Proinflammatory Effects in *Ex Vivo* Human Lung Tissue of Respirable Smoke Extracts from Indoor Cooking in Nepal

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Abstract

Rationale: Exposure to biomass smoke is believed to increase the risk of developing chronic obstructive pulmonary disease. However, little is known about the mechanisms underlying responses to biomass smoke in human lungs.

Objectives: This study had two objectives: first, to quantify "real-life" exposures to particulate matter <2 μm in diameter ($PM_{2.5}$) and carbon monoxide (CO) measured during cooking on stoves in rural areas of Nepal in different geographical settings; and second, to assess the effect of biomass smoke extracts on inflammatory responses in *ex vivo* human lung tissue.

Methods: Personal exposures to $PM_{2.5}$ and indoor near-stove CO concentrations were measured during cooking on a range of stoves in 103 households in 4 different Nepalese villages situated at altitudes between ∼100 and 4,000 m above sea level. Inflammatory profiles to smoke extracts collected in the field were assessed by incubating extracts with human lung tissue fragments and subsequent Luminex analysis.

Results: In households using traditional cooking stoves, the overall mean personal exposure to $PM_{2.5}$ during cooking was 276.1 μg/m^3 (standard deviation [SD], 265 μg/m^3), and indoor CO concentration was 16.3 ppm (SD, 19.65 ppm). The overall mean $PM_{2.5}$ exposure was reduced by 51% ($P = 0.04$) in households using biomass fuel in improved cook stoves, and 80% ($P < 0.0001$) in households using liquefied petroleum gas. Similarly, the indoor CO concentration was reduced by 72% ($P < 0.001$) and 86% ($P < 0.0001$) in households using improved cook stoves and liquefied petroleum gas, respectively. Significant increases occurred in 7 of the 17 analytes measured after biomass smoke extract stimulation of human lung tissue (IL-8 [interleukin-8], IL-6, TNF-α [tumor necrosis factor-α], IL-1β, CCL2, CCL3, and CCL13).

Conclusions: High levels of real-life exposures to $PM_{2.5}$ and CO occur during cooking events in rural Nepal. These exposures induce lung inflammation *ex vivo*, which may partially explain the increased risk of chronic obstructive pulmonary disease in these communities.

Keywords: household air pollution; exposure; smoke extract; lung inflammation; cytokine

(Received in original form November 12, 2019; accepted in final form February 5, 2020)

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Supported by a research grant from the University of Nottingham Global Challenge Research fund support. The field studies were partially supported by core fund of the International Centre for Integrated Mountain Development. I.P.H. is a National Institute for Health Research Senior Investigator.

Author Contributions: B.K., P.S.M., and S.P.P. conducted exposure monitoring and sample collection in field. B.K., D.T., and C.K.B. collected tissue samples and performed all tissue culture work. B.K. and A.P.H. performed cytokine screening Luminex assay. B.K., P.S.M., I.S., S.P.P., and I.P.H. conceived the study, designed experiments, analyzed data, performed statistical analyses, and wrote the manuscript.

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This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org.

Ann Am Thorac Soc Vol 17, No 6, pp 688–698, Jun 2020

DOI: 10.1513/AnnalsATS.201911-827OC
Internet address: www.atsjournals.org

Inhalation of particulate matter of <2.5 μm diameter ($PM_{2.5}$) from sources, such as tobacco smoke, vehicle exhausts, and factory emissions, is known to cause lung inflammation (1, 2). Extensive epidemiological evidence also shows that high levels of exposure to $PM_{2.5}$ are associated with increased risk of developing chronic obstructive pulmonary disease (COPD), a disease characterized by lung inflammation. Although COPD is a major cause of morbidity and mortality globally (3), COPD morbidity and mortality varies across regions. Over 90% of deaths attributed to COPD occur in low- and

