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Short-term differences in cardiac function following controlled exposure to cookstove air pollution: The subclinical tests on volunteers exposed to smoke (STOVES) study

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envint.2020.106254>.

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Abstract

Background: Exposure to household air pollution from solid fuel combustion for cooking and heating is an important risk factor for premature death and disability worldwide. Current evidence supports an association of ambient air pollution with cardiovascular disease but is limited for household air pollution and for cardiac function. Controlled exposure studies can complement evidence provided by field studies.

Objectives: To investigate effects of short-term, controlled exposures to emissions from five cookstoves on measures of cardiac function.

Methods: Forty-eight healthy adults (46% female; 20–36 years) participated in six, 2-h exposures ('treatments'), including emissions from five cookstoves and a filtered-air control. Target fine particulate matter (PM_{2.5}) exposure-concentrations per treatment were: control, 0 µg/m³; liquefied petroleum gas, 10 µg/m³; gasifier, 35 µg/m³; fan rocket, 100 µg/m³; rocket elbow, 250 µg/m³; and three stone fire, 500 µg/m³. Participants were treated in a set (pre-randomized) sequence as groups of 4 to minimize order bias and time-varying confounders. Heart rate variability (HRV) and cardiac repolarization metrics were calculated as 5-min means immediately and at 3 h following treatment, for analysis in linear mixed-effects models comparing cookstove to control.

Results: Short-term differences in SDNN (standard deviation of duration of all NN intervals) and VLF (very-low frequency power) existed for several cookstoves compared to control. While all cookstoves compared to control followed a similar trend for SDNN, the greatest effect was seen immediately following three stone fire ($\beta = -0.13$ ms { % }; 95% confidence interval = $-0.22, -0.03\%$), which reversed in direction at 3 h (0.03%; $-0.06, 0.13\%$). VLF results were similar in direction and timing to SDNN; however, other HRV or cardiac repolarization results were not similar to those for SDNN.

Discussion: We observed some evidence of short-term, effects on HRV immediately following cookstove treatments compared to control. Our results suggest that cookstoves with lower PM_{2.5} emissions are potentially capable of affecting cardiac function, similar to stoves emitting higher PM_{2.5} emissions.

Keywords

Cookstove; Fine particulate matter; Household air pollution; Healthy adult; Heart rate variability; Cardiac repolarization

1. Introduction

Air pollution is considered by the Global Burden of Disease Study to be the largest environmental contributor to premature death and disability (Stanaway et al., 2018). The study estimated a loss of 60 million disability-adjusted life years from individual exposure to household fine particulate matter (2.5 µm diameter; PM_{2.5}) emitted from combustion of

solid fuels (Stanaway et al., 2018). This burden of disease is particularly high in low- and middle-income countries, where a substantial proportion of the population relies on solid-fuel combustion for domestic energy purposes (cooking, heating, lighting) (Bonjour et al., 2013). Cardiovascular disorders, such as myocardial infarction and stroke, are a large contributor to the mortality attributed to PM_{2.5} exposure (Brook, Newby and Rajagopalan, 2018). Intervention studies seek to reduce PM_{2.5} exposure concentrations emitted from solid-fuel combustion by introducing improved cookstove designs (Thomas et al., 2015) and subsequently reducing the potential for an associated health burden (Lai et al., 2019).

Despite these efforts, scientific knowledge is lacking on the short- and long-term effects of household air pollution and its role in cardiovascular disease. While there are a number of field studies evaluating blood pressure (McCracken et al., 2007; Baumgartner et al., 2011, 2014), 2018; Clark et al., 2011, 2013; Dutta and Ray, 2012; Alexander et al., 2015, 2017; Neupane et al., 2015; Burroughs Pena et al., 2015; Norris et al., 2016), there is currently only one published field study on cardiac function and household air pollution (McCracken et al., 2011). That one field study, an intervention to reduce woodsmoke-derived household air pollution by switching cookstove type among users, provided mixed results for the impact on cardiac function: ST segment depression was improved with the intervention, but not high frequency (HF) or low frequency (LF) measures of heart rate variability (HRV) (McCracken et al., 2011).

Measurement of HRV provides an indication of change in the autonomic nervous system that can affect cardiac function and risk of adverse cardiovascular outcomes (Pope and Dockery, 2006). Chronically decreased HRV is associated with an increased risk of adverse cardiovascular events (Thayer, Yamamoto and Brosschot, 2010). Decreased HRV following exposure to ambient air pollution, as reflected by a decreased standard deviation of the time interval between heart contractions (SDNN), has been observed in multiple studies using ambulatory measures (Weichenthal, 2012; Weichenthal et al., 2014; Cole-Hunter et al., 2016, 2018; U.S. EPA, 2019). Cardiac repolarization can be measured to indicate short-term changes in the risk of cardiovascular-related disorders (Dekker et al., 2004). For example, ventricular repolarization (T-wave complexity) informs on the current state of the myocardium, and changes reflect vulnerability for cardiovascular morbidity and mortality (Zareba, Nomura and Couderc, 2001). Changes in cardiac repolarization (most commonly prolongation of heart rate-corrected QT interval, QTc) have been associated with short-term (hours to days) exposure to PM_{2.5} in ambient (observational) (Baja et al., 2010; Liao et al., 2010; Xu et al., 2019) and concentrated (controlled) (Devlin et al., 2014) atmospheres.

Field-based observational studies evaluating the health effects of exposure to household air pollution may be limited due to a number of factors, such as potential confounding by a subject's ability to afford a lower-emissions stove. While controlled exposure studies do not necessarily represent real-world conditions, they may complement field studies in that the exposure event may be isolated and more carefully controlled (as a 'treatment'). There are remarkably few controlled exposure studies for solid-fuel combustion emissions, and fewer on cardiac outcomes. One such study of diluted wood smoke (among healthy, non-smoking participants) clearly showed immediate reductions in HRV (e.g., SDNN) up to the end of the 1-h post-exposure period following treatment (2-h PM_{2.5} exposure concentration mean: 314

$\mu\text{g}/\text{m}^3$) (Unosson et al., 2013). Meanwhile, another similar study suggested no such short-term (immediate) changes in other HRV metrics either immediately or at 20 h following treatment (3-h $\text{PM}_{2.5}$ exposure concentration mean: $485 \mu\text{g}/\text{m}^3$), compared to control (Ghio et al., 2012).

Accordingly, the objective of the analysis presented here was to investigate short-term effects on cardiac function of healthy human adults following controlled exposure to air pollution emitted from various cookstove technologies. This analysis is part of the larger ‘STOVES’ (*Sub-clinical Tests On Volunteers Exposed to Smoke*) study. Importantly, cardiac function was not the primary outcome in this study, meaning that the study was not specifically designed (powered) to investigate a concentration–response relationship across cookstoves and this analysis is exploratory rather than addressing a predefined hypothesis.

2. Methods

The STOVES study methods and procedures have been published previously (Fedak et al., 2019, 2020; Walker et al., 2020b); however, below is a brief overview of those methods and the introduction of specific methods for this analysis.

2.1. Eligibility criteria

Young, healthy, never-smoker adults (18–35 years) of a normal to overweight body mass index ($19\text{--}28 \text{ kg}/\text{m}^2$) and weight greater than 49 kg (110 lb) were invited to participate in this study. A recruitment questionnaire was administered by study staff interview to determine participant health and eligibility status, followed by a physical exam conducted by a study physician at screening.

