lipids and inflammatory biomarkers in 48 young, healthy participants following controlled exposures to air pollution emitted from cookstoves. Our study, referred to as the Subclinical Tests on Volunteers Exposed to Smoke (STOVES) Study, implemented a crossover design with six controlled treatments consisting of air pollution emitted from five cookstove technologies and a filtered air control. We report differences in serum lipids (total cholesterol, HDL, LDL, and triglycerides) and inflammatory biomarkers (C-reactive protein [CRP], serum amyloid A [SAA], intercellular adhesion molecule-1 [ICAM-1], vascular cell adhesion molecule-1 [VCAM-1], interleukin-6 [IL-6], interleukin-8 [IL-8], and tumor necrosis factor-alpha [TNF-α]) for each cookstove treatment compared to control at three post-treatment time points (0-hours, 3-hours, and 24-hours). Other outcomes assessed in the STOVES Study are reported separately (Fedak et al. 2019, 2020; Walker et al. 2020).

MATERIALS AND METHODS

Study design and participants

The STOVES study implemented a 6×6 Latin square crossover design. The Latin square included six sequences of six treatments to air pollution emitted from five different cookstove technologies and a filtered air control (Figure S1). The design was highly structured so that each treatment followed every other treatment only once across the six treatment sequences, limiting the impact of potential confounders that may have varied with time (e.g., ambient air pollution or temperature). In addition, other potential confounding factors (e.g., participant sex or age) were limited by each participant serving as their own control in the crossover design. The 2-hour treatments each had a target level of $PM_{2.5}$ and were administered with a washout period of at least 2 weeks between treatments within a sequence.

Participants (n=48) were recruited from the Fort Collins, Colorado area starting in September of 2016. Participants were eligible for the study if they were between 18 and 35 years of age at the time of recruitment, had body mass index (BMI) between 18 and 29 kg/m^2, had no history of smoking, no regular air pollution exposures (above ambient levels), were not pregnant, and had no history of chronic disease that could impact the effect of the treatments on the study outcomes or put the participant at higher risk by participating in the study (e.g., chronic cardiovascular, respiratory, or metabolic disease). After passing eligibility screening, participants were assigned to a treatment sequence (Figure S1). Due to the complex treatment schedule and the demanding time requirement for participants, random assignment to the treatment sequences was generally not possible. However, the strength of the study design was not in random assignment to treatment sequences, but in the structure and organization of the treatments within the sequences as described above. As such, sequence assignment was primarily based upon the best alignment of each participant's personal schedule with the treatment sequence schedules. Eight participants were assigned to each treatment sequence and followed the same unique sequence of six treatments. After treatment sequences were completed, participants were allowed to return for out-of-sequence makeup sessions if they missed a scheduled session.

All study procedures were approved by the Institutional Review Board at Colorado State University. Participants provided written consent for all study procedures and were monetarily compensated for each completed session at the amount of $175.00 USD. A bonus incentive of $225.00 USD was included if participants completed all 6 study sessions in the assigned order.

Study sessions

Each study session spanned a period of approximately 27 hours during which participants underwent an assigned treatment with four separate health assessments (Figure S2). The health assessments took place at baseline (pre-treatment), 0 hours post-, 3 hours post-, and 24 hours post-treatment. At the start of each study session, a cardiologist visited each participant to ensure they were healthy enough to participate by discussing any current or recent illness, inflammatory, or allergic reactions on the part of the participant. Based on

this discussion, the cardiologist made a final decision regarding each participant's ability to participate in a given session. Each study session followed the same sequence of events: a baseline health assessment, the assigned 2-hour treatment, the 0-hour post-treatment health assessment, a 3-hour period in which participants remained in the testing facility building, a 3-hour post-treatment health assessment, an 18-hour period in which participants left the testing facility building, and a 24-hour post-treatment health assessment. In total, participants spent approximately 9 hours at the testing facility during each study session.

Since diet can impact non-fasting blood lipids (Langsted and Nordestgaard 2019), we asked participants to eat a consistent, low-fat diet and refrain from alcohol and caffeine during the 24 hours leading up to each study session until after the 24-hour post-treatment health assessment. To encourage consistency in diet while participants were at the testing facility, we provided a low-fat, low-cholesterol lunch after the 0-hour post-treatment health assessment that was consistent across all study sessions. Participants were also asked to refrain from using medications starting 3 days prior to each study session until after the 24-hour post-treatment health assessment.

Health assessments and study outcomes

Participants completed a series of health measurements following a 10-minute rest period in supine position (Figure S2). Blood samples were collected via venipuncture at the end of each health assessment by a trained phlebotomist. For blood lipids, samples were collected into SST tubes (BD Diagnostics, USA), inverted 5 times, allowed to clot for at least 30 minutes, and then centrifuged for 10 minutes at 1300 relative centrifugal force (Model MP4R, International Equipment Company, USA) to separate the serum from the clot. Samples were then left at room temperature and collected at the end of the study day by a local laboratory for analysis (Cobas 8000, Roche Diagnostics, USA).

The inflammatory markers were from two kits to assess inflammation and vascular injury in humans (Meso Scale Diagnostics LLC, USA). Blood samples were collected into CPT tubes (BD Diagnostics, USA), inverted 8–10 times, and centrifuged for 20 minutes at 1800 relative centrifugal force. Aliquots of plasma (500 μL) from the sample were stored in a −80°C freezer until analysis. Analyses for the cytokine panel (V-PLEX Human Proinflammatory Panel II, Meso Scale Diagnostics LLC, USA) and vascular injury panel (V-PLEX Vascular Injury Panel 2 Human, Meso Scale Diagnostics LLC, USA) were run as singlets following manufacturer protocols (MESO QuickPlex SQ 120, Meso Scale Diagnostics LLC, USA).