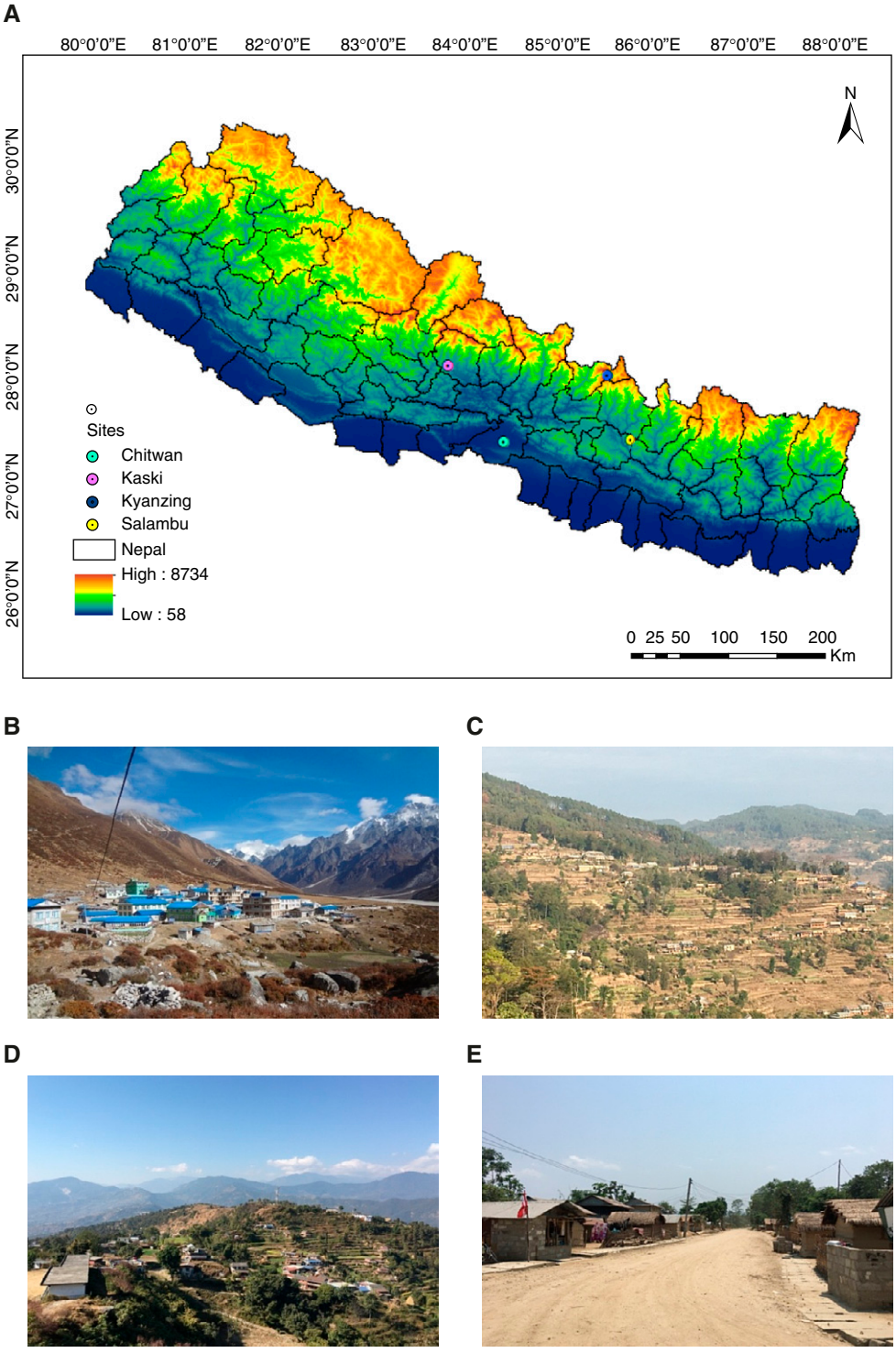

Figure 1. Study locations: (*A*) map of Nepal with elevation and location of all four monitoring site villages; (*B*) Kyanzing Kharka, Langtang (4,000 m above sea level [masl]); (*C*) Salambu, MajhiFeda (1,800 masl); (*D*) Bhujel Gaun, Pokhara (1,200 masl); and (*E*) Simreni Village, Chitwan (200 masl).

middle-income countries (LMICs) (4) and, in LMICs, a significant contribution to disease burden is believed to come from exposure to poor air quality, either outdoors or indoors (5–7). In LMICs, one major source of indoor air pollution is cooking using biomass on traditional stoves in poorly ventilated houses (8).

Despite the epidemiological evidence that household air pollution (HAP) contributes to respiratory symptoms and increased risk for COPD (9, 10), little is known of the mechanisms underlying the biological effects of inhaling products of biomass combustion (11). A few *in vitro* studies have shown increased markers of inflammation in different cell lines stimulated with biomass smoke extracts (12–14). However, the data on the effects of biomass smoke exposures are limited compared with the extensive data on the effects of exposure to tobacco smoke extracts (15, 16), and no data are available in human lung tissue *ex vivo*.

Approximately half of the world's population, and more than 90% of households in rural parts of LMICs, use solid biomass fuel as a primary source of energy for cooking (17, 18). In Nepal, 80% of the population of 30 million live in rural settings, and >80% use locally sourced wood, crop residues, or dried dung as a cooking source (19). Most cooking takes place on traditional cook stoves (TCSs), which are three-stone– or two-pot–style open fires in rooms without a chimney or proper ventilation, and this would be predicted to cause high levels of exposure to $PM_{2.5}$, carbon monoxide (CO) and other health-damaging pollutants (20). The emissions of fine particulate matter, in poorly ventilated dwellings using inefficient stoves can reach up to 100-fold higher than the air quality standard set by the World Health Organization (WHO) (21). Two recent studies using static sampling conducted in the Sarlahi and Janakpur districts of Nepal have found a daily average indoor $PM_{2.5}$ concentration of 1,376 $\mu g/m^3$ (22) and a 48-hour average $PM_{2.5}$ concentration of 417.6 $\mu g/m^3$ (23). Consistently higher concentrations of indoor air pollutants in households using biomass fuel has been reported in similar studies in other countries (24–27). After several national initiatives in Nepal, improved cook stoves (ICSs) have been trialled in some villages: these have an improved combustion system and/or vent fumes through a chimney (28). According to the Central Bureau of Statistics, Nepal, ~21% of households use liquefied petroleum gas (LPG) stoves (29). Preliminary work performed in Nepal has shown reductions in levels of HAP with use of these ICSs and LPG stoves, though the reduction achieved varied between studies (30–32). Although in Nepal and other parts of South Asia studies have been done examining indoor exposures, none of these studies have investigated lung inflammatory responses (33–35).

The aims of this study were therefore twofold: namely, to define the range of personal exposures encountered by individuals cooking on both TCSs and ICSs in real life in Nepal, and to investigate the potential proinflammatory consequences of these exposures using *ex vivo* human lung explants as a model system.

Methods

Site Description

This study was conducted in four different rural or semirural settings representing different geographical regions in Nepal (Figure 1). The sites were Simreni village in Chitwan (~200 m above sea level [masl]), Bhujel Gaun in Pokhara (~1,200 masl), Salambu in Majhi Feda (~1,800 masl), and Kyanzing Kharka in Langtang (~4,000 masl) (*see* SECTION E1 in the online supplement for a more detailed site description).

Exposure Monitoring

Real-life exposure to $PM_{2.5}$ and CO concentration was monitored in a total of 103 households from all four villages, with different stove designs: details are presented in Table 1. The exposure monitoring was performed for a single cooking episode in each household throughout the cooking period; however, a short-term noncooking period exposure concentration was also measured in each household. The cooking period varied in each household in each monitoring site with the average (± standard deviation [SD]) of 78 (±15.9) minutes, 79 (±24.2) minutes, and 68 (±15.2) minutes in Salambu, Pokhara, and Chitwan, respectively. As stoves in Kyanzing remained in use for 16–17 hours per day, the monitoring period was fixed to 120 minutes in each household to represent the cooking period. Non–cooking period exposure was monitored for a total of 40 minutes in each household with a continuous 20-minute exposure before cooking commenced and 20 minutes after cooking ended. Real-time personal exposures to $PM_{2.5}$ were measured using an Aerosol Mass Monitor (Aerocet831; Met One Instrument, Inc.) (36, 37), which was attached directly to the person involved in cooking. The cook was asked to carry an Aerocet on their back, and its inlet connected by an adjustable tube placed near the cook's breathing zone. An indoor air quality (IAQ) meter (Advancedsense Pro IAQ; Graywolf Sensing Solutions) was used to monitor real-time indoor CO exposures. The monitoring was performed for cooking and noncooking periods by placing the IAQ meter ~1 m from the stove and ~1.5 m off the floor to capture direct, real-time CO exposures. Exhaled breath CO concentration of people involved in cooking was also measured as a biomarker of CO exposure. Personal exhaled breath CO concentrations were monitored using a micro CO meter (CareFusion) (38). The concentration of CO in exhaled breath for each individual was taken at three different time points in three monitoring sites, except in Kyanzing. The first measurement was taken before cooking started, the second measurement was taken during cooking, and the third was taken immediately after

Table 1. Number of households sampled in each monitoring site

Monitoring Site	Total Household Sampled	Cooking Methods		
		Biomass (TCS)	Biomass (ICS)	LPG
Kyanzing	14	5	9	N/A
Salambu	25	17	8	N/A
Pokhara	27	24	N/A	3
Chitwan	37	30	N/A	7
Total	103	76	17	10

Definition of abbreviations: ICS = improved cook stove; LPG = liquefied petroleum gas; N/A = not applicable; TCS = traditional cook stove.
N/A: Cooking method was not used in the respective site.

cooking. For each phase, measurements were taken three times, and the mean of three readings was calculated. In Kyanzing, exhaled-breath CO concentration was measured in individual using TCSs and ICSs. All equipment details and calibration studies can be found in the Section E2.