The following exclusion criteria were applied: prolonged exposure to commute-/traffic-related air pollution (defined by living outside of 20 miles [$\sim 32 \text{ km}$] from study location); regular exposure to smoke, dust, fumes, solvents, or regularly burned candles or incense within the last 3 months; history of heart disease, diabetes, or any chronic inflammatory or respiratory disease (such as asthma, arthritis, or severe allergies); ear or abdominal/thoracic surgery in the last month; spirometry value $<70\%$ of predicted value for age/sex; abnormal complete blood count, comprehensive metabolic panel, or lipid panel; diagnosis of kidney disease, systemic sclerosis, or recent diagnosis of cancer (i.e., current disease or have been in remission for <6 months); central intravenous line or port, or pacemaker; had a mastectomy; pregnancy, be breast-feeding, or planning a pregnancy within the next 6 months; regular intake of statins, anti-inflammatory medication, or certain other medications (as determined by study cardiologist); use of certain recreational drugs (including methamphetamines, cocaine, tetrahydrocannabinol, barbiturates, phencyclidine, amphetamines, opiates) within the last 3 months; history of claustrophobia; or, allergy to latex.

2.2. Study design

As described previously (Fedak et al., 2019), we used a cross-over repeated-measures design to evaluate five different cookstoves (‘treatments’), plus one filtered air treatment as a control, for their impact on HRV and repolarization. The different cookstoves were selected

to represent widely available technologies that span a broad range of pollutant emissions. As previously reported, a sample size of 48 participants was chosen based primarily on statistical power calculations to detect changes in cardiovascular outcomes (Fedak et al., 2019). We enhanced statistical power by setting target PM_{2.5} exposure concentration ranges to be narrow and not over-lapping between different cookstove treatments.

Potential confounding factors were controlled for by study design (age, sex, temporally-varying factors) and by study protocol; participants served as their own control in analyses. As per protocol, participants were instructed to maintain a diet restricted to low-fat and low-cholesterol food for the 48-h period around each treatment (from the 24-h period prior to treatment until the end of the 24-h period following treatment). To facilitate this, consistent nourishment (i.e., diet-restricted lunch and snacks, and isotonic drinks, along with water) were provided to participants at the study site. For this same period, participants were instructed to abstain from all medications and dietary supplements (unless approved by the study physician). Further, for the 24-h period prior to treatment through the end of the 24-h follow up measure, participants were instructed to abstain from high-intensity exercise, alcohol, and caffeine. Deviations from this protocol were reported in the self-administered follow-up questionnaire (see Questionnaires, below).

All study procedures were approved by the Institutional Review Board of Colorado State University. Written informed consent was obtained from all study participants.

2.3. Study location

The study was conducted at the Powerhouse Energy Campus building of Colorado State University, Fort Collins, Colorado, USA. Treatments were administered in a Simulated Environmental Testing (SET) facility (see Controlled exposure treatments, below). The study schedule period ran from October 2016 to January 2018, with three “rounds” of participants (Round 1: October 2016 to April 2017; Round 2: April 2017 to August 2017; Round 3: August 2017 to January 2018) largely aligned to university semesters and covering the seasons of autumn/fall, spring, and summer.

2.4. Study session protocol

Study participants completed six treatment sessions involving a 7-h day including a 2-h treatment period. During the entire 7 h, participants were asked to avoid physical exertion (e.g., by using study building elevator instead of stairs to travel between floors). Cardiac function was measured at three time-points: immediately before, immediately after, and 3 h after this treatment period. Other health endpoints were measured at these time-points, and also at approximately 24 h post treatment (Fedak et al., 2019, 2020; Walker et al., 2020b). A total of four participants were scheduled to start participation on the same day of week and started at 30 min intervals, either 0730 h, 0800 h, 0830 h or 0900 h (maintained across sessions; e.g., Participant A participated in six sessions always starting on a Monday at 0800 h), with at least 2 weeks between sessions for a given participant. The six unique treatment orders (sequences) are deterministic and follow the Williams square design (Williams, 1949) – see Fig. 1 of Walker et al. (2020a, 2020b) for treatment sequences. In brief, the Williams square is a specialized balanced Latin square crossover. Each participant receives each of the

six treatments. Individuals are randomly assigned to one of the six sequences. If a session was missed by a participant due to illness or unforeseen circumstances, that session (treatment type, 'make-up' session) was attempted at the end of each round.

A trained technician monitored PM_{2.5}, carbon monoxide, and oxygen concentrations, along with temperature and atmospheric pressure within the SET facility during operation for treatment periods. Real-time feedback of the SET facility system allowed levels of PM_{2.5}, the exposure parameter of interest, to be maintained within pre-determined target ranges for each treatment.

Cookstove air pollution was diluted with HEPA (high-efficiency particulate air)-filtered air and the resultant mixture was drawn into the SET facility exposure chamber. The volumetric rate of flow for these airstreams was modulated (control system adjusted) to maintain PM_{2.5} target levels within the SET. PM_{2.5} was measured at 1-s intervals to avoid deviations greater than 5% from target as a 3-min rolling average throughout the treatment period. In the case of elevated concentrations, more filtered air was delivered, and vice versa. This dynamic delivery system was designed to provide similar levels of noise, relative humidity, and temperature (potential confounding variables) across treatments.

2.5. Controlled exposure treatments

Five different cookstove technologies were chosen to represent a range of emission levels that may be experienced with cookstove use in a real-world environment (World Health Organization, 2014; Pope et al., 2017). Cookstove emissions were administered as treatments within the SET facility housing up to four participants simultaneously, sitting at individual corners, in a main area of 2.7 m high by 3.5 m wide and 2.8 m long. The chamber main area had an anteroom to allow participant egress/ingress without affecting treatment concentrations. The chamber was also connected via piping to a fume hood which collected cookstove emissions and diluted PM_{2.5} concentrations to a target treatment range. Participants were assigned to treatment order at random.

A filtered-air treatment was administered as a control (targeted at 0 µg/m³ of PM_{2.5}). The cookstoves, in order of increasing target PM_{2.5} exposure concentration representing respective technologies, were: liquefied petroleum gas (LPG), 10 µg/m³; gasifier, 35 µg/m³; fan rocket, 100 µg/m³; rocket elbow, 250 µg/m³, and; three-stone fire, 500 µg/m³. Target exposure concentrations were based a Monte-Carlo emissions/dispersion model (L'Orange et al., 2015) that utilized published emission factors for each stove-type (Jetter et al., 2012; L'Orange et al., 2012). Stoves were selected so that emission levels would be distinct and resultant exposure levels would span the range of WHO Interim Target Levels for household air pollution (World Health Organization, 2014). Emissions concentrations seen in homes for a given cookstove technology can vary greatly and depend on factors such as stove operation, home size, and air exchange rate. The target concentrations used in this study were set to represent levels that could realistically be found in homes using a given technology. Initial concentration ranges were estimated using emissions rates (Jetter et al., 2012; Bilsback et al., 2019) and a box-model approach to estimate concentration (L'Orange et al., 2015; Johnson et al., 2011). Target setpoints were then selected to fall within (or close to within) the estimations resulting from the box model. Continuous PM_{2.5} concentration

was monitored in the SET using an aerosol monitor (DustTrak DRX 8533, TSI Incorporated, USA) that was calibrated to gravimetric PM_{2.5} filter samples collected for the full duration of each treatment (Pallflex Fiber Film T60A20; sample flow rate of 6 L/min using a Leland Legacy [SKC Inc, Eighty Four PA] sampling pump with integrated flow control). Additionally, concentrations of carbon monoxide and oxygen were measured continuously using nondispersive infrared spectrometry (Ultramat 23, Siemens AG, Munich, Germany) and paramagnetic analysis (Ultramat/Oxymat 6, Siemens AG, Munich, Germany), respectively. Background levels of gases were determined by monitoring in the same way for at least 5 min prior to conducting the treatment component of the study. Gas samples were drawn from a wall port and through a HEPA-filter. All continuous air quality measurements were made at a frequency of 1 s and subsequently averaged to a 3-min period. Additional characterization of the pollutant emissions, by stove treatment, was conducted outside of the participant treatment sessions; results for these characterizations are reported in the supplemental material of a related publication (Fedak et al., 2019).

