Original Article

# Household Fuel Use and Biomarkers of Inflammation and Respiratory Illness among Rural South African Women

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**Running Title:** Household fuel use and biomarkers of inflammation

**Abstract**

Though literature suggests a positive association between use of biomass fuel for cooking and inflammation, few studies among women in rural South Africa exist. We included 415 women from the South African Study of Women and Babies (SOWB), recruited from 2010-2011. We obtained demographics, general medical history and usual source of cooking fuel (wood, electricity) via baseline questionnaire. A nurse obtained height, weight, blood pressure, and blood samples. We measured plasma concentrations of a suite of inflammatory markers (e.g., interleukins, tumor necrosis factor-α, C-reactive protein). We assessed associations between cooking fuel and biomarkers of inflammation and respiratory symptoms/illness using crude and adjusted linear and logistic regression models. We found little evidence of an association between fuel-use and biomarkers of inflammation, pre-hypertension/hypertension, or respiratory illnesses. Though imprecise, we found 41% (95% confidence interval (CI)=0.72-2.77) higher odds of self-reported wheezing/chest tightness among wood-users compared with electricity-users. Though studies among other populations report positive findings between biomass fuel use and inflammation, it is possible that women in the present study experience lower exposures to household air pollution given the cleaner burning nature of wood compared with other biomass fuels (e.g., coal, dung).

**Key Words:** biomass, household fuel, biomarkers of inflammation, respiratory

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## Highlights

- Few studies assessing the relationship in women from rural South Africa.
- Little evidence of association between wood fuel and inflammatory markers.
- Limited evidence of relation of wood use with respiratory symptoms or hypertension.
- Possible lower household air pollution exposures compared to previous studies.

## 1. INTRODUCTION

Much of the world's population uses biomass fuel (i.e., burned plant and animal material such as wood, charcoal, agricultural residue, or animal dung) as a primary source of energy for heating and cooking, particularly in low and middle income countries. It has been estimated that more than 60% of households in Africa rely on solid fuels for cooking (1, 2). According to the World Health Organization (WHO), fuels such as kerosene are the cleanest burning while animal dung, twigs and grass are among the most polluting; wood falls in the middle in terms of efficiency and polluting potential (3). The use of simple open stoves or fires to burn biomass fuels coupled with incomplete combustion can result in substantial emissions of pollutants including carbon monoxide, particulate matter, and volatile organic compounds (e.g., benzene, benzo(a)pyrene) (4-6). Poor ventilation may also contribute to high concentrations of indoor pollution in households, which rely on biomass fuel for cooking and heating (7, 8). Women and children may be particularly affected by household air pollution from burning biomass fuels due to the increased time spent in the cooking area by these groups (6).

The use of solid fuel accounts for an estimated 4.6% of disability adjusted life-years lost globally (9), primarily due to respiratory tract infections in children less than five years of age and chronic obstructive pulmonary disease (COPD) in adult women (2). The posited mechanism through which exposure to biomass-related household air pollution affects health is through inflammation and oxidative stress (7). The pro-inflammatory effects of woodsmoke exposure have been demonstrated in vitro (10) and experimental studies have shown short-term controlled inhalation exposure of woodsmoke can induce pulmonary and systemic inflammation (11-13).

Epidemiologic studies indicate that individuals exposed to biomass smoke have increased risk of respiratory symptoms and illnesses (14-17), including evidence of respiratory tract infections among individuals exposed to biomass smoke in Kenya (18) and among South African children living in homes where polluting fuel sources are used for heating and cooking (19). Studies also find more frequent respiratory symptoms or illness among women using traditional stoves compared to women using improved cookstoves (20, 21). Furthermore, studies in Central America and South Asia suggest cooking with biomass fuels is associated with increased levels of: biomarkers of endothelial inflammation (i.e., intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) (22) and pro-inflammatory biomarkers (i.e., interleukin-6 (IL-6), interleukin-8 (IL-8), tumor necrosis factor-alpha (TNF-α), and C reactive protein (CRP)) (16, 23, 24).