Smoke Extract Collection

A total of 52 smoke extract samples, including 33 samples from biomass combustion in TCSs, 10 samples from biomass combustion in ICSs, and 6 samples from LPG, were collected from the four villages. From each monitoring site, ambient air samples were also collected using the same methods. Full details can be found in Section E3.

Endotoxin Quantification

Endotoxin concentration present in the smoke sample collected from different locations was quantified using a Limulus Amebocyte lysate assay (PierceLAL Chromogenic Endotoxin Quantification Kit; ThermoFisher Scientific) (39, 40). *See* Section E4 for more detail. Airborne endotoxin concentrations are known to vary depending on the type of fuel used, cow dung and agricultural residue being higher sources of endotoxin than fuel wood (41). Households in this study in all monitoring sites primarily used fuel wood for cooking.

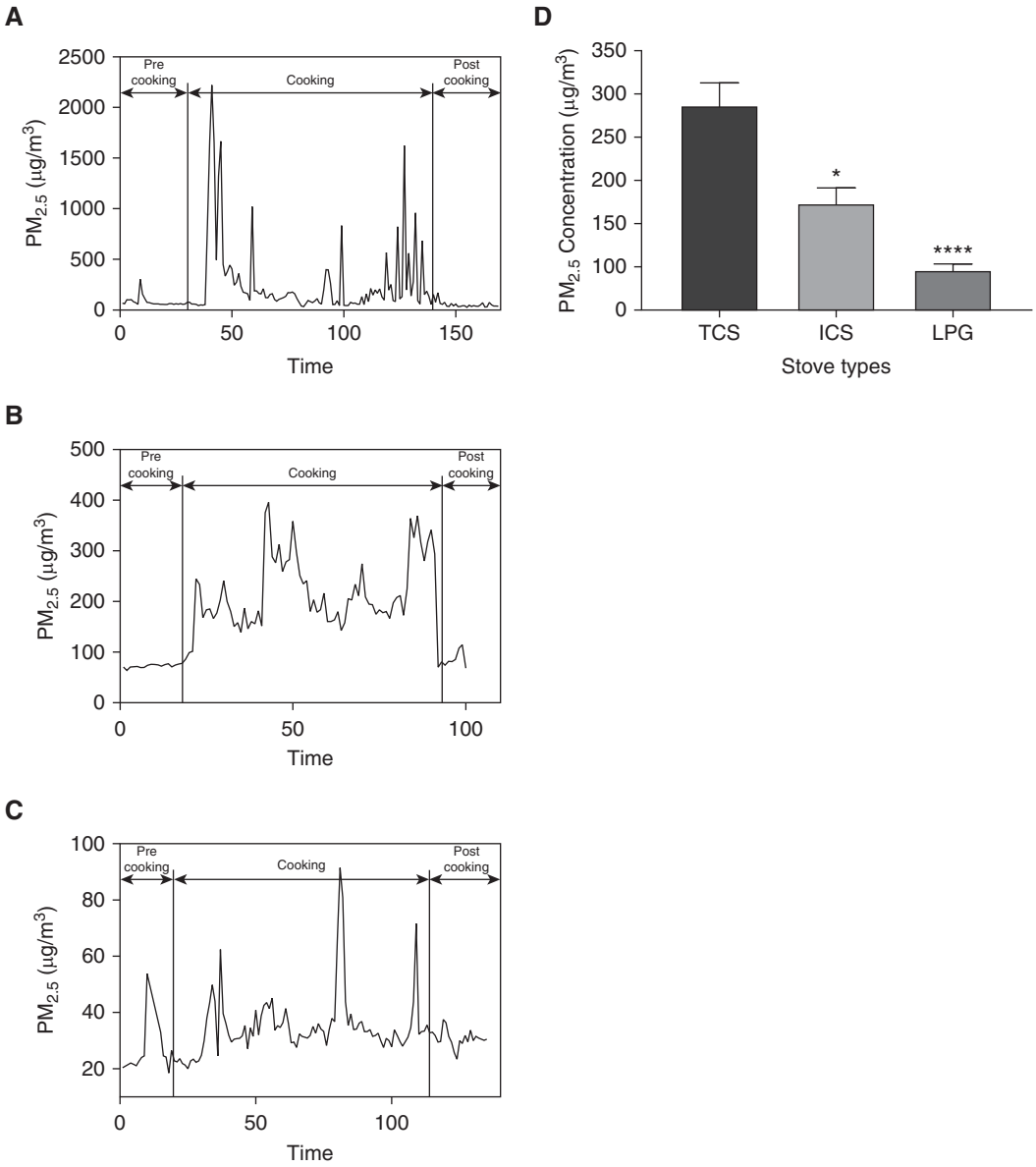

Figure 2. Representative temporal variation in real-life personal exposure to particulate matter <2 μm in diameter ($PM_{2.5}$). (*A–C*) Minute-to-minute variations in $PM_{2.5}$ exposure using biomass fuel in traditional cook stoves (TCSs) (*A*), biomass fuel in improved cook stoves (ICSs) (*B*), and liquefied petroleum gas (LPG) stove (*C*) in one representative house. (*D*) Mean $PM_{2.5}$ exposure during cooking using biomass in TCSs and ICSs, and using LPG stove. The data in *D* represent mean (±SEM) of all-households mean data over total cooking period for TCSs ($n = 76$), ICSs ($n = 17$), and LPG ($n = 10$). The mean exposure when using ICSs and LPG was compared with mean exposure using TCSs. Significance test was performed by Kruskal-Wallis test followed by Dunn's multiple comparison test. $^{*}P < 0.05$ and $^{****}P < 0.0001$. SEM = standard error of mean.

Endotoxin concentrations in smoke extract samples collected in homes using biomass fuel and LPG stoves were also measured.