Ambient pollution and meteorological information for Fort Collins were collected from regional monitors. For the 24-h period prior to each treatment, hourly measures of PM_{2.5} were downloaded from the U.S. Environmental Protection Agency's Air Quality Data API (U.S. Environmental Protection Agency, 2018); meteorological data (relative humidity, temperature) were obtained from the Colorado State University Atmospheric Science Department's Christman Field Weather Station (Colorado State University, 2018).

2.6. Measures of short-term differences in cardiac function

To monitor cardiac function (electrocardiography; ECG), an ambulatory 12-lead digital Holter recorder (H12+, Mortara Instruments Inc., Milwaukee, WI, USA) approved by the US Food and Drug Administration (US FDA, 2005) was applied to participants by a trained technician at the beginning of each study day. Participants were asked to remove hair prior to arriving, however a shaver was provided for this purpose. The skin receiving Holter lead electrodes were further prepped (cleaned of oils and dead skin cells) by an alcohol wipe and abrasive paste. Individual electrode signal strength was checked prior to initiating Holter recording. The Holter recorder stored raw continuous data (sampled at 1000 Hz) on a high-frequency memory card.

While the Holter recorded cardiac function continuously, measurement periods at each of the 3 time-points lasted 5 min (marked by study staff pushing the Holter 'event' button) and followed a 10-min rest period all while participants laid supine. The Holter was removed following measurement at the 3-h after-treatment time-point.

Raw continuous data were extracted from Holter memory cards and a full suite of cardiac function parameters were calculated by a trained research nurse using Holter manufacturer software (Mortara Instruments Inc.). The following quality-control procedure steps were implemented: (1) the trained research nurse, blinded to the exposure randomization, manually edited the sequence of ECG complexes to ensure proper labeling of each QRS complex and to check that ECG traces correspond to biologically plausible gross observations of the participant; (2) the measurement time-points recorded by the internal clock of the device (pushing the Holter 'event' button) were cross-referenced and verified by

the technician-noted time recorded at the start and end of exposure, and the start and end of measurement time-points, and; (3) all parameters were plotted to determine sample distribution and existence of measurement outliers.

Prepared HRV metrics include both frequency- and time-domain measures. Frequency-domain measures are VLF (very-low frequency: 0.0033–0.04 Hz), LF (low frequency: 0.04–0.15 Hz), and HF (high frequency: 0.15–0.4 Hz) power, and the ratio of LF to HF (LFHFR). Time-domain measures are SDNN (standard deviation of the duration of all NN intervals), pNN50 (percent of consecutive NN intervals that differ by more than 50 ms), and RMSSD (square root of the mean of the squares of the differences between adjacent NN intervals). Prepared cardiac repolarization metrics include P-complexity (representing P-wave/atrial depolarization), QRS-complexity (representing QRS-wave/ventricular depolarization), T-complexity (representing T-wave/ventricular repolarization), and QTc (average length of QT interval, the period from start of QRS-wave to end of T-wave, corrected for heart rate by dividing by the square root of RR interval).

2.7. Questionnaires

To capture information on potential confounders, participants self-administered the following questionnaires: (1) a questionnaire collecting time-invariant information on participant demographic characteristics (e.g., race, ethnicity) once during the study; (2) a questionnaire collecting information on the preceding 24 h, including acute cardiac/allergy symptoms (as a potential health response, and for potential acute care concerns for an adverse response to the treatment), alcohol and caffeine consumption, diet, exposures to smoke and other air pollution, medication use, physical activity, sleep duration, and their mode of travel to the study facility; (3) a questionnaire collecting information on the preceding few hours (between time-points), including behavior (e.g., caffeine consumption), at each of the three time-points on each study day (alongside cardiac function measurements).

2.8. Statistical analysis

All statistical analyses were performed in R (Version 3.5.0, The R Foundation for Statistical Computing). Descriptive statistics for treatment and health outcome parameters were mean, standard deviation, minimum, and maximum values.

All cardiac function measurements were analyzed as 5-min averages, guided by previous work (Baja et al., 2010; Shields et al., 2013; Cole-Hunter et al., 2016, 2018). LF and HF power were normalized for heart rate (i.e., converted to LFn and HF_n, as normalized units [n.u.] respectively) according to previous work (Voss et al., 2015). Model residuals were evaluated for normality, heteroscedasticity, and potential outliers (i.e., meeting assumptions for linear models, to determine if transformations were needed) by diagnostic plots (i.e., QQ and residuals vs fitted values). Prior to modelling, all measurement parameters were transformed using the natural logarithm (presented as “percent difference from control”), except for pNN50, which was square-root transformed due to the presence of zero values (presented as “difference from control”).

Linear mixed-effect models (*lmer* function in R) were used to compare cardiac function measurements between exposure and control treatments per time-point (Bates et al., 2015). The primary models contained a fixed categorical term for cookstove treatment type (rather than a continuous term for PM_{2.5} emission/exposure concentration), a fixed continuous term for baseline (immediately before treatment) measurement value, a random intercept for participant, and a random intercept for date of the controlled exposure. We included the baseline term to account for variations in the outcomes between treatment levels at the beginning of each study day (i.e., variations unrelated to the controlled exposures) (Vickers and Altman, 2001). We included the term for participant to account for repeated measures within each participant. We included the term for date to account for correlation that may occur between participants who were part of the same study session. A sensitivity analysis was performed on a subset of the dataset, including only participants who completed their treatments as originally scheduled (i.e., ‘in-sequence’); models in these analyses included terms for sequence and session number.

Ambient PM_{2.5}, relative humidity, and temperature values over the prior 24 h were calculated as mean, standard deviation, and range (minimum, maximum), and compared across treatments by Kruskal-Wallis rank sum tests. PM_{2.5} was the only ambient pollutant investigated as it was the only treatment pollutant measured, and only for the prior 24 h as this coincides with other potential confounder information collected (e.g., caffeine consumption, physical activity).

In addition, we conducted sensitivity analyses to assess for potential confounding separately by ambient concentrations of PM_{2.5}, ambient relative humidity, and ambient temperature (averaged for the 24-hr period prior to treatment) as untransformed covariates added to the primary model. Univariate analyses by treatment were previously conducted to determine self-reported frequency of participant non-compliance with restricted use of alcohol, caffeine and medication, as well as self-reported mode of transport to study facility and sleep quantity (Fedak et al., 2019).

