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Inflammatory Markers in Blood and Exhaled Air after Short-Term Exposure to Cooking Fumes

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Objectives: Cooking fumes contain aldehydes, alkanoic acids, polycyclic aromatic hydrocarbons, and heterocyclic compounds. The inhalation of cooking fumes entails a risk of deleterious health effects. The aim of this study was to see if the inhalation of cooking fumes alters the expression of inflammatory reactions in the bronchial mucosa and its subsequent systemic inflammatory response in blood biomarkers.

Methods: Twenty-four healthy volunteers stayed in a model kitchen on two different occasions for 2 or 4 h. On the first occasion, there was only exposure to normal air, and on the second, there was exposure to controlled levels of cooking fumes. On each occasion, samples of blood, exhaled air, and exhaled breath condensate (EBC) were taken three times in 24 h and inflammatory markers were measured from all samples.

Results: There was an increase in the concentration of the *d*-dimer in blood from 0.27 to 0.28 mg ml^{-1} on the morning after exposure to cooking fumes compared with the levels the morning before (*P*-value = 0.004). There was also a trend of an increase in interleukin (IL)-6 in blood, ethane in exhaled air, and IL-1β in EBC after exposure to cooking fumes. In a subanalysis of 12 subjects, there was also an increase in the levels of ethane—from 2.83 parts per billion (ppb) on the morning before exposure to cooking fumes to 3.53 ppb on the morning after exposure (*P* = 0.013)—and IL-1β—from 1.04 on the morning before exposure to cooking fumes to 1.39 pg ml^{-1} immediately after (*P* = 0.024).

Conclusion: In our experimental setting, we were able to unveil only small changes in the levels of inflammatory markers in exhaled air and in blood after short-term exposure to moderate concentrations of cooking fumes.

Keywords: cooking fume; exposure; human experiment; inflammation; inhalation

INTRODUCTION

When food is prepared, often at temperatures up to 300 °C, carbohydrates, proteins, and fat are degraded into potentially harmful substances, such as aldehydes and alkanoic acids (Vainiotalo and Matveinen 1993; Robinson *et al*. 2006).

Cooking fumes, especially from frying, contain fine and ultrafine particles (UFP) and several specific agents (Svendsen *et al*. 2002; Wallace, Emmerich, and Howard-Reed 2004; Afshari, Matson, and Ekberg 2005; Sjaastad and Svendsen 2008; Sjaastad *et al*. 2010). Earlier studies have

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shown that frying mainly produces particles of sizes <0.5 μm (Abt *et al*. 2000; Sjaastad and Svendsen 2008) and it seems as if the level of UFP is higher during frying on a gas stove compared to frying over an electric stove (Sjaastad *et al*. 2010). The different chemical substances identified in cooking fumes include aldehydes, polycyclic aromatic hydrocarbons (PAHs), heterocyclic amines, aromatic amines, and alkanoic acids (Vainiotalo and Matveinen 1993; Svendsen *et al*. 2002; Robinson *et al*. 2006; Sjaastad and Svendsen 2008; Sjaastad *et al*. 2010). Personal measurements performed during the frying of beefsteak on both gas and electric stoves have shown that cooks are exposed to PAHs, though in low concentrations (Sjaastad and Svendsen 2009; Sjaastad *et al*. 2010). Cooking with oils rich in polyunsaturated fatty acids at high temperatures also generates various higher aldehyde species, i.e. aldehydes with a higher number of carbon atoms, such as *trans*-2-alkenals, *trans*,*trans*-alka-2,4-dienals and *n*-alkanals, arising from the fragmentation of conjugated hydroperoxy diene precursors (Gertz 2000). Both short-chain aldehydes and higher aldehydes have been detected in the breathing zone of cooks, both in restaurants and during the normal domestic frying of beefsteak (Svendsen *et al*. 2002; Sjaastad and Svendsen 2008, 2009; Sjaastad *et al*. 2010). The levels of the specific agents and particles, however, do not correlate well when measurements are done during the preparation of different types of food (Sjaastad and Svendsen 2009; Sjaastad *et al*. 2010). Both the brand and the age of the cooking oil may also have implications for the composition of the emission (Sjaastad *et al*. 2010). This implies that measuring "total particles" is not a good indicator of exposure to potentially harmful components in cooking fumes. It is still unknown which specific components in cooking fumes may contribute to an increased risk of specific diseases like asthma and cancer.