Human Lung Tissue Processing

Human parenchymal lung tissue was obtained with informed, written consent from patients undergoing lung resection surgery from the Papworth Hospital Tissue Bank. The majority of donors (11/14) were ex-smokers (quit smoking ⩾5 yr prior), two individuals were current smokers, and two individuals were never smokers. There were nine males and five females with a mean age of 62.6 (±9.3) years. The methods for tissue sample processing and mediator assays are presented in Section E5, and demographics of all donor patients are presented in Table E2.

Statistical Analysis

All data were normalized using wet tissue weights in individual experiments. Data are expressed as means (±SEM). All data were analyzed using GraphPad Prism Software (Version 7; GraphPad Software Inc.). All statistical significance tests were performed using *t* test for comparison of two variables and using analysis of variance for

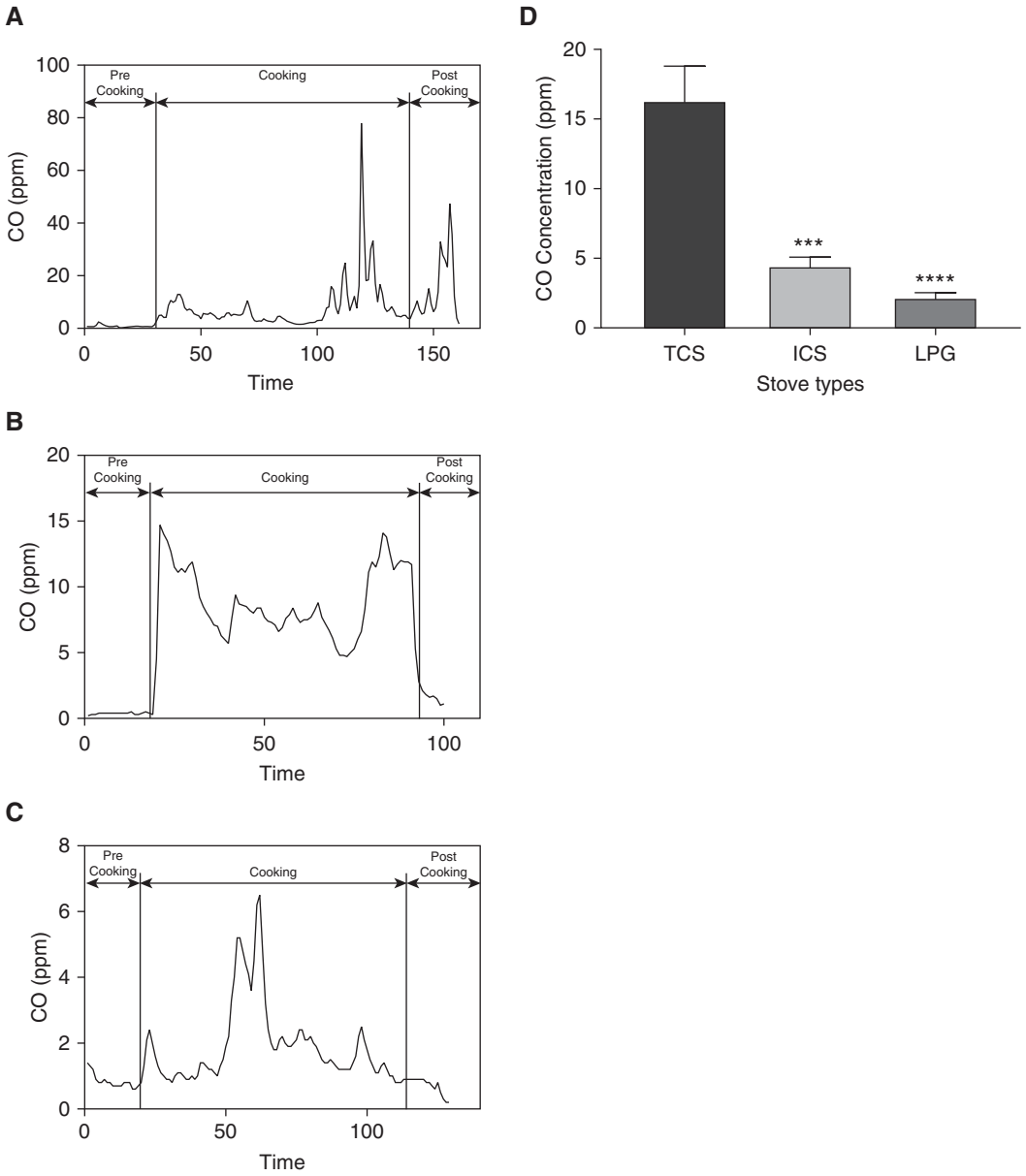

Figure 3. Representative temporal variation in real-life carbon monoxide (CO) exposure. (*A–C*) Minute-to-minute variations in CO exposure using biomass fuel in traditional cook stoves (TCSs) (*A*), biomass fuel in improved cook stoves (ICSs) (*B*), and liquefied petroleum gas (LPG) stove (*C*) in one representative house. (*D*) Mean CO concentration during cooking using biomass in TCSs and ICSs and using LPG. The data in (*D*) represent mean (±SEM) of all-households mean data over total cooking period for TCSs ($n = 76$), ICSs ($n = 17$), and LPG ($n = 10$). The mean exposure when using ICSs and LPG was compared with mean exposure using TCSs. Significance test was performed by Kruskal-Wallis test followed by Dunn's multiple comparison test. ***$P < 0.001$ and ****$P < 0.0001$. SEM = standard error of mean.

comparison of three or more variables (a value of $P < 0.05$ being considered as significant).

Ethical Approval

Ethical approval for lung tissue studies was obtained from Papworth Hospital Research Tissue Bank (ethics reference 08/H0304/56 + 5). Field-based exposure monitoring work was conducted under the ethical approval of the research proposal entitled "Epidemiological response to air pollution exposure in Nepal," granted by the Nepal Health Research Council (reg. no. 395/2016).

Results

Indoor Air Pollutant Exposures during Cooking in "Real-Life" Rural Nepal

Representative $PM_{2.5}$ and CO profiles showing the variation seen during cooking periods using biomass fuel in TCSs, biomass fuel in ICSs, and LPG are shown in Figures 2 and 3, respectively. There was considerable variability in exposure levels to both pollutants through the cooking period. Both $PM_{2.5}$ and CO levels registered high concentrations more than once during cooking, with the variation in exposure levels in individual households being dependent on the cooking practices followed. Greater variation in the peak concentrations were noted in households using TCSs compared with households using ICSs or LPG stoves, and levels were also higher in homes using TCSs.