Controlled exposure treatments

The six controlled treatments (with $PM_{2.5}$ target levels) included filtered air control (0 $\mu g/m^3$), liquefied petroleum gas (LPG; 10 $\mu g/m^3$), gasifier (35 $\mu g/m^3$; fuel of pine wood chips), forced-draft fan rocket elbow (referred to as "fan rocket"; 100 $\mu g/m^3$; fuel of pine wood sticks), natural-draft rocket elbow (referred to as "rocket elbow"; 250 $\mu g/m^3$; fuel of pine wood sticks), and three stone fire (500 $\mu g/m^3$; fuel of pine wood sticks). The cookstoves used for the treatments were selected to represent a spectrum of cookstove technologies commonly used around the world. Treatments were administered using a controlled exposure facility called the Simulated Environmental Testing (SET) facility.

Details on the SET facility operation have been published previously (Fedak et al. 2019). Briefly, study personnel operated the cookstoves in a total-capture fume hood (located adjacent to the SET) during the treatments. Emissions from the fume hood were mixed with high-efficiency particulate air (HEPA) filtered air to reach the target concentration for each respective treatment and directed into the SET through a mixing plenum. Flow of dilution and pollution air were automated to keep $PM_{2.5}$ concentrations in the SET near target values (LabVIEW™, v15.0 32-bit, National Instruments, USA). $PM_{2.5}$ (DustTrak DRX 8533, TSI Incorporated, USA), carbon monoxide and oxygen (Siemens Ultramat 6E gas analyzer, Siemens AG, Germany), and humidity and temperature (Omega HX94BC transmitter and Type K thermocouple, OMEGA Engineering, USA) were monitored in real time within the SET facility during the controlled treatments.

Participants were monitored throughout the treatments to ensure their well-being. Nursing staff remotely measured blood pressure, heart rate, and oxygen saturation every 15 minutes while participants were inside the SET facility.

In separate tests, we characterized additional pollutant concentrations ($PM_{2.5}$ mass, particle number size distributions [10 nm to 500 nm], $PM_{2.5}$ elemental and organic carbon, nitrogen oxide, nitrogen dioxide, and carbonyls) inside the SET facility for each of the six treatments. Detailed methods and results for the additional pollutant characterization are published elsewhere (Fedak et al. 2019).

Questionnaires and potential confounders

We administered a demographic questionnaire during each participant's first study session. In addition, we administered questionnaires at the beginning of each study day to collect information on potential confounders and adherence to study protocols. Prior to the baseline health assessment of each study session, participants were asked to report their mode of transportation to the study facility, frequency of alcohol and caffeine consumption, outside smoke exposures, medication use, physical activity, and sleep quality during the previous 24 hours. Participants answered the same questionnaire prior to the 24-hour post-treatment health assessment regarding the period between the 3-hour post- and 24-hour post-treatment health assessments. Participants also recorded their dietary intake during the morning prior to both the baseline and 24-hour post-treatment health assessments, as well as during the period between the treatment and the 3-hour post-treatment health assessment. Questionnaires were entered electronically into tablets using a statistical survey web app (LimeSurvey version 2.0). Ambient temperature and $PM_{2.5}$ concentrations were collected from local monitors and also considered as potential confounders in secondary analyses (Colorado State University 2018; U.S. Environmental Protection Agency 2018).

Statistical analysis

We used R version 3.5.0 (The R Project for Statistical Computing) for data cleaning, visualization, and analysis. We calculated summary statistics (mean, standard deviation [sd], minimum, median, maximum) for participant characteristics and health outcomes at baseline. We also estimated mean $PM_{2.5}$ and carbon monoxide concentrations for each

treatment by averaging the mean concentrations from each individual participant's 2-hour treatment.

We used the lme4 (Bates et al. 2015) and lmerTest (Kuznetsova et al. 2017) packages to fit linear mixed models to our data. Our models included a fixed effect for the categorical treatment (stove type), a fixed linear effect of baseline outcome measurement (to account for outcome variations at the beginning of each study day that were unrelated to the treatments), a random intercept for participant (to account for correlation of the repeated measures within each participant), and a random intercept for date of the treatment (to account for potential correlation between observations for participants who were part of the same study session). We used separate models for each outcome and each post-treatment time point (0, 3, and 24 hours) to assess differences in the outcomes for each cookstove treatment compared to control.

We performed sensitivity analyses using a dataset that did not include out-of-sequence makeup visits. These analyses used the Latin square terms for sequence and visit as additional fixed effects in the models. We also conducted additional sensitivity analyses that included potential confounders as covariates. Further details on sensitivity analyses are available in the Supplemental Materials. Diagnostic plots (i.e. QQ plots and residuals vs fitted values plots) were evaluated for all models to determine if linear model assumptions were met.

RESULTS

Participants

Participants (n = 48; 26 males and 22 females) largely identified as non-Hispanic white (42/48 participants), had mean age at baseline of 28 years (sd = 4), and had mean BMI at baseline of 23 kg/m^2 (sd = 2) (Table 1). Twenty-six of the 48 participants missed at least one treatment in their assigned sequence due to illness or unplanned scheduling conflicts. However, including out-of-sequence makeup sessions, 45 participants completed at least five treatments and 39 participants completed all six treatments (Table 1). Overall, there was 7% missing data after accounting for missed study sessions and missing observations due to blood collection and lab processing errors.