Previous studies have shown that cooks show an increased prevalence of respiratory distress, increased mortality from airway disease (Svendsen, Sjaastad, and Sivertsen 2003; Borgan 2009), and increased risk of ischemic heart disease (Sjögren, Barlow, and Weiner 2009). A link between the inhalation of respiratory irritants and increased blood coagulability was suggested >15 years ago. Several subsequent studies have found increments in inflammatory markers after various inhalation exposures (Sjögren *et al*. 1999; Corradi *et al*. 2002; Hilt *et al*. 2002; Barregard *et al*. 2006; Barreto *et al*. 2006; Boyce *et al*. 2006; Caglieri *et al*. 2006; Barregard *et al*. 2008; Samet *et al*. 2009). Exposure to cooking fumes has also been associated with an increased risk of respiratory cancer (Coggon *et al*. 1986; Zhong *et al*. 1999; Yang *et al*. 2000; Zhou *et al*. 2000). Based on 'limited evidence in humans, and sufficient evidence in experimental animals', the International Agency for Research on Cancer (IARC) recently classified 'emissions from high temperature frying' to be probably carcinogenic to humans (IARC 2010).

Oxidative stress and inflammatory processes have been suggested to be central parts of the mechanism behind the deleterious effects of cooking fumes (Tung *et al*. 2001; Chang, Lo, and Lin 2005; Wang *et al*. 2010). Such reactions can be assessed both in blood and in exhaled air. The measurement of inflammatory markers in exhaled air provides a possibility of examining the status of the respiratory tract with non-invasive procedures that can be repeated at short intervals (Gergelova *et al*. 2008). Exhaled air consists of a gaseous phase that contains volatile substances, such as nitric oxide (NO) and hydrocarbons, and vapour with epithelial lining fluid that can be captured in an exhaled breath condensate (EBC; Mutlu *et al*. 2001).

The measurement of exhaled nitric oxide (eNO) is a method of assessing airway inflammation, which has proved useful for monitoring patients with asthma [American Thoracic Society and the European Respiratory Society (ATS/ERS) 2005; Corradi and Mutti 2005]. The level of the aliphatic hydrocarbon ethane in exhaled air is seen as a marker of free radical-induced lipid peroxidation (Kneepkens, Lepage, and Roy 1994). Clinically, this has been used to monitor airway reactions in smokers (Puri *et al*. 2008) and to follow patients with interstitial lung disease (Kanoh, Kobayashi, and Motoyoshi 2005).

The levels of interleukin-1β (IL-1β), leukotriene B_4 (LTB_4) and 8-isoprostane in EBC are considered to reflect inflammatory reactions in the airways (Horvath et al. 2005). IL-1β is an important early-response cytokine and was, therefore, chosen as an interesting inflammatory biomarker for analysis. LTB_4 is linked to neutrophil-driven inflammation and 8-isoprostane is a marker of oxidative stress, which is a central part of the airway reaction to cooking fumes. EBC has been previously used for not only disease surveillance mainly but also evaluation of the effects of exposure to xenobiotics in a few studies (Corradi *et al*. 2002; Barreto *et al*. 2006; Boyce *et al*. 2006; Caglieri *et al*. 2006; Barregard *et al*. 2008)

For preventive purposes, we find it worthwhile to try to refine non-invasive methods for the early detection of adverse respiratory and systemic effects in subjects exposed to xenobiotics. The aim of this

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study was to see whether the inhalation of cooking fumes alters the expression of inflammatory reactions in the bronchial mucosa and its subsequent systemic inflammatory response in blood biomarkers.

SUBJECTS AND METHODS

Subjects

There were 24 non-smoking students without any current respiratory diseases who volunteered to participate in the study. They were divided into two groups (A and B) of 12 subjects each, and they were examined on two different occasions under slightly different conditions. Lung function measurements in the same groups have been reported earlier (Svedahl *et al*. 2009).