Beyond LFn and HF_n (normalizing LF and HF for heart rate), models were not adjusted for heart rate nor mean arterial blood pressure since Holter recording occurred while the participant was at rest (laying supine). Moreover, with the exception of LFn and HF_n, adjusting HRV for heart rate may remove meaningful variance that can be attributable to autonomic and neurophysiological phenomena (de Geus et al., 2019).

3. Results

3.1. Participant sample

In total, 48 participants (22 females, 26 males) were included in the analyses due to satisfying inclusion criteria and participating in at least two of the six scheduled treatments. The mean body mass index was 23.4 kg/m² (standard deviation: 2.2 kg/m²; range: 19.4–28.7 kg/m²), and the mean age was 27.5 years (3.6 years; 20.5–36.1 years) at onset of participation. Baseline (pre-treatment) levels of cardiac autonomic activity parameters, presented in Table 1, were within a normal, healthy range (Agelink et al., 2001).

Including allotted make-up sessions, all 6 controlled exposures (treatments) were completed by 39 (81% of total) participants, while at least 5 treatments were completed by 45 (94% of total) participants. Among the 288 participant-treatments that were scheduled (48 participants \times 6 treatments), 272 (94%) were included in the primary analysis; 261 (91%) were included in the secondary (in-sequence sensitivity) analysis. Fifteen participant-treatments were missed due to either known but unavoidable scheduling conflicts or unforeseen circumstances (e.g., acute illness) and an inability to make up sessions within the allotted scheduling period. Additionally, one participant-treatment was not included due to a recording error on the Holter device. Within the 816 (272 participant-treatments \times 3 time-points) possible data points, 5 are missing due to scheduling conflicts for participants (resulting in them leaving a study day without completing the three-hour follow-up time-point). This results in 811 (94% of a possible 864 [48 participants \times 6 treatments \times 3 time-points]) data points included in analyses.

3.2. Treatment conditions

Mean exposure levels (see Table 2) across the cookstove technologies tested ranged from 462 $\mu\text{g}/\text{m}^3$ for the three stone fire (interquartile range, IQR: 76 $\mu\text{g}/\text{m}^3$) to 8 $\mu\text{g}/\text{m}^3$ for the LPG cookstove (IQR: 4 $\mu\text{g}/\text{m}^3$); the mean exposure for the filtered-air control was 0.5 $\mu\text{g}/\text{m}^3$ (IQR: 0.4 $\mu\text{g}/\text{m}^3$). In general, each cookstove category closely matched (although was typically lower than) the target exposure level, with the largest difference of achieved versus target level occurring for the gasifier (11 $\mu\text{g}/\text{m}^3$; 31% below target) and the smallest difference for the rocket elbow (9 $\mu\text{g}/\text{m}^3$; 3.6% above target).

Within a treatment session, a participant's (corresponding 2-h) interquartile range of real-time (1-s resolution) measurements of $\text{PM}_{2.5}$ exposure indicated that target concentration levels did not vary substantially across the 2-h period, resulting in non-overlapping average exposure concentration ranges between different cookstoves (Table 2).

Mean ambient levels of $\text{PM}_{2.5}$, relative humidity, and temperature in the 24-h period prior to treatments were substantially different for some treatments. For example, mean $\text{PM}_{2.5}$ and temperature for this 24-h period was a few units higher for fan-rocket compared to other treatments (Table 3).

3.3. Short-term differences in cardiac function following cookstove treatments compared to control

3.3.1. Primary model outcomes—Results from our primary models indicate differences in overall HRV (as indicated by SDNN), but not cardiac repolarization, at some time points for some cookstoves. All model outcomes reported below are for cookstove treatments in comparison to control (filtered air) treatment at the same time point.

3.3.1.1. Heart rate variability. All HRV primary model outcomes are presented in Fig. 1, with numerical values provided in Supplemental Table A1.

We observed a general trend for SDNN (as an overall indicator of HRV) to be lower immediately, and higher 3 h, following all cookstove treatments compared to control. The cookstoves eliciting the greatest short-term difference in SDNN following treatment

compared to control, in order of decreasing magnitude of difference, were three stone fire, gasifier, LPG and fan rocket (equally), and rocket elbow. General trends for VLF were similar to those for SDNN, however not for any of the other frequency-domain HRV parameters following cookstove compared to control. See Fig. 1 and Supplemental Table A1. Specifically, we observed lower SDNN immediately following treatment for three stone fire compared to control ($\beta = -0.13\%$, 95% CI = $-0.22, -0.03\%$) and gasifier compared to control ($\beta = -0.12\%$, 95% CI = $-0.22, -0.02\%$).

We observed higher SDNN at 3 h following treatment for LPG compared to control ($\beta = 0.14\%$, 95% CI = $0.04, 0.23\%$) and rocket elbow compared to control ($\beta = 0.15\%$, 95% CI = $0.05, 0.24\%$). We also observed higher RMSSD ($\beta = 0.15\%$, 95% CI = $0.03, 0.27\%$) and pNN50 ($\beta = 4.34\%$, 95% CI = $0.00, 8.69\%$) at 3 h following rocket elbow compared to control. We did not note any differences in other time-domain HRV parameters. In the frequency domain of HRV at 3 h following rocket elbow compared to control, we observed both higher HFn ($\beta = 0.27\%$, 95% CI = $0.04, 0.51\%$) and VLF ($\beta = 0.38\%$, 95% CI = $0.11, 0.64\%$). See Supplemental Table A1 for all frequency-domain primary model results.

3.3.1.2. Cardiac repolarization.: All cardiac repolarization primary model outcome values are presented in Supplemental Table A1.

Overall, we observed little evidence of an association between cookstove treatments and cardiac repolarization. However, we observed QTc to be higher at 3 h following rocket elbow treatment compared to control ($\beta = 0.003\%$; 95% CI = $0, 0.007\%$). See Fig. 1 and Supplemental Table A1. P-complexity was higher immediately following gasifier treatment compared to control ($\beta = 0.10\%$; 95% CI = $0.03, 0.17\%$). T-complexity was also higher immediately following LPG treatment compared to control ($\beta = 0.06\%$; 95% CI = $0.01, 0.12\%$). Other cardiac repolarization endpoints were not different among cookstove treatments compared to control.

3.3.2. Sensitivity analyses—We did not observe evidence of differences compared to the primary results when adjusting for the 24-h (pre-treatment) mean levels of ambient PM_{2.5}, temperature, or humidity (see Supplemental material text and Tables A2, A3 and A4, respectively). Some notable differences were (at either 0 or 3 h) following fan rocket and rocket elbow treatments, for example: SDNN 3 h following fan rocket treatment compared to control when adjusted for ambient PM_{2.5} ($\beta = 0.08\%$; $-0.04, 0.19\%$) versus unadjusted (primary model) ($\beta = 0.10\%$; $0.00, 0.20\%$); SDNN immediately following fan rocket treatment compared to control when adjusted for ambient temperature (-0.12% ; $-0.22, -0.01\%$) versus unadjusted (primary model) (-0.07% ; $-0.17, 0.03\%$). See Supplemental Tables A1–A3.

Further, we did not observe evidence of differences when analyses were performed on a subset of the dataset containing only those participants who completed their schedule in sequence, including terms for sequence and session number: the biggest difference was seen for VLF 3 h following three-stone fire treatment compared to control among the subset (0.28% ; $0.02, 0.53\%$) versus the full data set (0.22% ; $-0.03, 0.48\%$) (see Supplemental Table A1 and A5).