Though research from multiple developing countries provides indication of a positive association between cooking with biomass fuels and biomarkers of inflammation, less is known about this purported association among South African women, where just over 40% of the population cooks primarily with wood, generally in a separate kitchen building (25). The variances in cultural practices around cooking when compared to the Indian and Central American populations could be the cause for the possibly lower levels of exposure in the African women. Therefore, the aim of the present study was to investigate the relation between cooking with wood and biomarkers of inflammation and respiratory symptoms and illnesses among reproductive-aged women living in rural South Africa.

## 2. METHODS

Data for this analysis are from the South African Study of Women and Babies (SOWB), a study of DDT (dichlorodiphenyltrichloroethylene) exposure and clinically-recognized pregnancy loss (26)(26). From 2010-2011, the SOWB study enrolled women from eight villages in the Thulamela Municipality of the Vhembe district in the Limpopo Province of South Africa. Eligibility criteria for SOWB included: aged 20-30 years, not currently using contraception, regular menstrual periods (unless breastfeeding), negative spot pregnancy test, no history of infertility and no medical or other condition which would prevent pregnancy. SOWB was approved by the institutional review boards of the University of Pretoria, South Africa and the National Institute of Environmental Health Sciences (NIEHS).

Consenting, eligible women completed an interviewer-administered questionnaire on demographics, lifestyle, reproductive and medical history, and usual cooking practices. At baseline, women also underwent a short physical exam where anthropometric measurements were obtained in triplicate, including height and weight, from which body mass index (BMI) was calculated. Participants' blood pressure was measured at baseline in triplicate and lastly, the women provided a blood sample. Of the 442 women initially enrolled in the SOWB study, 15 were later found ineligible due to age (n=3) or not living in one of the eight study villages (n=12), leaving 427 eligible women. For the present analysis, we excluded one woman who did not have a blood specimen and six women who did not have information on cooking fuel use. Further, five women who reported current smoking were excluded. This left 415 women for the present analysis.

The main exposure metric explored in this study was women's self-reported usual cooking fuel (wood vs. electricity). Additional information on location (indoors versus outdoors) of cooking was also assessed among women who reported using wood for cooking. These women were asked: "Most of the time do you cook inside or outside?" Based on responses to this question, a secondary exposure variable was created with the following categories: electricity users, wood users-mostly outdoors, and wood users-mostly indoors.

The primary dependent variables of interest were biomarkers of inflammation. Plasma samples were aliquoted and stored at -80 degrees Centigrade until analyzed. The samples were then assayed for: Interleukin-1 β (IL-1β), Interleukin-6 (IL-6), Interleukin 8 (IL-6) and Tumor Necrosis factor-α (TNF-α ) using the Human Pro-Inflammatory II 4-Plex Ultra-Sensitive Kit; and for C-Reactive Protein (CRP), Serum amyloid A (SAA), Vascular cell adhesion molecule-1 (VCAM-1) and Intercellular adhesion molecule 1 (ICAM-1) using the Human Vascular Injury Panel II kit, both from MSD (Meso Scale Discovery, Gaithersburg, Maryland, USA) according to manufacturer's protocols. Briefly, a 96-well plate that had been pre-coated with capture antibodies on spatially distinct spots was blocked with blocking solution for one hour at room temperature with constant shaking at 700 rpm. After washing three times with phosphate buffered saline-tween (PBS-T) buffer, samples and standards were added to the appropriate wells and the plate was incubated for two hours at room temperature with constant shaking. The plate was washed again as mentioned above, detection antibody was added to each well, and the plate was again incubated at room temperature with constant shaking (two hours for the Pro-Inflammatory panel plate and one hour for the Human Vascular Injury Panel plate). After washing the

plate three times with (PBS-T), 150 µl of 1x Read Buffer was added to each well and the plate was immediately analyzed on the Sector Imager 2400 System (MSD). The instrument measures intensity of emitted light to afford a quantitative measure of Pro-Inflammatory or Vascular Injury Panel analytes in the sample. CRP, SAA, ICAM-1 and VCAM-1 were reported in units of mg/L, and IL-1β, IL-6, IL-8 and TNF-α were reported in units of pg/ml. No measurements were less than the limit of detection and values were log-transformed prior to analysis.