Exposure model

On the same weekday of two consecutive weeks, the study subjects spent time in a model kitchen and had samples taken. For all the subjects, the first week was spent without any exposure other than to normal air, and the second week, with exposure to generated cooking fumes. The cubic content of the kitchen was 56 m^3 (2.5 × 4 × 5.6 m). The door and the window were kept closed, and the only ventilation was a kitchen hood with a capacity to extract up to 600 m^3 h^{-1}. In their second period in the model kitchen, all subjects were exposed to controlled levels of cooking fumes during the pan frying of beef on an electric hob for the first group of 12(Group A) and on a gas hob for the second group of 12(Group B). The concentration of cooking fumes was monitored with an MIE pDR-1200 optical aerosol monitor (Thermo Andersen, Inc., Smyrna, GA, USA) located on a table ~1.5 m from the cooking device and set to register the concentration of PM5 particulate matter in which 50% of particles have an aerodynamic diameter of less than 5 μm aerosols. The levels were kept at 8–10 mg m^{-3} for Group A and at 10–14 mg m^{-3} for Group B by adjusting the quantity of beef in the pan, the extraction level of the kitchen hood, and the effect level of the hotplate or the gas burner. Four subjects participated each time on the days of the experiment. The subjects in Group A stayed in the kitchen for 2 h, and on the day with exposure to cooking fumes, each person performed the frying on three occasions for approximately 15 min each time. The subjects in Group B stayed in the kitchen for 4 h, and on the day with exposure to cooking fumes, each person performed the frying on three occasions for approximately 25 min each time. Table 1 summarizes the time frame of the occasions when subjects from Groups A and B stayed in the kitchen and when samples were taken.

During the stay in the kitchen on the exposure day, each person was equipped with a sampling device for the measurement of personal exposure to total particulate matter. For the sampling, pre-weighed, double Gelman AE glass fibre filters (37 mm) were placed in a closed face, clear styrene, acrylonitrile cassette connected to a pump (Casella Vortex Standard 2 personal air-sampling pump, Casella CEL, Bedford, UK) and set at an air flow of 2 l min^{-1}. The filters were placed on the right shoulder of the participant. Before and after sampling, the filters were conditioned in an exicator for 24 h. The filters were analysed gravimetrically, using a Mettler balance (0.01 mg resolution). An inner calibration was performed on the balance before every weighing. Blank filters were included in the analysis in order to control for deviations caused by temperature or humidity.

Sampling of biomarkers

Three samples of blood and exhaled air were taken from the participants during two consecutive 24-h periods with one week in between. The first period with sampling was without any other exposure than normal air, and during the second period, the participants were exposed to controlled levels of cooking fumes. On both occasions, the three sampling points in time were (i) 0: the morning before entering the kitchen, (ii) 1: when leaving the kitchen after 2 (Group A) or 4 h (Group B), and (iii) 2: 24 h after entering the kitchen. The programme of the participants on the unexposed days was exactly the same as on the days with exposure in regard to location and activities, except for the cooking activities. This facilitated an evaluation of the subjects as their own controls, making it possible to compare each subject's development in terms of the levels of biological markers in exhaled air and blood during a period with short-term exposure to cooking fumes with the development in levels of biological markers from a period without such exposure.

EBC was collected using a breath condenser (ECoScreen; Jaeger, Wurzburg, Germany). The subjects rinsed their mouth with water. In a sitting position, wearing a noseclip, they breathed tidally for 15 min through a two-way non-rebreathing valve, which also served as a saliva trap. In order to avoid loss of molecules from inflammatory markers due to adhesion to the walls, the tubes were coated with 1% bovine serum albumin and 0.01% Tween 20 for 30 min according to previous optimization procedures (Tufvesson and Bjermer 2006). The EBC samples were immediately frozen at –70 °C. Owing to low concentrations in EBC, samples were concentrated [5 times (IL-1β) and 10 times (LTB_4 and

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Table 1. Timing of the exposure and the sampling of exhaled breath, exhaled breath condensate (EBC), and blood in the subjects who first entered the exposure chamber on each of the days of the experiments and a comparison of the timing for Groups A and B.