The overall mean $PM_{2.5}$ exposures from all households using TCSs from all sites showed that the population using biomass fuel in Nepal is exposed to high concentrations of indoor $PM_{2.5}$ (overall mean, 276.1 $\mu g/m^3$). There was a significant reduction in mean $PM_{2.5}$ exposure concentration in households using biomass fuel in ICSs (51% reduction, $P = 0.04$) and in households using LPG (80% reduction, $P < 0.0001$) (Figure 2D). Similarly, the population using biomass fuel in TCSs was exposed to higher concentrations of CO (overall mean, 16.3 ppm). These CO concentrations were significantly reduced in households using biomass fuel in ICSs (72% reduction, $P = 0.0002$) and in households using LPG (86% reduction, $P < 0.0001$) (Figure 3D). However, even the reduced exposures to $PM_{2.5}$ using either ICSs or LPG remained higher than the WHO safe recommended concentration of 25 $\mu g/m^3$ (21). The exposure levels for CO using both ICSs and LPG remained below the WHO guidelines.

The mean $PM_{2.5}$ and CO exposures calculated across the whole cooking period in households using TCSs were significantly higher (~4.6-fold for $PM_{2.5}$, $P < 0.0001$ and ~8-fold for CO; $P < 0.0001$) than the non–cooking-period exposures (Figures 4A and 4B). The overall mean $PM_{2.5}$ and CO exposures in the household using ICSs was 2.8-fold ($P < 0.001$) and 3.4-fold ($P < 0.0001$) higher in cooking periods compared with the noncooking periods. The overall mean $PM_{2.5}$ exposures in households using LPG were not significantly different ($P = 0.84$) between the cooking and noncooking periods; however, the overall mean cooking period CO exposures were approximately 1.5-fold ($P = 0.02$) higher than non–cooking-period exposures.

Differences in mean $PM_{2.5}$ and CO exposures during cooking and noncooking periods in each monitoring site using different stoves, along with indoor temperature and relative humidity, are presented in Table 2. The overall exposures to mean $PM_{2.5}$ was significantly higher in the higher-altitude regions in Langtang (746.1 $\mu g/m^3$) and Salambu (500.6 $\mu g/m^3$) than in lower-altitude regions in Pokhara (211.9 $\mu g/m^3$) and Chitwan (121.9 $\mu g/m^3$). The reductions in $PM_{2.5}$ exposures when ICSs was used instead of TCSs were 88% ($P < 0.0001$) and 59% ($P < 0.01$) in Kyanzing and Salambu, respectively, whereas the percent reductions in CO exposures were 78% ($P < 0.001$) and 72% ($P < 0.05$), respectively. Similarly, the reductions in $PM_{2.5}$ exposures in households using LPG in Pokhara and Chitwan were significant compared with TCSs: 75% ($P < 0.01$) in Pokhara and 55% ($P < 0.01$) in Chitwan. The percent reductions in CO exposures in households using LPG in Pokhara and Chitwan were 85% ($P < 0.05$) and 79% ($P < 0.001$), respectively.

The concentration of CO in exhaled breath was measured to estimate changes in exhaled breath CO levels due to short-term exposure to HAP. The pattern of exhaled-breath CO variations between different cooking phases in households using TCSs in three monitoring sites are presented in Figures 5A–5C. For all subjects using TCSs

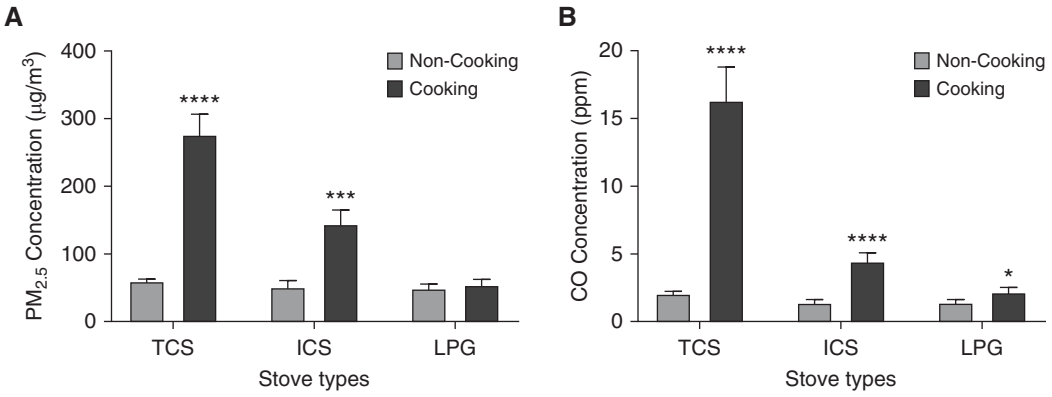

Figure 4. Cooking and noncooking exposure variation among different cook stove use across all sites. (*A*) Particulate matter <2.5 μm in diameter ($PM_{2.5}$) exposure, and (*B*) carbon monoxide (CO) exposure. The data shown are mean (±SEM) of all households from all sites with traditional cook stoves (TCSs; $n = 76$), improved cook stoves (ICSs; $n = 17$), and liquefied petroleum gas (LPG; $n = 10$). The variation between two periods for each stove was assessed using Mann-Whitney *t* test. *$P < 0.05$, ***$P < 0.001$, and ****$P < 0.0001$. SEM = standard error of mean.

Table 2. Cooking and noncooking-period exposure concentration in Kyanzing, Salambu, Pokhara, and Chitwan