In addition to the inflammatory biomarkers described above, we were also interested in the association between cooking fuel and respiratory symptoms and illnesses, and blood pressure. Respiratory symptoms included breathlessness (i.e., "When you work hard, do you feel you have less breath compared to other people your age"; yes/no) and wheezing/chest tightness (i.e., "During the last year have you had wheezing or tightness of your chest", yes/no). Self-report of tuberculosis, pneumonia, and asthma at age 16 or older were grouped together to represent respiratory illness. For this analysis, we collapsed the mean of the systolic blood pressure (SBP) and diastolic blood pressure (DBP) measurements into a dichotomous variable: normal blood pressure (i.e., SBP <120 mm Hg and DBP <80 mm Hg) versus pre-hypertension/ hypertension) (SBP >=120 mm Hg or DBP >=80 mm Hg). We used guidelines reported in the the Seventh Report of the Joint National Committee to additionally include women with pre-hypertension (27) as only a small number of women in this study (n=15, 3.8%) had clinical hypertension according to the standard cutoff (i.e., SBP ≥ 140 mm Hg or DBP ≥ 80 mm Hg).

We conducted separate linear regression models for each inflammatory marker. Age (years) was included in the regression models as an *a priori* covariate. Other variables we explored in the models included: body mass index (BMI; kg/m$^2$), years of education (<11, 11, 12, >12), gravidity (number of pregnancies; 0, 1, >2), regular coffee consumption (i.e., “at least once per week for six months or longer”; yes/no), passive smoking (i.e. “in the past 12 months, has anyone smoked at least one cigarette/day for six months or more near you?”; yes/no), consumption of alcohol (ever/ never), primary source of drinking water (public tap vs. water piped to yard/home), and any medication taken in the previous 24 hours (yes/no). To arrive at a common set of adjustment variables, we included in all models those covariates that were statistically significantly ($p<0.20$) associated with a minimum of three of the inflammatory biomarkers. Based on this criterion, in addition to age, we included gravidity, caffeine consumption, passive smoking, and source of drinking water in all fully-adjusted models. We also examined the impact of location of cooking (inside versus outside) through a sensitivity analysis using a 3-category exposure variable (electricity-user, wood-user mostly inside, wood-user mostly outside). Regression coefficients (β) and the 95% confidence intervals (95% CI) are reported. The secondary analysis of the association between usual cooking fuel and respiratory symptoms, illness, and blood pressure was conducted using logistic regression models. In these analyses, odds ratios (ORs) and 95% CI are reported. As a second sensitivity analysis, we analyzed systolic and diastolic blood pressure as continuous variables.

All analyses were conducted in SAS (version 9.4; SAS Institute Inc., Cary, NC, USA).

## 3. **RESULTS**

The median age of study participants was 24 years (interquartile range (IQR) = 22, 26) and the median BMI was 24.7 kg/m$^2$ (IQR = 21.5, 28.3 kg/m$^2$) [Table 1]. Nearly a quarter (24.1%) of the women had less than 11 years of education and 30.9% reported at least two pregnancies. While most (93.7%) women reported caffeine consumption in the previous six months, few women reported exposure to passive smoking in the previous year (22.4%) or ever alcohol use (16.1%). More than one-third (39.5%) of the women reported a public water tap as their main water source. Compared with electricity users, a larger proportion of wood users had less than 11 years of education (30.4% versus 18%) and reported using a public tap as their main water sources (44.6% versus 34.6%). Nearly half (49.2%) of the women reported primarily cooking with wood and among these, more than half (62.8%, data not shown) reported cooking mostly indoors. Overall, few women reported having a respiratory illness (5.3%), feeling breathless (13.7%) or having wheezing/chest tightness (9.4%) and one-third (33.3%) of women were classified as having abnormal blood pressure (data not shown). Of those women who cook with wood indoors, only 2.3% reported not having any ventilation in their kitchen (data not shown).