4. Discussion

We observed evidence of short-term differences in some metrics of cardiac function following short-term exposures to cookstove air pollution compared to filtered air among healthy adults. We observed these short-term differences across the suite of cookstove types studied, with the differences not following an increasing monotonic concentration–response for fine particulate matter (PM_{2.5}). The results suggest that stoves emitting relatively low amounts of PM_{2.5} could still be capable of producing short-term changes in some HRV metrics, similar to the effect from stoves emitting higher amounts of PM_{2.5}. In general, the strongest evidence of a short-term difference occurred in the HRV time-domain parameter of SDNN (which is a metric indicative of overall HRV) and the HRV frequency-domain parameter of VLF. Across cookstoves, the strongest effect on cardiac function occurred following the rocket elbow cookstove, which was the second highest categorical PM_{2.5} exposure concentration (250 µg/m³). We observed differences in the direction and magnitude of change for parameters between time-points (from immediate to 3-h following exposure).

A recent meta-analysis of two controlled human exposure studies and two epidemiological panel studies found consistent evidence of a short-term pathophysiological (HRV) response (<6 h) following short-term exposures to ambient-sourced PM_{2.5} and UFP (Breitner et al., 2019). Similar to our results, they found responses varied across time-points – direction of response was not consistent and magnitude was not monotonic. While the reviewed studies found a general decrease and recovery (increase) in SDNN, two studies (‘REHAB’, ‘UPDIABETES’) saw the same general decrease immediately (first hour) following exposure; the remaining two studies (‘Augsburg Panel’, ‘UPCON’) found the opposite (Breitner et al., 2019). Like our study, none of the evaluated studies found evidence of changes in RMSSD or T-complexity within the first few hours following exposure.

A chronic lowering of HRV indicates an imbalance in cardiac autonomic function and an increased risk of adverse cardiovascular events including myocardial infarction and stroke (Thayer et al., 2010). The higher SDNN at 3 h following treatment (compared to control) across three of the five cookstove types suggests a short-term recovery (return to baseline value) or compensation (increase above baseline value) in cardiac autonomic function following exposure, as seen in similar previous analyses among healthy individuals (Magari et al., 2002; Wu et al., 2011; Huang et al., 2013) and coronary artery disease patients (Zanobetti et al., 2010).

Specifically, while Magari et al. (2002) report decreased SDNN up to a PM_{2.5} moving average period of 3 h, a recovery and eventual compensation was seen from the 4-h to 9-h period. Similarly, while statistically insignificant, Zanobetti et al. (2010) report decreased SDNN up to a PM_{2.5} moving average of 2 h, and compensation by the (following) 48-h period. Huang et al. (2013) also report decreased but recovered SDNN up to a (maximum) 1-h moving average period, possibly too brief to see compensation. However, Wu et al. (2011) report some reduction in SDNN and other HRV parameters across increasing moving average periods (from 0.5 to 4 h), however less sign of recovery and no compensation.

Some studies have shown only short-term increases (without first a decrease) in SDNN following PM exposure (Riediker et al., 2004; Shields et al., 2013), such as within 30 min (Shields et al., 2013) suggesting that cardiac autonomic activity may be affected immediately upon sensing PM deposition in the lower respiratory system (Rhoden et al., 2005).

Interestingly, Wu et al.'s (2010) study of real-time traffic-related PM_{2.5} exposure showed that short-term (30-min) exposures at lower concentrations (<50 µg/m³) increased SDNN, while higher concentrations decreased SDNN. Most of our (cookstove-emitted) PM_{2.5} exposure concentrations were greater than this reported turning point; however, we still observed decreased SDNN following our lowest categorical concentration (10 µg/m³), potentially explained by our longer (2-h) exposure period and therefore higher accumulated PM_{2.5} dose. Our observation suggests that effects on HRV, sometimes in opposite directions, may be explained now only by timing of exposure in relation to outcome but also by a non-monotonic exposure-response relationship (discussed below).

Observing differences for SDNN and VLF, while not other HRV metrics, following exposure may make sense as VLF is highly correlated with SDNN and is predominately modulated by parasympathetic activity through the baroreceptor reflex (Shaffer and Ginsberg, 2017). VLF is more strongly associated with all-cause mortality than other frequency domain measures (LF, HF), and low VLF has been associated with increased inflammation (physiological stress response) and cardiovascular health risk (Shaffer and Ginsberg, 2017). The absence of difference we observed in most of the suite of HRV or cardiac repolarization parameters, however, challenges the interpretation of our findings.

4.1. PM_{2.5} concentration-response

One could hypothesize that the physiological response to cookstove smoke exposure would have a monotonic relationship with PM_{2.5} concentration, in that larger concentrations elicit larger responses. In this study, the highest target concentration was approximately 50 times higher than the lowest target (i.e., 500 versus 10 µg/m³). However, we did not observe a proportionally greater magnitude of cardiac autonomic response following this greater concentration compared to lesser or the lowest concentration treatment. The difference in SDNN we observed immediately after gasifier (PM_{2.5}: 35 µg/m³) compared to control was similar to that observed immediately after three stone fire compared to control, despite the latter treatment having over 10 times the concentration of PM_{2.5}. Similarly, the difference in SDNN we observed immediately after rocket elbow (PM_{2.5}: 250 µg/m³) compared to control was lower in magnitude to that observed immediately after fan rocket (100 µg/m³) compared to control, despite the latter delivering less than half the PM_{2.5} concentration of the former. This non-monotonic concentration–response relationship is consistent with our previous analyses (Fedak et al., 2019, 2020; Walker et al., 2020a, 2020b) and similar to our observations for other time-domain and frequency-domain measures of HRV, as well as cardiac repolarization. Short-term physiological effects may follow a threshold-response whereby any PM_{2.5} concentration level produces a binary response. Such a threshold-response is not suggested by previous studies evaluating short-term exposures (U.S. EPA,

2019). However, most of those studies are observational and not controlled human exposure studies.

4.2. Composition of cookstove emissions profiles

Improved cookstoves (e.g., rocket elbow) may substantially reduce emission concentrations of PM_{2.5} compared to traditional cookstoves (e.g., three stone fire), however other combustion-related emissions such as UFP have been seen to increase (de la Sota et al., 2018). Pollutants besides PM_{2.5} can vary in emission level depending on fuel or cookstove type used (Bilsback et al., 2019). In our previously published results, we see that concentrations of emissions such as carbon monoxide generally increased with cookstove-determined PM_{2.5} target concentrations (Fedak et al., 2019). However, compared to other treatments: ultrafine particle (UFP; particle aerodynamic diameter < 0.1 μm) and carbonyl concentrations were higher for LPG treatment; nitric oxide concentrations were highest for fan rocket treatment, although comparable to rocket elbow treatment, and; elemental carbon concentrations were higher for rocket elbow treatments (Fedak et al., 2019).

Studies on UFP emissions from cookstoves are scarce (Patel et al., 2016), yet UFP are considered an important exposure in addition to PM_{2.5} when studying adverse health effects (Chan et al., 2004; Pan et al., 2018). Our cookstove emission characterization test results do not point to a particle-size differentiation (difference in particle number concentrations, dominated by UFP) between cookstove treatments, despite some cookstoves (fan rocket, rocket elbow, three stone fire) burning the same fuel but at different rates to achieve different target PM_{2.5} concentrations (PM_{2.5}: 100, 250, 500 μg/m³, respectively) (Fedak et al., 2019). However, our characterization measurements saw that the rocket elbow, while emitting half of the concentration of PM_{2.5} compared to three stone fire, emitted more than double the concentration (~100 μg/m³) of elemental carbon as well as the highest concentration of nitrous oxides (NO_x/NO₂) and acetone compared to all cookstoves tested (Fedak et al., 2019).