	TCS			ICS		
	Cooking	Noncooking	*P* Value	Cooking	Noncooking	*P* Value
Kyanzing						
T, °C	14.1 (2.3)	13.75 (6.18)	0.73	18.01 (6.23)	20.19 (8.2)	0.6
RH, %	42.1 (3.4)	37.8 (11.6)	0.55	34.67 (10.8)	29.57 (13.8)	0.15
$PM_{2.5}$, μg/m^3	746.1 (318.9)	21.3 (16)	0.007	91.27 (70.11)	16.78 (15.3)	0.001
CO, ppm	12.6 (4.7)	1.15 (0.69)	0.015	2.68 (1.47)	1.11 (0.58)	0.001
Salambu						
T, °C	20.65 (4.68)	18.04 (4.09)	0.15	17.86 (2.3)	14.3 (3.18)	0.4
RH, %	42.91 (9.92)	47.1 (9.51)	0.21	48.6 (7.72)	58.23 (7.9)	0.12
$PM_{2.5}$, μg/m^3	500.6 (314.6)	104.45 (101.15)	0.0001	203.25 (61.42)	84.8 (20.1)	0.0002
CO, ppm	25.78 (30.4)	3.28 (3.38)	0.0001	7.06 (2.48)	2.81 (3.27)	0.01
Pokhara						
T, °C	16.6 (3.35)	16.3 (4.22)	0.33	13.8 (2.83)	11.7 (0.14)	0.71
RH, %	40.05 (9.51)	35.77 (8.62)	0.20	48.76 (6.9)	44.55 (2.05)	0.52
$PM_{2.5}$, μg/m^3	211.93 (133.44)	44 (15.85)	0.0001	52.4 (14.5)	33.4 (0.4)	0.1
CO, ppm	19.08 (21.07)	1.9 (1.78)	0.0001	2.96 (1.7)	0.81 (0.16)	0.1
Chitwan						
T, °C	27.4 (3.870	27.98 (7.52)	0.97	31.09 (3.45)	30.8 (3.4)	0.81
RH, %	59.3 (11.7)	59.39 (11.05)	0.94	55.06 (9.25)	56.6 (9.1)	0.87
$PM_{2.5}$, μg/m^3	121.9 (57.8)	79.48 (48.9)	0.001	54.68 (31.02)	53.4 (20.3)	0.71
CO, ppm	9.08 (6.98)	5.37 (7.91)	0.01	1.85 (0.94)	1.6 (0.81)	0.24

Definition of abbreviations: CO = carbon monoxide; ICS = improved cook stove; $PM_{2.5}$ = particulate matter <2.5 μm in diameter; ppm = parts per million; RH = relative humidity; SD = standard deviation; T = temperature; TCS = traditional cook stove.
Differences were assessed using Mann-Whitney tests. The data shown are mean (±SD) of all households in each monitoring sites.

in all sites, exhaled-breath CO was elevated over baseline during cooking-period observations. Postcooking levels were lower than the cooking-period level; however, the levels were still higher than the levels noted in precooking observations. The overall mean exhaled CO levels in individuals using ICSs in Kyanzing showed a reduction of 75% ($P < 0.0001$) compared with individuals using TCSs (Figure 5D). Use of LPG in Chitwan showed that exhaled CO levels were not significantly elevated during cooking (Figure 5E) as compared with noncooking levels.

Human Lung Tissue Cytokine Responses

Ex vivo human lung tissue responded to LPS stimulation by producing 14 quantifiable signals out of the 17 inflammatory analytes assayed across all donors (data not shown), in keeping with our previously published data (42). Increased levels of seven mediators were detected in the supernatants from lung tissue stimulated with biomass smoke extract samples (Figure 6). The relative magnitude of cytokine responses varied between LPS and biomass smoke extract stimulation (Table 3).

Figure 6 and Table 3 show overall means for all samples collected from all monitoring sites in households using TCSs. The inflammatory profiles observed in lung tissue stimulated with site-specific biomass smoke extracts were quantitatively similar across all sites (Figure E4). There was a trend toward a positive response from tissue treated with ambient air samples, but no response was statistically significant.

Impact of Improved Stove Designs and Clean Fuel on Cytokine Responses

Having demonstrated that biomass smoke extracts collected from cooking with TCSs in a real-life setting induced a proinflammatory response in human lung tissue, and, given that use of ICSs and LPG reduced exposures by up to 51% and 80%, respectively, we investigated smoke extracts from cooking with ICSs and LPG on inflammatory responses in human lung tissue. The smoke samples derived from cooking with ICSs still induced an inflammatory response in human lung tissue (Figures 7A and 7B) for six of the seven analytes. Interestingly, the smoke extracts derived from cooking with LPG also induced quantifiable responses, although only increases for IL-8 and TNF-α were statistically significant over the untreated basal condition (although a smaller number of samples was available for this comparison).

Endotoxin Concentration in Indoor Air Sample

The levels of endotoxin in the indoor smoke extract samples collected during cooking using different cooking fuels were analyzed. Endotoxin was present in all samples. The highest levels of endotoxin were measured in samples using biomass fuel (5.49 ± 2.86 EU/ml); levels in samples using LPG were lower (2.14 ± 1.31 EU/ml). We also looked for potential correlations between the level of endotoxin present in samples and cytokine expression from stimulated *ex vivo* lung tissue. Expression levels of IL-8 ($r = 0.46$, $P = 0.01$) and CCL2 ($r = 0.38$, $P = 0.04$) were moderately correlated with endotoxin levels, but, although r values remained positive, no significant correlations were seen between the expression of IL-6 ($r = 0.25$, $P = 0.18$), IL-1β ($r = 0.35$, $P = 0.06$), and CCL3 ($r = 0.24$, $P = 0.2$) and endotoxin levels.

Discussion

The main aims of the studies described in this article were to measure real-life personal