Controlled exposure treatments

Mean $PM_{2.5}$ exposure concentrations for each treatment were generally close to the target concentrations for the respective treatments (Table 2). The three highest treatments, with target $PM_{2.5}$ concentrations of 500, 250, and 100 $\mu g/m^3$, had mean concentrations that were less than 9% from the target. The gasifier treatment (target of 35 $\mu g/m^3$) had a mean $PM_{2.5}$ concentration of 46 $\mu g/m^3$, the LPG treatment (target of 10 $\mu g/m^3$) had a mean $PM_{2.5}$ concentration of 8 $\mu g/m^3$, and the filtered air control treatment (target of 0 $\mu g/m^3$) had a mean $PM_{2.5}$ concentration of less than 1 $\mu g/m^3$. Carbon monoxide, which did not have a target level for each treatment, generally increased as target $PM_{2.5}$ concentrations increased and had mean mixing ratios of less than 10 ppm for each treatment (Table 2).

Characterization of additional pollutants in the SET has described previously (Fedak et al. 2019). In general, concentrations of the additional pollutants increased as $PM_{2.5}$ target concentrations for the treatments increased.

Blood lipids and inflammatory biomarkers

Total cholesterol, LDL, HDL, TNF-α, ICAM-1, and VCAM-1 met linear model assumptions evaluated by assessing QQ plots and residuals vs fitted-values plots. Triglycerides, IL-6, IL-8, SAA, and CRP were natural log-transformed to meet model assumptions for linear regression; results for these transformed outcomes are presented as percent changes for ease of interpretation.

Baseline values of blood lipids were within normal ranges for young, healthy adults (American College of Cardiology 2018). Baseline values of inflammatory biomarkers were similar to those measured during previous controlled exposure studies with healthy adults (Barregard et al. 2006; Stockfelt et al. 2013). There were some differences in the outcomes between the treatments at baseline (i.e. pre-treatment; Table S1); however, differences between each cookstove treatment and control at baseline were generally small in magnitude and not clinically meaningful. We also observed some differences in outcomes at baseline by participant sex, such as higher HDL and LDL and lower triglycerides in females compared to males (Table 1); however, sex of the participant did not impact the effect of the treatment on the measured outcomes (See supplemental figures). Correlations (Tables S3 and S4) between the outcomes were generally low (i.e. between −0.5 and 0.5) with the exception of total cholesterol and LDL (correlation = 0.91) and CRP and SAA (correlation = 0.58).

Model estimates and 95% confidence intervals (CI) for the difference between each treatment and control at the three post-treatment time points are presented in Tables 3 and 4 and Figures 1 and 2. For total cholesterol, LDL, HDL, IL-6, IL-8, and TNF-α, we observed no meaningful differences compared to control for all cookstove treatments at any post-treatment time point (Tables 3 and 4; Figure 1). Although estimates for these outcomes did vary in magnitude, we observed no consistent trends of higher or lower values for any specific treatment compared to control or at any particular post-treatment time point.

CRP and SAA were higher than control 24 hours after the three stone fire treatment yet were generally consistent with no meaningful differences compared to control for other treatments or at other time points (Table 4, Figure 2). For example, the difference between the LPG treatment and control for CRP at the 24-hour post treatment time point was 5.6% (95% CI: −9.5, 23.2) and the difference between the three stone fire treatment and control for CRP at the 24-hour post treatment time point was 16.1% (95% CI: 0.8, 33.7). Results for SAA followed a similar pattern to CRP, although with wider confidence intervals.

Triglycerides were marginally higher 24 hours after the cookstove treatments compared to control; however, the magnitude of the differences varied, confidence intervals were wide, none of the differences were statistically significant (p-value < 0.05), and the rocket elbow treatment was not different from control (Table 3; Figure 1). For example, at 24 hours post-treatment, triglycerides were 8.6% higher after the LPG treatment compared to control (95% CI: −3.6, 22.4) and 12.1% higher after the three stone fire treatment compared to

control (95% CI: −0.5, 26.2). There were no meaningful differences in triglycerides for any treatment compared to control at the 0-hour and 3-hour post-treatment time points.

There were no meaningful differences at the 0-hour post-treatment timepoint for ICAM-1 or VCAM-1 for any treatment compared to control. ICAM-1 was higher than control for each cookstove treatment at the 3-hour post-treatment time point, although the magnitude of the differences was lower and not statistically significant following the fan rocket and rocket elbow treatments (Table 4, Figure 2). For example, at the 3-hour post-treatment time point, the difference between the LPG treatment and control was 15.6 ng/mL (95% CI: 3.8, 27.4) and the difference between the three stone fire treatment and control was 13.1 ng/mL (95% CI: 2.0, 24.3). ICAM-1 was also marginally higher 24 hours after the LPG, gasifier, and three stone fire treatments compared to control, although not for the fan rocket or rocket elbow treatments. Results for VCAM-1 followed a similar trend as ICAM-1, although estimates for VCAM-1 were generally smaller in magnitude and not statistically significant (p-value < 0.05).

Results from sensitivity analyses are presented in the Supplemental Materials. None of the sensitivity analyses or inclusion of potential confounders resulted in meaningfully different model estimates compared to the primary model estimates presented in Tables 3 and 4 and Figures 1 and 2.

DISCUSSION

We assessed the impact of air pollution emitted from multiple cookstove technologies on blood lipids and inflammatory biomarkers in a controlled exposure setting. We observed higher ICAM-1 and VCAM-1 3 hours after the cookstove treatments compared to control, although not all of the differences were statistically significant and the magnitude of the effects varied across treatment type. We also observed higher CRP, SAA, and triglycerides 24 hours after the three stone fire treatment compared to control, although differences for these outcomes following other treatments and at other time points were variable and had wide confidence intervals. There were no consistent patterns of higher or lower values across the cookstove treatments for total cholesterol, HDL, LDL, IL-6, IL-8, or TNF-α at any post-treatment time point. Sensitivity analyses using potential confounders and subsets of the data had similar results to the primary analyses.