**Table 1**. Distribution of selected characteristics of 415 women aged 20-30 years in the South African Study of Women and Babies (SOWB), 2011

| | All Women (n = 415) | Electricity-Users (n = 211) | Wood-Users (n = 204) |
|---|---|---|---|
| Age (years) [median (IQR)] | 24 (22, 26) | 23 (21, 26) | 24 (22, 26.5) |
| BMI (kg/m$^2$) [median (IQR)] | 24.7 (21.5, 28.3) | 24.8 (21.5, 28.3) | 24.6 (21.6, 28.3) |
| Education (years) [n (%)] | | | |
| <11 | 100 (24.1) | 38 (18.0) | 62 (30.4) |
| 11 | 117 (28.2) | 56 (26.5) | 61 (29.9) |
| 12 | 127 (30.6) | 70 (33.2) | 57 (27.9) |
| >12 | 71 (17.1) | 47 (22.3) | 24 (11.8) |
| Gravidity [n (%)] | | | |
| 0 | 79 (19.0) | 41 (19.4) | 38 (18.6) |
| 1 | 208 (50.1) | 111 (52.6) | 97 (47.5) |
| ≥2 | 128 (30.9) | 59 (28.0) | 69 (33.8) |
| Coffee Consumption [n (%)] | | | |
| No | 26 (6.3) | 13 (6.2) | 13 (6.4) |
| Yes | 389 (93.7) | 198 (93.8) | 191 (93.6) |
| Passive Smoking [n (%)] | | | |
| No | 322 (77.6) | 162 (76.8) | 160 (78.4) |
| Yes | 93 (22.4) | 49 (23.2) | 44 (21.6) |
| Alcohol Consumption [n (%)] | | | |
| No | 348 (83.9) | 170 (80.6) | 178 (87.3) |
| Yes | 67 (16.1) | 41 (19.4) | 26 (12.7) |
| Primary Source of Drinking Water [n (%)] | | | |
| Public tap | 164 (39.5) | 73 (34.6) | 91 (44.6) |
| Piped to yard/home | 251 (60.5) | 138 (65.4) | 113 (55.4) |

With a few exceptions, median levels of the inflammatory biomarkers were similar among women using electricity or wood for cooking [Table 2]. We did observe higher median TNF-α levels among wood-users (9.4 pg/ml) compared with electricity-users (8.0 pg/ml). Median SAA and CRP levels were each higher among electricity-users (SAA: 17.1 mg/L; CRP: 8.7 mg/L) than wood-users (SAA: 12.3 mg/L; CRP: 7.3 mg/L). We did not observe any statistically significant differences in median levels of biomarkers of inflammation between the two groups.

**Table 2**. Distribution of biomarkers of inflammation[a] among 415 non-smoking women in the South African Study of Women and Babies (SOWB), 2010