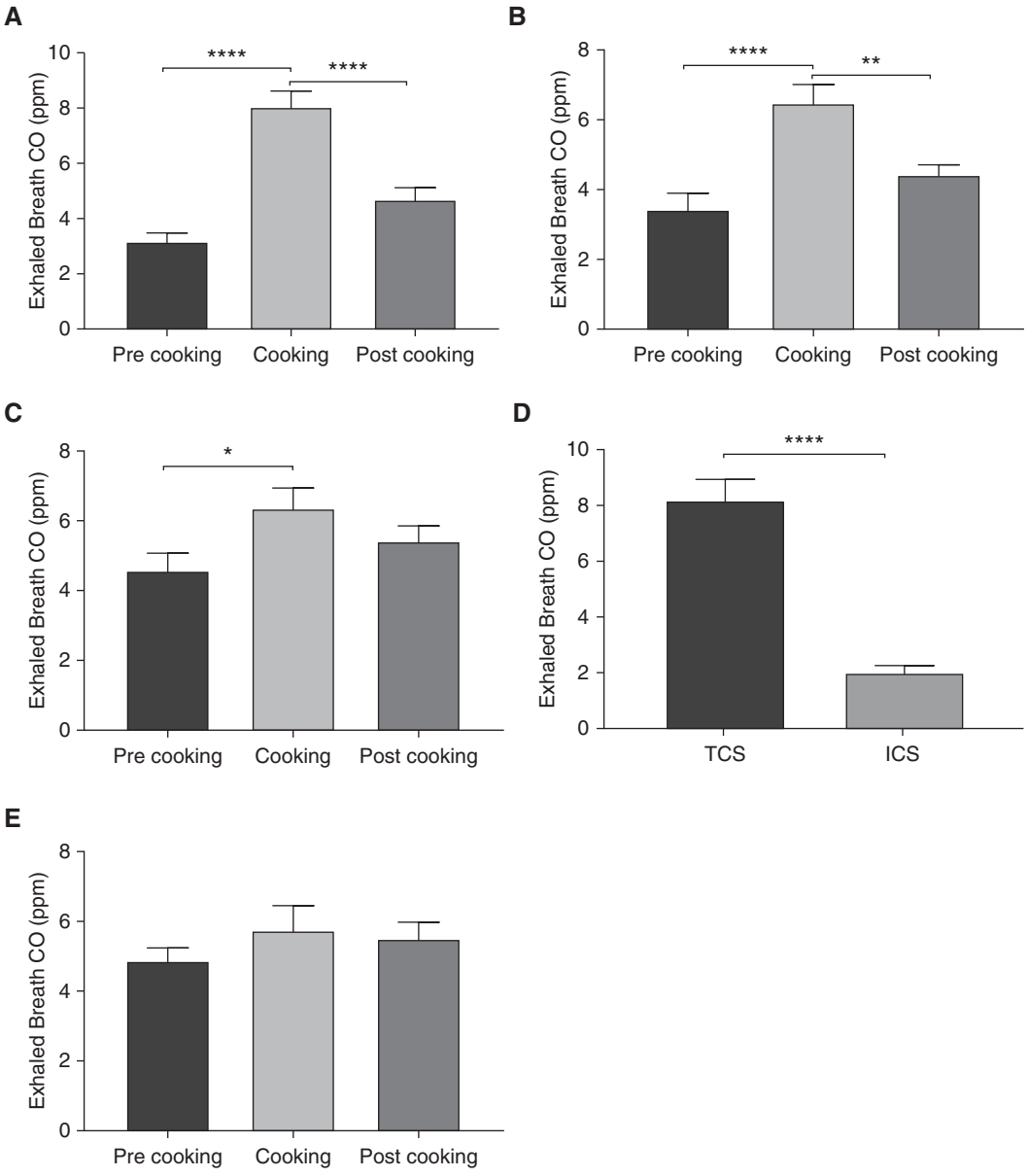

Figure 5. Exhaled breath carbon monoxide (CO) concentrations at the start of cooking, during cooking, and after cooking using different cook stoves. (*A*) Salambu using traditional cook stoves (TCSs; $n = 17$), (*B*) Pokhara using TCSs ($n = 19$), (*C*) Chitwan using TCSs ($n = 33$), (*D*) Kyanzing using TCSs ($n = 11$) and improved cook stoves (ICSs) ($n = 18$), and (*E*) Chitwan using liquefied petroleum gas (LPG; $n = 10$). The variation in exhaled breath CO concentration for each monitoring phase was assessed using one-way analysis of variance Friedman test followed by Dunn's multiple comparisons test. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{****}P < 0.0001$. The data shown are mean (±SEM) of all individuals. SEM = standard error of mean.

exposure to $PM_{2.5}$ and indoor near-stove CO concentration during indoor cooking in rural Nepal, and to gain insight into the potential effects of exposure of human lung tissue to respirable material generated from burning biomass. We studied four different sites in rural Nepal, chosen because they were in different altitude and climate regions. Although there were some differences in the absolute exposures observed between different households and in different geographical regions, the main conclusion that can be drawn from this work is that, generally, high levels of $PM_{2.5}$ exposure were seen while cooking on traditional stoves, in a range that is likely to be harmful to human health. The exposures observed in this study in different locations were between 5- and 29-fold higher than 24-hour WHO standards for indoor $PM_{2.5}$ (25 $\mu g/m^3$) (21). Very few previous studies performed in Nepal have attempted to measure personal exposure to indoor $PM_{2.5}$, although previous studies using gravimetric sampling have also shown high concentrations of indoor $PM_{2.5}$ in households using biomass fuel, consistent with our findings (23, 31). Personal exposures to $PM_{2.5}$ observed in this study were generally comparable to those

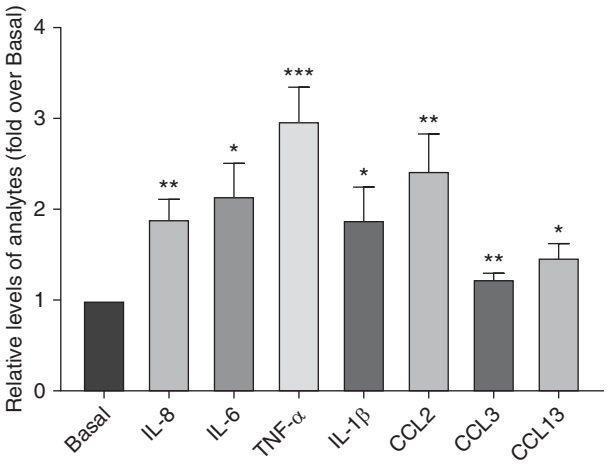

Figure 6. The inflammatory response of *ex vivo* human lung tissue after smoke extracts from traditional cook stove (TCS) stimulation. Biomass smoke stimulation showed an effect on cultured human tissue explant by significantly inducing seven analytes. The data represent mean fold increases in five independent donor experiments using biomass samples from all four monitoring sites (mean of 20 data points), and are shown as mean (±SEM). Raw data were normalized using wet tissue mass for each analyte before computing fold stimulation, and elevated levels between smoke extract–treated and untreated basal condition were checked in the raw data using Wilcoxon matched paired test. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$. CCL = C-C chemokine ligand; IL = interleukin; SEM = standard error of mean; TNF-α = tumor necrosis factor-α.

observed elsewhere in studies in lowland South Asia (26, 43). Increased exposure to CO resulted in increased exhaled CO concentrations in individuals after cooking, demonstrating that exposure to higher levels of CO in the indoor atmosphere resulted in increased levels in the lung. The high levels of $PM_{2.5}$ and CO seen in the cooking environment in the majority of rural households in Nepal are due to cooking taking place indoors, typically using traditional stove designs with no flue, and in poorly ventilated rooms. Typical households would undertake this activity either twice or three times a day, with cooking periods being up to 2 hours each. Cooking is mainly done by women, although children are often also present in the room, and these exposures are likely to be to be an important cause of chronic respiratory disease, especially in Nepalese women.

The use of improved stove designs (with a flue to vent smoke out of the room) has been found, in other settings, to be potentially effective in reducing indoor exposures (44, 45). The reduction in real-life indoor exposure with ICSs in this study was around 70%, which is in line with reductions reported in a previous study conducted in Nepal using gravimetric sampling (30). However, the exposures seen with ICSs remain well above the threshold level recommended by WHO and other organizations.