Our results add to the limited evidence describing the impact of cookstove-emitted air pollution on biomarkers of inflammation and blood lipids. Only one study to date has assessed household air pollution and blood lipids; no associations were observed between household air pollution exposures and total cholesterol, HDL, LDL, and triglycerides in a cross-sectional study of primary household cooks in rural Honduras (Rajkumar et al. 2019). In contrast, associations have been observed between inflammatory biomarkers and household air pollution in field settings. Higher serum levels of ICAM-1, VCAM-1, and E-selectin were observed in biomass fuel users compared to clean fuel users in Peru (Caravedo et al. 2016). In India, biomass fuel users had higher serum levels of IL-6, IL-8, TNF-α, and CRP compared to clean fuel users (Dutta et al. 2012).

Controlled exposure studies have assessed inflammatory biomarkers following exposure to wood smoke; however, findings in these studies have shown few changes in inflammatory markers, and sample sizes have been limited to 20 or fewer participants (Barregard et al. 2006; Forchhammer et al. 2012; Ghio et al. 2012; Riddervold et al. 2012; Stockfelt et al. 2013; Bonlokke et al. 2014). A study with 13 adult participants observed higher SAA at 0, 3, and 20 hours after wood smoke exposures compared to clean air, although no meaningful changes were observed for CRP, IL-6, or TNF-α (Barregard et al. 2006). Another study with 10 adult participants reported higher neutrophils and cytokine IL-1β following controlled exposure to wood smoke particles (Ghio et al. 2012). Subsequent controlled-exposure studies have observed no meaningful differences in inflammatory biomarkers following wood smoke exposures compared to filtered air (Forchhammer et al. 2012; Riddervold et al. 2012; Stockfelt et al. 2013; Bonlokke et al. 2014). Our results may be different than those from previous studies due to differences in study design, participant characteristics and sample size, or due to the type and duration of the exposures of interest.

The specific mechanisms through which air pollution initiates proinflammatory and atherogenic pathways are likely initiated following inhalation of PM that leads to localized inflammation in the lung and a subsequent systemic inflammatory response and oxidative stress (Brook et al. 2010; Li et al. 2012; Bai and Sun 2016). Circulating inflammatory cytokines can lead to a cascade of inflammatory events including endothelial injury and dysfunction, expression of adhesion molecules, and disruption of endothelial permeability (Gonzalez and Selwyn 2003; Bai and Sun 2016). In addition to their impact of the vascular endothelium, cytokines also stimulate the liver to increase production of CRP and SAA, both acute indicators of systemic inflammation that have strong associations with cardiovascular events and mortality (Chait et al. 2005). These events are at the core of the atherogenic process and vascular dysfunction; they highlight the interaction between blood lipids and inflammatory biomarkers in the development of atherosclerosis and cardiovascular disease.

Although our results provide some evidence of the potential physiological pathways described above, there were also instances when the outcomes we assessed were not different between the cookstove treatments and control. A weakness in our study is that we were logistically limited to collecting outcome measurements at a small number of post-treatment time points, and only up to 24 hours post-treatment. It is possible that there were differences in some health outcomes at other times, which may explain why we saw differences in some biomarkers (e.g. ICAM-1, VCAM-1) but not others (e.g. IL-6, IL-8, LDL, and HDL). Future studies with a similar design could use our results to inform when they collect health measurements and could improve on our design by assessing health outcomes at more post-treatment time points (e.g., 36 or 48 hours post-treatment). An additional consideration is that we used non-fasting measurements, and blood lipids can vary acutely depending on dietary fat intake; however, there is evidence that non-fasting lipids predict cardiovascular disease as well as lipids assessed in a fasting state (Langsted and Nordestgaard 2019). We also asked participants to eat low-fat meals during the 24 hours leading up to each study session until after the 24-hour post-treatment follow-up, and to eat a consistent diet across study sessions. Although participants were not entirely consistent in adhering to study dietary protocol, there were generally no patterns in dietary intake associated with a particular treatment (Table S2). Sensitivity analyses also indicated that

consuming higher fat food items or being less consistent in dietary consumption across treatments did not impact the results.

In some instances, the differences compared to control were similar in magnitude across the treatments (i.e. the difference for LPG was similar to that of the three stone fire). These results are consistent with the impact of the treatments on blood pressure (Fedak et al. 2019), pulse wave velocity, and central pulse pressure (Walker et al. 2020) from the same study. Our characterization of additional pollutants for each treatment did not provide an explanation for these trends: based on the pollutants we were able to measure, no single pollutant had a concentration (different from control) of similar magnitude across all of the treatments (Fedak et al. 2019). Instead of a single pollutant causing the observed results, it is possible that each cookstove in our study emitted a unique, complex mixture of pollutants that had a similar impact on the health outcomes. Alternatively, our results may be indicating that the health impact of short-term exposures to any level of particulate matter air pollution may have a threshold and elicit similar responses across exposure levels, at least for the subclinical endpoints that we evaluated in this study.

Our results have limited generalizability with regard to the numerous cookstove designs, fuels, and stove-use practices seen throughout the world. However, the internal validity of the crossover design in our study is much stronger than the observational studies typically used in household air pollution research. We encourage readers to interpret our results as complementary to field studies that have higher external validity but may be more subject to biased results. Potential confounders that typically impact an observational study were unlikely to be associated with the individual treatments in our study, so the impact of confounding on our results was limited; results from sensitivity analyses were not meaningfully different from the primary model results and helped confirm this. The statistical models we used in our analyses additionally helped control for confounding: by including a term for the baseline health outcome prior to each treatment, we were able to account for potential time-variant confounders that may have varied at random between study days. The mixed-model approach that used a random intercept for each individual participant helped control for potential time-invariant confounders that did not change within person throughout our study. In addition, field studies that randomly assign interventions can realistically only assess one type of cookstove at a time within a population, while we have assessed a wider spectrum of cookstove technologies than any previous study to date.