| | All Women (n=415) | Electricity-users (n=211) | Wood-users (n=204) |
|---|---|---|---|
| Biomarker | Median (IQR) | Median (IQR) | Median (IQR) |
| IL-1β | 0.6 (0.3, 1.4) | 1.03 (0.30, 1.30) | 0.68 (0.34, 1.46) |
| IL-6 | 2.3 (1.3, 4.0) | 2.09 (1.25, 3.66) | 2.42 (1.27, 4.29) |
| IL-8 | 2.0 (1.1, 4.6) | 2.12 (1.01, 4.08) | 1.97 (1.09, 4.78) |
| TNF-α | 8.6 (5.9, 14.1) | 7.98 (5.91, 14.12) | 9.38 (5.87, 14.15) |
| CRP | 7.6 (2.6, 23.5) | 8.66 (2.67, 24.85) | 7.25 (2.64, 20.74) |
| SAA | 15.3 (6.0, 39.5) | 17.13 (7.16, 42.41) | 12.29 (5.17, 33.15) |
| I-CAM | 0.8 (0.5, 1.3) | 0.78 (0.34, 1.33) | 0.77 (0.50, 1.32) |
| V-CAM | 1.2 (0.7, 1.96) | 1.22 (0.64, 2.04) | 1.17 (0.66, 1.81) |

CRP: C Reactive Protein; I-CAM: Intercellular Adhesion Molecule 1; IL-1β: Interleukin-1β; IL-6: Interleukin-6; IL-8: Interleukin-8;IQR: interquartile range; SAA: Serum Amyloid A; TNF-α: Tumor Necrosis Factor-α; V-CAM: Vascular Cell Adhesion Molecule

[a] IL-1 β, IL-6, IL-8 and TNF-α are reported in units of pg/ml; CRP, SAA, I-CAM, V-CAM are reported in units of mg/L

The crude and fully-adjusted associations between usual cooking fuel use and log-transformed biomarkers of inflammation were similar (Table 3). Most associations were negative and of small magnitude. However, we did observe that, compared with electricity users, women who cooked with wood had higher log-transformed concentrations of IL-1β (adjusted β = 0.17, -0.05, 0.39) and TNF-α (adjusted β = 0.09, 95% CI = -0.04, 0.21), though the estimates were imprecise. Results from the analyses examining the inflammatory effects separately among women who cooked with wood indoors outdoors (compared to electricity use) revealed similar results among the two groups, with the exception of SAA (data not shown). Compared with electricity-users, women who use wood indoors had decreased concentrations of SAA (adjusted β = -0.38, 95% CI = -0.68, -0.08) while no association was observed comparing wood users outdoors to electricity users (adjusted β = 0.07, 95% CI = -0.29, 0.43).

**Table 3**. Crude, and fully-adjusted[a] associations between cooking with wood (vs. electricity) and log transformed biomarkers of inflammation among 415 non-smoking women in the South African Study of Women and Babies (SOWB), 2010

| | Crude | Fully-adjusted |
|---|---|---|
| | β (95% CI) | β (95% CI) |
| IL-1β | 0.15 (-0.07, 0.37) | 0.17 (-0.05, 0.39) |
| IL-6 | 0.06(-0.11, 0.22) | 0.08 (-0.09, 0.25) |
| IL-8 | 0.08 (-0.12, 0.27) | 0.10 (-0.09, 0.29) |
| TNF-α | 0.07 (-0.05, 0.19) | 0.09 (-0.04, 0.21) |
| CRP | -0.06 (-0.38, 0.25) | -0.10 (-0.41, 0.21) |
| SAA | -0.21(-0.48, 0.05) | -0.21 (-0.48, 0.05) |
| I-CAM | -0.01 (-0.16, 0.13) | -0.01 ( -0.16, 0.13) |
| V-CAM | -0.03 (-0.18, 0.11) | -0.02 (-0.16, 0.12) |

CRP: C Reactive Protein; I-CAM: Intercellular Adhesion Molecule 1; IL-1β: Interleukin-1β; IL-6: Interleukin-6; IL-8: Interleukin-8; SAA: Serum Amyloid A; TNF-α: Tumor Necrosis Factor-α; V-CAM: Vascular Cell Adhesion Molecule
[a]Adjusted for age, gravidity, caffeine consumption, passive smoking and water source