Higher proportions of combustion-derived elemental carbon and chemical emissions may partly explain our observed stronger differences in effect on several HRV parameters (SDNN, RMSSD, pNN50, HFn, LFn, VLF) following rocket elbow compared to other treatments. Black carbon (measured as elemental carbon) is a component of PM_{2.5} believed to have higher toxicity than total PM_{2.5}, formed by incomplete combustion of solid fuel (Janssen et al., 2011, 2012). Recent evidence suggests that black carbon may adsorb and carry combustion-derived chemicals (such as complex hydrocarbons, carbonyls, including acetone) that interact with and modify the effect of PM mass on various sensitive tissues around the body (Janssen et al., 2011; Cassee et al., 2013; Niranjana and Thakur, 2017; Grady et al., 2018). A recent systematic review of cardiovascular endpoints including HRV stated that there is insufficient evidence to determine a difference in health effect between ambient black carbon and PM_{2.5} exposure (Karrane et al., 2019). Some evidence does exist, however, to suggest a greater effect of black carbon on HRV (Schwartz, 2005) and cardiac repolarization (Baja et al., 2010; Xu et al., 2019). Impaired cardiac function has been associated with short-term exposure to traffic-related NO₂ and NO_x in some studies (Shields et al., 2013; Brook, Newby and Rajagopalan, 2018), but not all (Cole-Hunter et al., 2016,

2018; Laeremans et al., 2018), suggesting further research is needed, particularly with cookstove-related emissions.

4.3. Limitations of our study

Our analysis only considers a single 5-min measurement at two (post-exposure) time-points, immediately following and 3 h following treatment. This limitation does not allow us to capture a complete description of the overall impact of exposure on cardiac function over time, including instantaneous and next-day effects corresponding to exposure. Our selected time-points may not have captured the period in which a full effect, and therefore a clear exposure-response across cookstoves, is seen. An alternative design to be considered for future research would involve an equivalent protocol for participant measurements within the exposure facility, allowing the capture of instantaneous exposure effects. Such a design was not feasible with the available exposure facility, due to the controlled and space-limited area, and consideration of participant comfort (remaining supine). Similarly, we did not capture next-day (approximately 20 h following treatment) effects due to the removal of the Holter at the end of the first study day. This removal was to minimize participant burden and due to the expectation, based on current literature, that effects would be more immediate rather than lagged.

The different target $PM_{2.5}$ concentration-exposure levels were set to be fairly representative of real-world cookstove technology emission/exposure levels that need to be addressed as intervention studies (World Health Organization, 2014; Pope et al., 2017), allowing some transferability of evidence to the field. However, using the same target $PM_{2.5}$ concentration level across treatments would have allowed identification of differences in health effects due to composition of pollution mixtures at the same $PM_{2.5}$ mass concentration and design/technology aspects (e. g., combustion efficiency, construction materials). As it is, both our combustion source (particle characteristics) and particle concentrations vary between experimental conditions, which reduces our ability to clearly interpret our findings compared to studies that examine across the same source or same concentration of particles. While our $PM_{2.5}$ exposure gradient is larger than most studies making similar observations in cardiac function (Magari et al., 2002; Gong et al., 2008; Baja et al., 2010; McCracken et al., 2011; Tong et al., 2012; Shields et al., 2013; Unosson et al., 2013), an inadequate concentration or dose may explain the lack of consistent or notable differences between treatments or at all in some metrics such as QRS-complexity. Our study design was such to discern differences of exposure from different cookstove technologies or fuels (i.e., whole system), reflecting real-world conditions, rather than $PM_{2.5}$ emission concentration alone or a clear exposure-response curve.

Due to the many cookstove technologies and cardiac function outcomes measured at multiple time-points, we must interpret our findings with caution due to multiple comparison tests being conducted. There may have been an issue with statistical power sufficiently capturing effects across the $PM_{2.5}$ exposure gradient. Importantly, we may not have been adequately powered to detect small changes in cardiac function.

Finally, our study population did not consist of real-world cookstove users, nor were measurements made in real-world conditions – participants were screened to exclude regular

air pollution exposures and underlying disease and were treated with intermittent rather than chronic exposure within a controlled environment. Moreover, our strict inclusion criteria, for the purpose of participant safety, limited the range of values for age, body mass index, smoking status, medical history, and other criteria which may modify measured effects. As such, our findings are not necessarily generalizable to real-world populations or environments of cookstove use. Underlying health conditions convey susceptibility for air pollution effects on the cardiac repolarization response (Xu et al., 2019). A similar exposure study design to ours, although focused on ambient UFP, also observed higher QTc and QRS-complexity among adults with metabolic syndrome, although the results were not statistically significant; stratifying the study population by a known genetic susceptibility (GSTM1) showed a greater (and statistically significant) increase in both metrics (Devlin et al., 2014).

4.4. Strengths of our study

To our knowledge, we have conducted the most comprehensive controlled human exposure study of cookstove emissions. We studied a relatively high number of cookstove technologies and cardiac autonomic activity endpoints. Moreover, we obtained a relatively high sample size of healthy adult participants that largely complied with consumption restrictions. We collected information on dietary intake of participants during participation, to note compliance of the requirement to restrict high-fat and high-cholesterol foods, which was satisfactory. The participant self-reported use of alcohol, caffeine, and medication was low throughout the study; bivariate analyses indicated no evidence of associations between these or other potentially confounding covariates (e.g., ambient PM_{2.5} and CO) and the various treatments (see Fedak et al., 2019 Data S2 and Tables S2–S11). Findings from controlled studies are more robust by the minimization of (or selection for) participant confounders (e.g., age, co-morbidities) and complement less internally valid observational studies of real-world use(r)s.

The main strength of our study comes from its design, being controlled by a specialized Latin (William's) square structure for maintaining participant procession within a group of four, consistently (pending participant availability) across study dates and treatments. This design balanced treatments and first-order carryover effects, minimizing potential confounding associated with time-invariant factors at the personal level with each participant receiving each treatment and time-variant factors (e.g., age, ambient conditions, caffeine or alcohol consumption) and study experience (comfort), and also seasonal variation in ambient conditions.

Further still, participants themselves were blinded to the order of exposure to minimize bias in perception of treatment and a potential stress response, in anticipation of higher exposures, reflected in cardiac autonomic activity.

Finally, the administration of our treatments was controlled to maintain pre-determined (target) PM_{2.5} concentration levels. This control allowed a categorical analysis of cookstove type to augment evidence for real-world health implications when and where that cookstove type is used. This control/analysis was made possible by the presence of a trained technician and an automated modulatory exposure-delivery system not possible in a field study. Our

controlled design avoided large fluctuations of exposure/emissions concentrations to help interpret our health responses, which is a major challenge in field studies due to the nature of cookstoves and combustion of solid fuel producing such fluctuations.