We next examined the potential for respirable biomass to produce proinflammatory effects in human lung tissue using biomass smoke extracts collected in the real-life setting. These extracts produced an inflammatory response in human lung tissue, although the magnitude of the response was less than that seen with previously investigated stimuli, such as LPS, which induces inflammation through Toll-like receptor 4 activation (42, 46, 47). Interestingly, both ICSs and LPG samples also produced an inflammatory response. One possible explanation for this is that ambient air pollution may be contributing to these responses, but there was no significant response in human lung tissue *ex vivo* to ambient air sample extracts (data not shown). It seems more likely, therefore, that the extracts contain additional inflammatory stimuli, such as volatile organic compounds, which are contributing to these responses. These data also suggest that further reductions in exposure, perhaps through source control by improved venting of fumes or better ventilation, will be required to prevent lung inflammation in real life.

Although the data presented here show clearly that high levels of personal exposure occur in rural Nepal during cooking using traditional techniques, and that these are likely to produce lung inflammation, there are some potential limitations of our work. First, it is difficult to accurately model true lung exposure, even with the approach that we have used for collecting samples in real life for human lung tissue experiments. Samples were stored at −20°C during transport, but it is still possible that some activity may have been lost during sample transfer. In addition, estimating the dilution factors used in our experiments may have resulted in underestimating true inflammatory effects. Samples were collected using a pump set at 3 L/min into a volume of 10 ml of medium: the final concentration that human lung tissue was exposed to was a 10% dilution of this extract. In the human lung, the actual tissue exposure will depend upon the dynamic equilibrium between inhaled and exhaled material, ventilation rates, the amount of lung lining fluid, and the effective volume of distribution of inhaled material. The volume

Table 3. LPS and biomass smoke extract responses in *ex vivo* human lung tissue

Analytes	IL-8	IL-6	TNF-α	IL-1β	CCL2	CCL3	CCL13
LPS stimulation fold change	7.56 (2.05)	5.86 (1.73)	196.8 (65.94)	49.45 (9.2)	6.8 (2.03)	12.93 (3.35)	4.62 (1.56)
Biomass stimulation fold change	1.89 (0.21)	2.15 (0.35)	2.97 (0.36)	1.88 (0.35)	2.9 (0.57)	1.23 (0.05)	1.47 (0.14)
% of LPS response	25	36.7	1.5	3.8	42.6	9.5	31.8

Definition of abbreviations: CCL = C-C chemokine ligand; IL = interleukin; LPS = lipopolysaccharide; SEM = standard error of the mean; TNF-α = tumor necrosis factor-α. Data are presented as mean (SEM) fold increases of each analyte after LPS and biomass stimulation ($n = 5$ independent donors experiments).

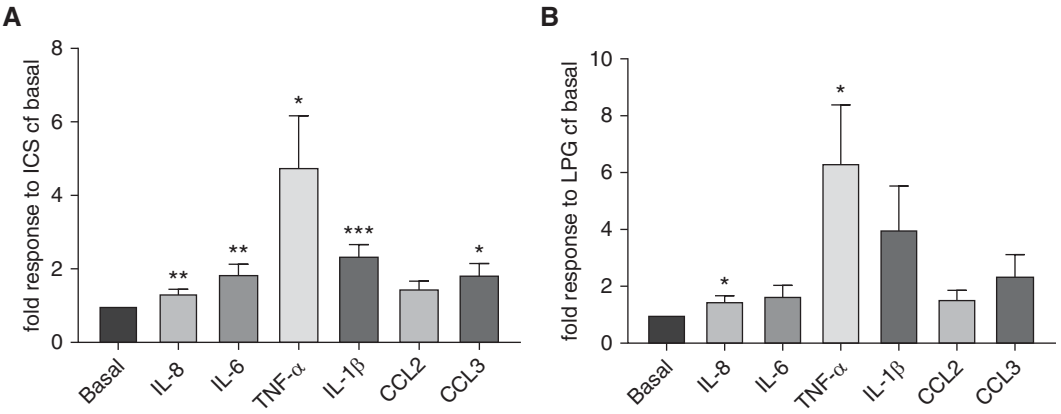

Figure 7. Comparison of concentration of analytes after stimulation with smoke extract from different cooking fuel sources. The inflammatory response seen are after stimulation with the samples from (*A*) biomass combustion in improved cook stoves (ICSs), and (*B*) liquefied petroleum gas (LPG) combustion. Data shown are the overall mean (±SEM) fold increase of 20 data points ($n = 5$ donors × 4 different ICS samples) and 10 data points ($n = 5$ donors × 2 different LPG samples). Raw data were normalized using wet tissue mass for each analyte before computing fold stimulation, and elevated levels between smoke extract–treated and untreated basal condition were checked in the raw data using Wilcoxon matched paired test. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$. CCL = C-C chemokine ligand; IL = interleukin; SEM = standard error of mean; TNF-α = tumor necrosis factor-α.

of the lung lining fluid is believed to be around 20–50 ml (48), but the true volume of distribution of the active components of inhaled biomass smoke will vary depending upon the physicochemical properties of the constituent being considered. It is possible, therefore, that we may have underestimated the true local exposure in the lung using the experimental design that we adopted. Nonetheless, we have tried to model real exposures as closely as possible.

As biomass smoke contains a wide range of potentially active compounds, including CO, volatile organic compounds, polycyclic aromatic hydrocarbons, aldehydes, free radicals, sulfur and nitrogen oxides, benzene, and particulate matter (5, 20), inflammatory responses will be driven by a range of different mechanisms. We measured endotoxin levels in our samples, and as would be expected, these were moderately elevated, and hence some of the responses could be driven directly through TLR activation. The observation that there were qualitative differences between LPS responses and biomass responses suggests that a range of pathways is likely to be involved.

In summary, we have shown, for the first time, that biomass smoke samples collected in a real-life environment from rural Nepal have proinflammatory effects in human lung tissue. These data support the need to reduce exposures to improve respiratory health in this setting, but suggest that additional methods other than those currently being trialed may be needed to reduce exposures to levels that will prevent lung inflammation from occurring in real-life settings. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

Acknowledgment: The authors give special thanks to all members of the Atmosphere Initiative at International Centre for Integrated Mountain Development for logistical support for all field work. The authors also thank members of Respiratory Medicine Division at University of Nottingham for helping in laboratory work and providing suggestions and comments on the initial version of the manuscript.

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