The generalizability of our results is also limited by the study population. The healthy, young adults in our study do not represent the wide spectrum of cookstove users around the world who follow countless cultural, dietary, and cookstove-use practices. In addition, our study design limited us to assessing short-term exposures to cookstove air pollution, whereas cookstove users are often exposed to cookstove air pollution daily over the course of their lives. Although the external validity of our study is limited, studies of this nature help us understand the underlying health impacts of household air pollution exposures. Atherosclerosis and advanced cardiovascular disease progress over the course of many years, yet the facilitating events take place acutely and repeatedly. Here and in previous publications from the present study, we have observed evidence that short-term exposures to particulate matter air pollution emitted from cookstoves can lead to acute cardiovascular

changes in young, healthy adults. Repeated particulate matter air pollution exposures may result in an underlying increase in cardiovascular disease risk (Brook et al. 2010), and in the case of higher triglycerides and inflammatory biomarkers, may lead to an increased risk of the progression of atherosclerosis (Gonzalez and Selwyn 2003; Libby and Ridker 2004; Talayero and Sacks 2011). The connection between the treatments in our study and cardiovascular disease risk is further supported by the differences we observed in hemodynamic indices. We have previously reported higher systolic blood pressure (Fedak et al. 2019), pulse wave velocity, and central pulse pressure (Walker et al. 2020) 24 hours after the treatments compared to control. Blood pressure, pulse wave velocity, and central pulse pressure are clinical indicators of cardiovascular disease and can change acutely through inflammatory pathways that impact endothelial function and vascular tone (Brook et al. 2010; Tomiyama and Yamashina 2010). Together, the results from our study show a consistent story that air pollution emitted from cookstoves is capable of acutely impacting multiple indicators of vascular function and cardiovascular disease risk. We recommend that future field studies assess the impact of cookstove interventions on biomarkers and indicators of vascular function to complement our findings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS:

We would like to acknowledge the participants and study personnel who made this research possible. We are grateful to the medical staff from Heart Center of the Rockies and the students who helped run the SET facility (Lizette Van Zyl, Evan Guiderra, Kathryne Gutekunst, and Danny Stringer). We are also grateful to Lisa Dailey, Tracey Montilla, and Julie Wood of the National Health and Environmental Effects Research Laboratory at the U.S. EPA for analysing the inflammatory markers reported in this manuscript.

The research described in this article has been reviewed by the National Health and Environmental Effects Research Laboratory, U.S. EPA, and approved for publication. The contents of this article should not be construed to represent Agency policy nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

FUNDING:

Research reported in this publication was supported by the National Institute of Environmental Health Sciences of the National Institutes of Health under award number R01ES023688. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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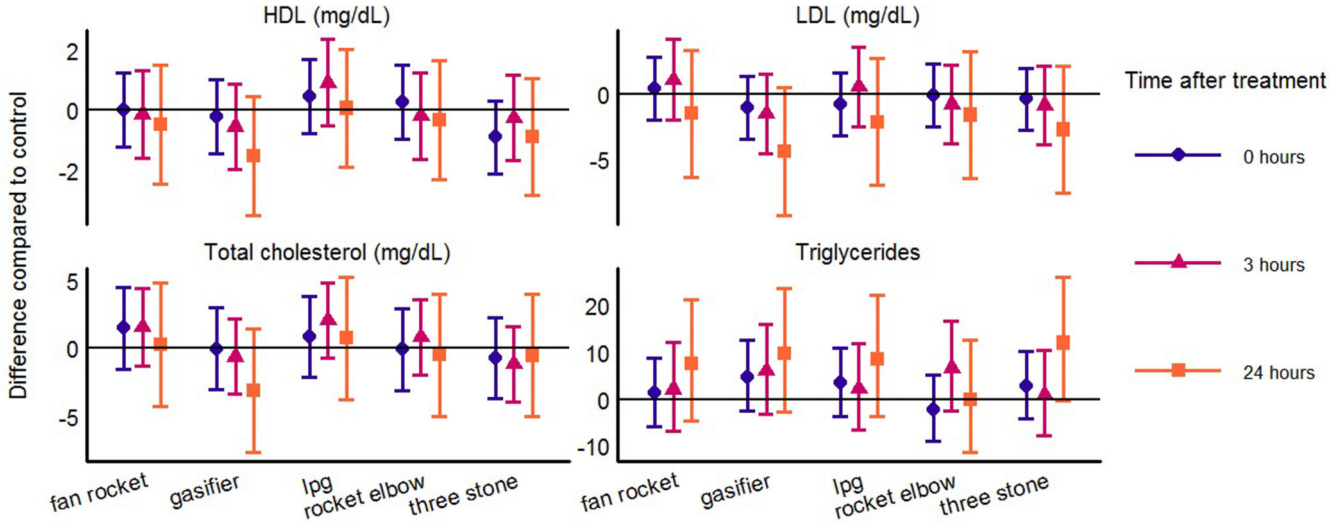

Figure 1:

Differences in lipid panel outcomes for each cookstove treatment compared to control at three post-treatment time points using linear mixed models*

HDL = high-density lipoprotein; LDL = low-density lipoprotein; LPG = liquefied petroleum gas

*Model terms include cookstove treatment level (fixed) + baseline health measurement (fixed) + date (random) + participant (random)