4.5. Conclusion

We find evidence to suggest that a short-term adverse difference in cardiac function, specifically in HRV (SDNN and VLF), can occur immediately following short-term exposures to even low or “improved” levels of cookstove-emitted PM_{2.5} among healthy adults. While the magnitude of our effect sizes was small, our findings point to a mechanism that may explain part of the public health burden from the global use of cookstoves. Exposure to household air pollution from cookstove emissions, including PM_{2.5}, contributes substantially to global air pollution-attributable deaths; growing evidence supports that long-term exposure to PM_{2.5} magnifies cardiovascular health risk by triggering short-term cardiovascular dysfunction (Brook et al., 2018).

Future work could address two principal challenges highlighted by our study. First, there is a need to assess associations across longer exposure lags due to opposing effects at different lags, by following participants with Holter monitors applied over a 24-h period. Secondly, there is a need to investigate further the issue of a non-monotonic exposure-response that may be confounded by co-pollutants, by varying exposure levels using the same cookstove/fuel. These designs could be assisted by bridging consistencies across field studies of cookstove emissions, as well as both observational and experimental studies of ambient air pollution exposure (e.g., considering exposure windows of shorter or longer duration).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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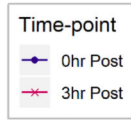
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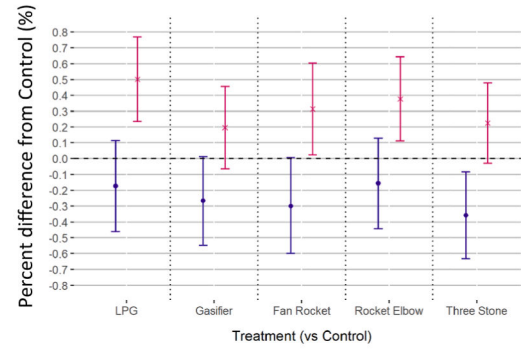
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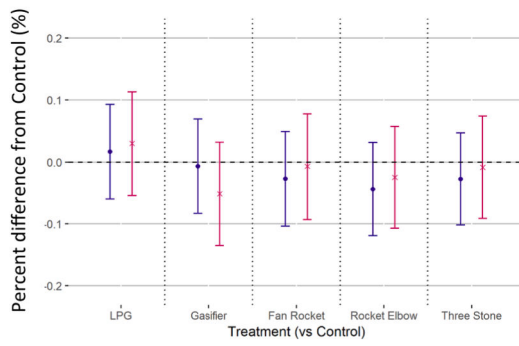
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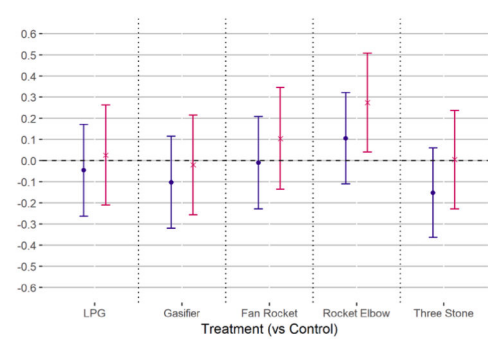
(a) VLF



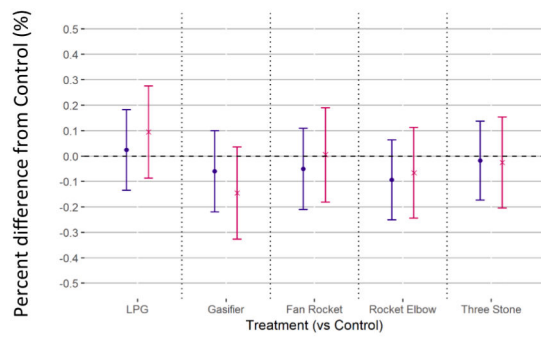
(b) LFn



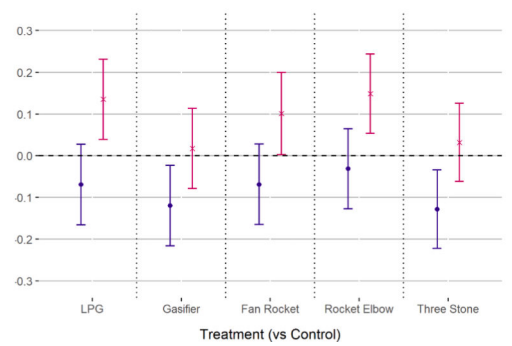
(c) HFN



(d) LFHFR



(e) SDNN



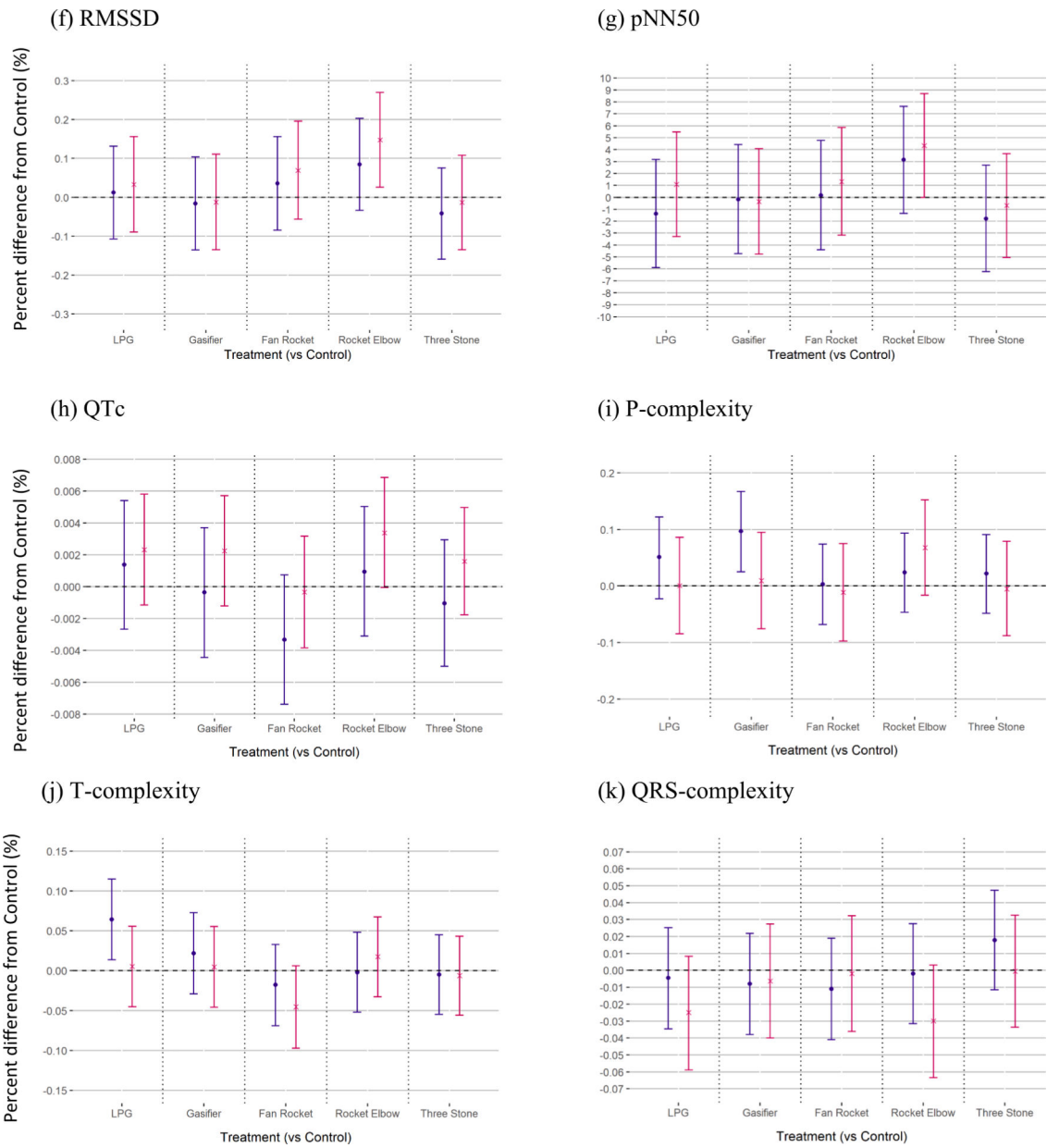


Fig. 1. Short-term differences in cardiac function following short-term exposure to cookstove air pollution compared to control.