Table 4 presents results from analyses of the associations between usual cooking fuel and respiratory symptoms and illnesses, and blood pressure. Compared with electricity-users, there was a suggestion of increased odds of self-reported wheezing/chest tightness among women who cook with wood (adjusted OR=1.41; 95% CI=0.72-2.77), although this estimate was imprecise. There was little evidence of an association between cooking with wood and self-reported breathlessness or respiratory illnesses, or pre-hypertension/hypertension. Due to small cell sizes, we did not analyze associations of wheezing or respiratory illnesses separately among women who cooked with wood primarily indoors or outdoors versus electricity users. However, compared with electricity users, we observed an indication of increased odds (though not statistically significant) of both breathlessness (adjusted OR=1.29, 95% CI=0.65, 2.56) and pre-hypertension/ hypertension (adjusted OR=1.29, 95% CI=0.80, 2.09) among women who

reported cooking with wood mostly indoors (data not shown). We found little evidence of an effect of using wood for cooking on blood pressure as continuous variables, (systolic β = -0.33, 95% CI = -2.37, 1.71; diastolic β = -0.21, 95% CI = -1.77, 1.35) (data not shown).

**Table 4**. Crude and fully-adjusted[a] association between selected medical conditions and fuel used for cooking among 415 non-smoking women in the South African Study of Women and Babies (SOWB), 2010.

| | n (%) | Crude OR (95% CI) | Fully-adjusted OR (95% CI) |
|---|---|---|---|
| Breathlessness | 57 (13.7) | 1.09 (0.62, 1.90) | 1.06 (0.60, 1.87) |
| Wheezing/chest tightness | 39 (9.4) | 1.44 (0.74, 2.81) | 1.41 (0.72, 2.77) |
| Respiratory Diseases[b] | 15 (3.6) | 0.63 (0.22, 1.82) | 0.64 (0.22, 1.85) |
| Pre-hypertension/Hypertension[c] | 138 (33.25) | 1.04 (0.69, 1.56) | 1.08 (0.71, 1.63) |

[a]Adjusted for age, gravidity, caffeine consumption, passive smoking, and water source
[b]Includes tuberculosis, pneumonia, and asthma
[c]Systolic BP > 120 mm Hg or Diastolic BP > 80 mm Hg.

## 4. **DISCUSSION**

Given the paucity of existing data, the goal of the study was to examine the relation between cooking with wood and biomarkers of inflammation and respiratory symptoms and illnesses among women whose level of exposure may have been lower than in previously studied populations. We found no statistically significant association between cooking with wood and any of the inflammatory biomarkers examined, with the exception of decreased SAA concentrations in women who used wood mostly indoors compared to electricity users. We found limited evidence of an association between cooking with wood and self-reported wheezing/chest tightness.

In contrast to our findings, previous studies have reported statistically significant differences in levels of inflammatory markers when comparing biomass vs. non-biomass users. For example, a study in India reported that women who cooked with biomass fuel

had 72% higher plasma levels of TNF-α and 62% higher plasma levels of IL-6 than women who cooked with liquefied petroleum gas (24). Similar results were reported in another study among Indian women, where the women who used biomass fuels for cooking were found to have significantly higher levels of TNF-α, IL-6, IL-8, and CRP (23). A study of Pakistani women reported higher mean serum concentrations of CRP among women cooking with biomass fuels in the home (1.9 mg/l) compared with non-exposed women (0.66 mg/l) (16). However, a small pilot study among women in rural China found no differences in CRP levels between women with high versus low exposure to particulate matter with aerodynamic diameter <2.5 μg ($PM_{2.5}$) (28). While our results were not statistically significant, we did report higher levels of TNF-α and IL-6 in women using wood, which was consistent with the Indian study. Interestingly, and in contrast to some previous findings, the median CRP levels in our population were lower among wood-users (7.3; IQR: 2.6, 20.7) compared to electricity users (8.7; IQR: 2.7, 24.9). We relied on blood biomarkers of inflammation, which are most cost effective in population studies.(29) However, pulmonary inflammatory markers (e.g. exhaled breath) would have represented a more specific assessment of the respiratory impact of household air pollution.(30)