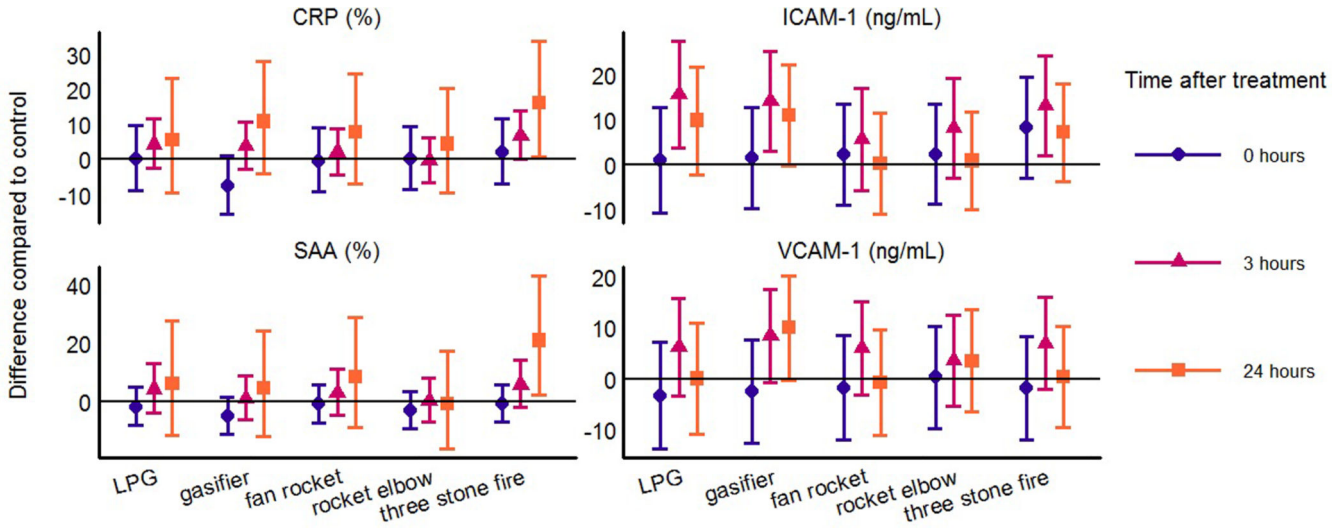

Figure 2:

Differences in vascular injury panel outcomes for each cookstove treatment compared to control at three post-treatment time points using linear mixed models*

CRP = C-reactive protein; SAA = serum amyloid A; ICAM-1 = intercellular adhesion molecule 1; VCAM-1 = vascular cell adhesion molecule 1; LPG = liquefied petroleum gas

*Model terms include cookstove treatment level (fixed) + baseline health measurement (fixed) + date (random) + participant (random)

Table 1:

Participant characteristics

Variable	All participants (n = 48)	Females (n = 22)	Males (n = 26)
	mean (sd), minimum, median, maximum		
Age at study start, years	28 (4), 21, 27, 36	27 (3), 23, 26, 33	28 (4), 21, 28, 36
Body mass index at study start, kg/m^2	23 (2), 19, 23, 29	23 (2), 20, 23, 29	23 (2), 19, 23, 26
Baseline* total cholesterol, mg/dL	170 (34), 91, 167, 299	182 (37), 138, 172, 299	159 (28), 91, 156, 211
Baseline* high density lipoprotein, mg/dL	60 (14), 37, 55, 93	66 (15), 46, 65, 93	54 (11), 37, 52, 80
Baseline* low density lipoprotein, mg/dL	87 (29), 30, 83, 190	95 (32), 49, 88, 190	79 (23), 30, 78, 136
Baseline* triglycerides, mg/dL	120 (64), 43, 102, 315	108 (61), 43, 101, 275	130 (66), 54, 104, 315
Baseline* interleukin-6, pg/mL	0.46 (0.16), 0.17, 0.44, 0.85	0.49 (0.17), 0.3, 0.45, 0.85	0.42 (0.14), 0.17, 0.43, 0.77
Baseline* interleukin-8, pg/mL	4.3 (1.4), 2.1, 4.1, 8.0	4.1 (1.5), 2.1, 3.6, 7.2	4.4 (1.2), 2.9, 4.2, 8.0
Baseline* tumor necrosis factor alpha, pg/mL	1.7 (0.5), 0.7, 1.6, 2.7	1.7 (0.6), 0.7, 1.6, 2.7	1.7 (0.5), 0.8, 1.7, 2.5
Baseline* serum amyloid A, mg/L	2.8 (3.5), 0.2, 1.4, 17.3	3.8 (4.1), 0.4, 2.5, 17.3	1.8 (2.4), 0.2, 1.0, 9.3
Baseline* C-reactive protein, mg/L	0.9 (1.1), 0.1, 0.4, 4.8	1.3 (1.4), 0.1, 0.7, 4.8	0.5 (0.4), 0.1, 0.4, 1.3
Baseline* soluble intercellular adhesion molecule 1, ng/mL	214, (44), 115, 205, 321	210 (42), 115, 212, 321	217 (46), 147, 201, 297
Baseline* soluble vascular cell adhesion molecule 1, ng/mL	257 (55), 134, 247, 394	246 (54), 134, 243, 394	268 (56), 183, 254, 359
	n (%)		
Non-Hispanic white ethnicity/race	42 (88)	18 (82)	24 (92)
Participants with data for all 6 treatments+	39 (81)	19 (86)	20 (77)
Participants with data for 5 or 6 treatments+	45 (94)	22 (100)	23 (88)

*Baseline means represent averages across all participants for the pre-treatment measurement of each participant's first study visit.

+Participant included if present for baseline health assessment, treatment, and at least one follow-up health assessment.

sd = standard deviation

Table 2:

SET facility 2-hour pollution concentrations compared to target levels of fine particulate matter

Treatment	Control	LPG	Gasifier	Fan rocket	Rocket elbow	Three stone fire
$PM_{2.5}$ target concentration	**0 μg/m^3**	**10 μg/m^3**	**35 μg/m^3**	**100 μg/m^3**	**250 μg/m^3**	**500 μg/m^3**
Participants with completed treatment, n	47	45	44	44	45	47
Mean (sd) $PM_{2.5}$ concentration, μg/m^3	1 (2)	8 (3)	46 (9)	95 (9)	254 (9)	462 (41)
Mean difference from target level, μg/m^3	1	−2	11	−5	4	−38
Maximum difference from target level, μg/m^3	9	7	42	23	26	133
Mean percent difference from target level, %		−18	31	−5	2	−8
Mean (sd) CO mixing ratio*, ppm	2 (2)	3 (1)	5 (3)	8 (2)	6 (2)	9 (4)

SET = Simulated Environmental Testing; LPG = liquefied petroleum gas; $PM_{2.5}$ = fine particulate matter; sd = standard deviation; CO = carbon monoxide

*CO did not have a target level; values represent the mean CO mixing ratio measured for each treatment.