Table 1

Baseline (pre-treatment) five-minute means of heart rate variability and cardiac repolarization.

Domain	Parameter	Unit	Base		0hr		3hr	
			n ^a	value*	n ^a	value*	n ^a	value*
HRV, Freq.	VLF	ms ²	253	2829 ± 3372 (65, 26186)	253	4214 ± 5976 (208, 49720)	253	3386 ± 4710 (164, 40928)
	LFn	n.u.	272	0.61 ± 0.17 (0.09, 0.90)	272	0.57 ± 0.17 (0.11, 0.89)	272	0.60 ± 0.17 (0.13, 0.89)
	HFn	n.u.	272	0.39 ± 0.17 (0.10, 0.91)	272	0.43 ± 0.17 (0.11, 0.89)	272	0.40 ± 0.17 (0.11, 0.87)
HRV, Time	LFHFR	1:1	272	2.13 ± 1.61 (0.10, 8.80)	272	1.73 ± 1.25 (0.12, 8.38)	272	2.07 ± 1.49 (0.15, 8.02)
	SDNN	ms	272	78.1 ± 38.3 (15, 265)	272	92.3 ± 43.8 (24, 270)	272	82.0 ± 38.7 (23, 243)
	RMSSD	ms	272	61.3 ± 43.8 (9, 280)	272	78.3 ± 47.6 (16, 285)	272	63.6 ± 40.8 (14, 267)
Cardiac repolar:	pNN50	%	272	28.2 ± 22.3 (0, 74)	272	37.3 ± 22.4 (0, 79)	272	30.5 ± 22.9 (0, 80)
	QTc	ms	272	417.3 ± 19.7 (376.0, 471.1)	272	416.3 ± 20.6 (378.5, 482.2)	272	418.2 ± 20.2 (379.4, 482.9)
	P-comp.	(unitless)	272	0.20 ± 0.10 (0.07, 0.73)	272	0.02 ± 0.11 (0.04, 0.78)	272	0.20 ± 0.12 (0.07, 0.82)
	QRS-comp.	(unitless)	272	0.45 ± 0.18 (0.08, 1.05)	272	0.46 ± 0.18 (0.09, 1.01)	272	0.45 ± 0.18 (0.09, 1.17)
	T-comp.	(unitless)	272	0.12 ± 0.05 (0.01, 0.38)	272	0.11 ± 0.05 (0.02, 0.30)	272	0.12 ± 0.05 (0.02, 0.33)

Abbreviations: S.D. = Standard deviation; n.u. = normalized units; VLF = Very low frequency (Power in range 0.0033–0.04 Hz); LFn = Low frequency (Power in range 0.04–0.15 Hz), corrected for heart rate; HFn = High frequency (Power in HF range 0.15–0.4 Hz), corrected for heart rate; LFHFR = Ratio of LF over HF; SDNN = Standard deviation of the duration of all NN intervals; RMSSD = Square root of the mean of the squares of the differences between adjacent NN intervals; pNN50 = Percent of consecutive NN intervals that differ by more than 50 ms; QTc = Interval of time between Q and T interval, corrected for heart rate; P-comp. = P-complexity (representing P-wave/atrial depolarization); QRS-comp. = QRS-complexity (representing QRS-wave/ventricular depolarization); T-comp. = T-complexity (representing T-wave/ventricular repolarization).

^aThe sample size (n) shown here is discrepant from the 288 expected (of 48 participants completing 6 treatments); while a total of 48 participants were included in analyses, not all participants could complete all treatments or had data available for all time-points – some treatments or time-points were missed due to participant absence or technical difficulties and the inability to reschedule repeats within the study period.

* Values are presented as mean (standard deviation [minimum..maximum]).

Table 2

Exposure concentration two-hour means of PM_{2.5} (and corresponding CO) according to treatment target levels, and differences from target: group time-weighted means (of 1-s measurements), and their associated (absolute and percentage) variations from target.

Treatment type	Number of Treatments ^a	Target Level, $\mu\text{g}/\text{m}^3$	Achieved Mean Level [IQR], $\mu\text{g}/\text{m}^3$	Difference of Achieved versus Target Level [IQR], $\mu\text{g}/\text{m}^3$ (%)	Corresponding Mean [SD] of CO* (ppm)
Control	47	0	0.5 [0.4]	0.9 [0.4] (NA)	2 [2]
LPG	45	10	8 [4]	3 [3] (- 26.8%)	3 [1]
Gasifier	43	35	46 [9]	11 [10] (+30.8%)	5 [3]
Fan rocket	44	100	95 [14]	11 [13] (- 10.9%)	8 [2]
Rocket elbow	45	250	255 [24]	9 [17] (+3.6%)	6 [2]
Three stone fire	47	500	462 [76]	42 [60] (- 8.5%)	9 [4]

Abbreviations: CO, carbon monoxide; IQR, interquartile range; LPG, liquefied petroleum gas; n, sample size; NA, not applicable.

^aNot all participants could complete all treatments – some treatments were missed due to participant absence or technical difficulties and the inability to reschedule repeats within the study period.

* Mean (SD); CO did not have a target concentration. This row is showing the measured mean CO concentration at each exposure level.

Table 3

Mean ambient conditions for the 24-h prior to treatment.

Treatment	Number of Days	PM _{2.5} , µg/m ³	Temperature, °C	Relative Humidity, %
All	81	7 ± 4 (1, 19)	10 ± 7 (- 8, 24)	49 ± 14 (23, 81)
Control	15	5 ± 3 (2, 13)	7 ± 6 (- 7, 20)	56 ± 13 (27, 80)
LPG	13	8 ± 5 (3, 19)	11 ± 5 (3, 24)	47 ± 11 (29, 67)
Gasifier	13	5 ± 3 (1, 11)	7 ± 7 (- 6, 14)	50 ± 12 (32, 69)
Fan rocket	14	9 ± 4 (2, 18) **	16 ± 6 ** (5, 23)	42 ± 13 (23, 69) **
Rocket elbow	14	6 ± 2 (3, 9)	13 ± 10 (- 8, 24)	44 ± 13 (27, 67)
Three stone fire	12	6 ± 3 (1, 12)	7 ± 5 (- 3, 15)	56 ± 14 (40, 81)

Ambient parameter values are presented as Mean ± S.D. (Minimum, Maximum). Abbreviations: S.D. = standard deviation; PM_{2.5} = fine particulate matter (particles of <2.5 µm diameter); LPG = liquified petroleum gas.

Kruskal-Wallis rank sum testing outcome; significantly different value compared to values of other treatments:

*
p < 0.05

**
p < 0.01.