In our study, women who reported cooking primarily with wood did not have statistically significant increased odds of breathlessness, respiratory disease (i.e., tuberculosis, pneumonia, and asthma), or pre-hypertension/hypertension. Unfortunately, we had too few women who reported specific diseases to analyze these separately. It is possible, therefore, that an association with a specific respiratory disease may have been missed. Consistent with other studies, we did find a positive association between using

wood for cooking and self-reported wheezing in the previous 12 months, though the estimate was imprecise.

In previous studies conducted in primarily South Asian countries, women using biomass fuel for cooking often used a mix of specific fuels, including coal, wood, and dung or crop residues. The biomass-users in the present study used primarily wood, which is considered a 'cleaner' burning fuel than other types of biomass (3). Thus, household air pollution exposures experienced by women in the SOWB may be lower than in other study populations. Due to the logistics and the high cost of conducting PM exposure measurements in the rural South African setting, we used the type of cooking fuel as an exposure surrogate. A study conducted in China found the 24-hr average $PM_4$ (i.e., particulate matter with aerodynamic diameter <4 μg) concentration in kitchens was lowest among households using primarily wood (164.0 $\mu g/m^3$) compared with those using either primarily crop residues (282.9 $\mu g/m^3$) or a combination of wood and crop residues (192.5 $\mu g/m^3$) (31). Estimates of $PM_{2.5}$ among households where wood is the primary fuel source are widely varied, and range nearly 8-fold, from 107 $\mu g/m^3$ to 901 $\mu g/m^3$ (32-38). Two studies of African populations cooking primarily with wood have been conducted among women in The Gambia and report average $PM_{2.5}$ concentrations on the lower end of this range: 312 $\mu g/m^3$ (37) and 395 $\mu g/m^3$ (38).

Differences in housing characteristics and cooking practices may also impact household air pollution concentrations among households using biomass fuels. In an Indian study, exposure levels in households which used a separate well-ventilated room to burn biomass for cooking were found to be lower than in households where cooking was done in the living area (39). Although 63% of the wood-users in the present study

report cooking mostly indoors, accounting for this factor did not change the results of our analyses. This could also be due to the almost universal use of some ventilation (smoke pipe or ventilated roof) in our study. Women in the present study were relatively young (median age=26 years). The mean or median ages of women included in other studies ranged from 36 to 45 (20, 23, 40, 41). Due to their younger age, women in this study likely had shorter lifetime duration of cooking, and therefore lower cumulative exposure to biomass-related household air pollution compared with women from other studies. Younger women may also be less prone to increased inflammatory activity due to unrelated chronic diseases (42, 43).

To our knowledge, the present study represents the first effort to examine whether women living in rural areas of the Vhembe district in Limpopo experience adverse health effects due to cooking with biomass fuel. While we found little evidence of such an association, it is possible that the women in the present study are not very highly exposed to household air pollution, despite reliance on biomass fuels for cooking and heating. Compared with other studies, where women may have been using a mixture of biomass fuels, biomass-users in the present study used only wood. Further, cooking practices (e.g., cooking mostly indoors vs. outdoors) and housing characteristics (e.g., type of ventilation) may differ across populations and serve to modify household air pollution exposures. Notwithstanding the present study's largely null findings, a large proportion of rural African women continue to rely on biomass fuels for cooking and heating, and while a great burden of disease has been attributed to the use of biomass fuels, there remains scarce information related to quantifying household air pollution exposures from cooking with biomass fuels among specific African populations. Thus, future efforts

should continue to provide education related to best cooking practices as well as research aimed at identifying potentially modifiable determinants of personal air pollution exposures among high-risk populations.

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6. **Conflicts of Interest:** None

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