Table 3:

Differences in blood lipids following 2-hour cookstove treatments compared to control at three post-treatment timepoints using linear mixed models*

Health measurement timepoint	Control	LPG	Gasifier	Fan rocket	Rocket elbow	Three stone fire
	Total cholesterol (mg/dL)					
	Mean (sd)	Difference compared to control (95% confidence interval)				
0-hour post-treatment	171 (32)	0.9 (−2.1, 3.9)	0.0 (−3.0, 3.0)	1.5 (−1.5, 4.5)	−0.1 (−3.1, 2.9)	−0.7 (−3.7, 2.3)
3-hour post-treatment	172 (33)	2.0 (−0.7, 4.8)	−0.6 (−3.4, 2.2)	1.6 (−1.3, 4.4)	0.8 (−1.9, 3.6)	−1.2 (−3.9, 1.6)
24-hour post-treatment	170 (34)	0.8 (−3.7, 5.3)	−3.1 (−7.7, 1.4)	0.3 (−4.3, 4.8)	−0.5 (−5.0, 4.0)	−0.5 (−5.0, 4.0)
	High density lipoprotein (mg/dL)					
	Mean (sd)	Difference compared to control (95% confidence interval)				
0-hour post-treatment	60 (16)	0.5 (−0.8, 1.7)	−0.2 (−1.4, 1.0)	0.0 (−1.2, 1.3)	0.3 (−1.0, 1.5)	−0.9 (−2.1, 0.3)
3-hour post-treatment	59 (15)	0.9 (−0.5, 2.3)	−0.5 (−2.0, 0.9)	−0.1 (−1.6, 1.3)	−0.2 (−1.6, 1.2)	−0.3 (−1.7, 1.2)
24-hour post-treatment	59 (14)	0.1 (−1.9, 2.0)	−1.5 (−3.5, 0.5)	−0.5 (−2.4, 1.5)	−0.3 (−2.3, 1.6)	−0.9 (−2.8, 1.1)
	Low density lipoprotein (mg/dL)					
	Mean (sd)	Difference compared to control (95% confidence interval)				
0-hour post-treatment	90 (29)	−0.8 (−3.2, 1.6)	−1.0 (−3.4, 1.4)	0.5 (−2.0, 2.9)	−0.1 (−2.5, 2.4)	−0.3 (−2.7, 2.0)
3-hour post-treatment	88 (30)	0.6 (−2.5, 3.6)	−1.5 (−4.5, 1.6)	1.1 (−2.0, 4.2)	−0.8 (−3.8, 2.3)	−0.8 (−3.9, 2.2)
24-hour post-treatment	91 (32)	−2.1 (−7.0, 2.7)	−4.4 (−9.2, 0.5)	−1.5 (−6.3, 3.4)	−1.6 (−6.4, 3.3)	−2.7 (−7.5, 2.2)
	Triglycerides (percent difference)+					
	Mean (sd)	Difference compared to control (95% confidence interval)				
0-hour post-treatment	104+ (59)	3.4 (−3.7, 11.0)	4.8 (−2.5, 12.6)	1.3 (−5.8, 8.9)	−2.2 (−9.0, 5.1)	2.7 (−4.2, 10.2)
3-hour post-treatment	123+ (64)	2.2 (−6.7, 11.9)	6.0 (−3.2, 16.0)	2.1 (−7.0, 12.1)	6.7 (−2.6, 16.8)	0.9 (−7.7, 10.4)
24-hour post-treatment	102+ (50)	8.6 (−3.6, 22.4)	9.7 (−2.8, 23.7)	7.6 (−4.7, 21.4)	−0.2 (−11.5, 12.6)	12.1 (−0.5, 26.2)

LPG = liquefied petroleum gas; sd = standard deviation

*Model terms include cookstove treatment level (fixed) + baseline health measurement (fixed) + date (random) + participant (random)

+Units for mean values during the control treatment: Triglycerides = mg/dL

Table 4:

Differences in inflammatory biomarkers following 2-hour cookstove treatments compared to control at three post-treatment timepoints using linear mixed models*

Health measurement timepoint	Control	LPG	Gasifier	Fan rocket	Rocket elbow	Three stone fire
	Interleukin-6 (percent difference)+					
	Mean (sd)	Difference compared to control (95% confidence interval)				
0-hour post-treatment	0.52ǂ (0.36)	−7.4 (−17.5, 4.0)	−10.0 (−19.5, 0.5)	2.4 (−8.4, 14.4)	−3.2 (−13.3, 8.0)	3.4 (−7.3, 15.4)
3-hour post-treatment	0.54ǂ (0.44)	2.8 (−13.3, 22.1)	−7.1 (−20.8, 9.0)	3.7 (−11.9, 22.0)	1.5 (−13.4, 18.8)	−2.0 (−16.4, 14.9)
24-hour post-treatment	0.53ǂ (0.35)	−6.5 (−23.9, 14.8)	−3.1 (−19.8, 17.2)	6.7 (−11.8, 29.1)	−3.3 (−19.7, 16.5)	1.0 (−16.2, 21.7)
	Interleukin-8 (percent difference)+					
	Mean (sd)	Difference compared to control (95% confidence interval)				
0-hour post-treatment	4.02ǂ (1.16)	−4.1 (−11.7, 4.2)	2.7 (−5.2, 11.3)	3.8 (−4.2, 12.5)	−1.9 (−9.3, 6.2)	−1.11 (−8.6, 7.0)
3-hour post-treatment	4.08ǂ (1.01)	−5.6 (−13.9, 3.5)	6.4 (−2.5, 16.1)	1.7 (−7.0, 11.1)	−4.9 (−12.7, 3.6)	−2.8 (−10.8, 5.9)
24-hour post-treatment	4.64ǂ (2.75)	1.5 (−7.6, 11.4)	0.2 (−8.1, 9.3)	3.6 (−5.1, 13.1)	−3.8 (−11.6, 4.8)	0.5 (−7.7, 9.4)
	Tumor necrosis factor alpha (pg/mL)					
	Mean (sd)	Difference compared to control (95% confidence interval)				
0-hour post-treatment	1.73 (0.65)	0.0 (−0.1, 0.1)	−0.1 (−0.2, 0.1)	0.0 (−0.1, 0.1)	−0.1 (−0.2, 0.1)	0.0 (−0.1, 0.1)
3-hour post-treatment	1.66 (0.63)	0.0 (−0.1, 0.1)	0.0 (−0.1, 0.1)	0.0 (−0.1, 0.1)	0.0 (−0.1, 0.1)	0.0 (−0.1, 0.1)
24-hour post-treatment	1.76 (0.59)	0.0 (−0.1, 0.1)	0.0 (−0.1, 0.1)	0.0 (−0.1, 0.1)	0.0 (−0.2, 0.1)	0.0 (−0.1, 0.1)
	Serum amyloid A (percent difference)+					
	Mean (sd)	Difference compared to control (95% confidence interval)				
0-hour post-treatment	2.66ǂ (2.53)	−1.9 (−8.3, 4.9)	−5.1 (−11.0, 1.3)	−0.9 (−7.2, 5.7)	−3.2 (−9.2, 3.3)	−0.7 (−6.8, 5.9)
3-hour post-treatment	2.59ǂ (2.48)	4.3 (−3.7, 13.0)	1.0 (−6.4, 8.9)	3.0 (−4.6, 11.2)	0.2 (−7.0, 8.0)	5.8 (−1.8, 14.0)
24-hour post-treatment	3.25ǂ (5.18)	6.2 (−11.7, 27.7)	4.7 (−11.8, 24.2)	8.5 (−8.7, 28.8)	−0.9 (−16.2, 17.2)	20.8 (2.1, 43.0)
	C-reactive protein (percent difference)+					
	Mean (sd)	Difference compared to control (95% confidence interval)				
0-hour post-treatment	0.85ǂ (1.23)	0.0 (−8.9, 9.9)	−7.7 (−15.7, 1.1)	−0.4 (−9.1, 9.1)	0.0 (−8.6, 9.5)	2.0 (−6.8, 11.6)
3-hour post-treatment	0.76ǂ (1.07)	4.3 (−2.5, 11.6)	3.8 (−2.6, 10.7)	2.1 (−4.4, 8.9)	−0.3 (−6.5, 6.2)	6.8 (0.2, 13.8)
24-hour post-treatment	1.09ǂ (1.95)	5.6 (−9.5, 23.2)	10.9 (−4.0, 28.1)	7.7 (−6.8, 24.6)	4.5 (−9.3, 20.3)	16.1 (0.8, 33.7)
	Soluble intercellular adhesion molecule 1 (ng/mL)					

Health measurement timepoint	Control	LPG	Gasifier	Fan rocket	Rocket elbow	Three stone fire
	Interleukin-6 (percent difference)[+]					
	Mean (sd)	Difference compared to control (95% confidence interval)				
	Mean (sd)	Difference compared to control (95% confidence interval)				
0-hour post-treatment	232 (52)	1.1 (−10.6, 12.7)	1.6 (−9.7, 12.8)	2.3 (−8.9, 13.6)	2.4 (−8.8, 13.5)	8.3 (−2.9, 19.6)
3-hour post-treatment	228 (48)	15.6 (3.8, 27.4)	14.1 (2.9, 25.4)	5.7 (−5.6, 17.1)	8.2 (−2.9, 19.3)	13.1 (2.0, 24.3)
24-hour post-treatment	234 (48)	9.9 (−2.1, 21.8)	11.0 (−0.2, 25.4)	0.2 (−11.0, 11.5)	0.9 (−10.0, 11.9)	7.2 (−3.7, 18.1)
	Soluble vascular cell adhesion molecule 1 (ng/mL)					
	Mean (sd)	Difference compared to control (95% confidence interval)				
0-hour post-treatment	264 (64)	−3.3 (−13.8, 7.2)	−2.4 (−12.6, 7.7)	−1.8 (−11.9, 8.4)	0.3 (−9.7, 10.4)	−1.8 (−11.9, 8.3)
3-hour post-treatment	258 (58)	6.3 (−3.3, 15.8)	8.5 (−0.7, 17.6)	6.0 (−3.2, 15.2)	3.5 (−5.5, 12.6)	6.9 (−2.1, 16.0)
24-hour post-treatment	266 (62)	0.0 (−10.9, 11.0)	10.0 (−0.2, 20.2)	−0.7 (−11.0, 9.6)	3.5 (−6.6, 13.5)	0.4 (−9.6, 10.4)

LPG = liquefied petroleum gas; sd = standard deviation

[*] Model terms include cookstove treatment level (fixed) + baseline health measurement (fixed) + date (random) + participant (random)

[+] Units for mean values during the control treatment: Interleukin 6 and Interleukin 8 = pg/mL, Serum amyloid A and C-reactive protein = mg/L