Action time	Sample number	Sampled	Subjectnumber	Time of the day	
				Group A(3 × 4 persons)	Group B(3 × 4 persons)
Morning before entering the kitchen	0	Exhaled breath, EBC, and blood	1	8:30	8:30
			2	8:45	8:45
			3	9:00	9:00
			4	9:15	9:15
Period in the kitchen (same day)			1	9:00–11:00,	9:00–13:00
			2	9:15–11:15,	9:15–13:15
			3	9:30–11:30,	9:30–13:30
			4	9:45–11:45	9:45–13:45
Immediately after leaving the kitchen(same day)	1	Exhaled breath, EBC, and blood	1	11:05	13:05
			2	11:20	13:20
			3	11:35	13:35
			4	11:50	13:50
Next morning	2	Exhaled breath, EBC, and blood	1	8:30	8:30
			2	8:45	8:45
			3	9:00	9:00
			4	9:15	9:15

8-isoprostane)] by freeze-drying and resolved in the respective assay buffer [as previously described (Tufvesson *et al*. 2010)]. The final concentrations were calculated from the specific freeze-dried volumes. LTB_4 and 8-isoprostane were analysed using the EIA kit from Cayman Chemical (Ann Arbor, MI) with a detection limit of 6 and 2.7 pg ml^{-1} respectively. IL-1β was measured using Quantikine HS from R&D Systems (Minneapolis, MN) with a detection limit of 0.05 pg ml^{-1}.

Measurements of eNO were performed by using a Logan LR 2000 chemiluminescence analyser (Logan Research Ltd, UK) in accordance with recommendations by the ATS/ERS (2005). The subjects were in a sitting position, exhaling from total lung capacity to residual volume against a resistance of 4–5 cm water, aiming at a flow rate of 250 ml s^{-1}, aided by a biofeedback monitor. For each subject, the mean of three plateau levels from acceptable eNO curves was registered in parts per billion (ppb).

Ethane in exhaled air was sampled in a 50-ml polypropylene syringe (Terumo) graded to contain 60 ml. A bacteria/virus filter (Icor, leda, Norway) and a non-rebreathing valve were connected to an exhaled air reservoir. The subjects were asked to perform three deep breaths through the mouthpiece mounted on the reservoir. Ethane samples were taken from the reservoir in three parallels. The samples were kept in a refrigerator and analysed within 24 h. Samples of background air were taken at each sampling time. The samples were analysed using a gas chromatograph (HRGC Mega 2 Model 8530, Fisons Instruments S.p.A. Milan, Italy) with a flame ionization detector and two capillary columns [GC-Q, 30 m, internal diameter 0.53 mm (J&W Scientific, Folsom, CA] using a cryofocusing technique described elsewhere (Dale *et al*. 2003).

IL-6 level in serum was measured with the commercial ELISA kit Human Interleukin-6 UltraSensitive (Biosource, Belgium). After standard incubations, the optical density was measured at 450 nm (Wallac Victor^3™ 1420 Multilabel Counter, Perkin Elmer, Shelton, CT, USA). Fibrinogen and *d*-dimer in blood were analysed at the Department of Clinical Chemistry at the St Olavs University Hospital of Trondheim. The fibrinogen concentration in plasma was measured by a Fibri-Prest automated by the clotting method of Clauss (Clauss 1957). *D*-dimer was measured by a latex-enhanced immunoturbidimetric method with an automated chemical analysis system (Roche modular-P, Mannheim, Germany) according to the protocols of the manufacturer.

Statistical analysis

We used mixed effects modelling with a random intercept to study the association between exposure

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to cooking fumes and the various outcomes. To account for the tendency of log normality, the data was log-transformed and the results presented as geometric means with a 95% confidence interval. In the model, exposure and baseline measurements were included as covariates together with a time variable, and measurements at Occasions 1 and 2 were included as the outcome variable. The data analysis was performed using Stata for Windows version 11. The analysis was performed both separately for the Groups A and B and combined for the two groups, and the chosen significance level was 5%.

Ethical considerations

The study was approved by the ethical committee for medical research in Central Norway. Participation was voluntary and all persons gave their informed consent prior to their inclusion in the study. Written information about the project was given to every participant, also stating that he/she could withdraw from the study at any time. All participants received an allowance for their participation.

RESULTS

Some background variables and mean measured concentrations of cooking fumes on the day with exposure for the participants are given in Table 2. The levels of personal exposure to particles measured by gravimetric analysis on the exposure days ranged from 13.8 to 32.9 mg m^{-3} for Group A and from 31.2 to 54.9 mg m^{-3} for Group B. In regard to current medication, one subject in Group A used contraceptives, and in Group B, two subjects were on contraceptives, two used antihistamines, and one used insulin. No other medication was reported.

Table 3 shows the geometric mean concentrations for inflammatory markers in blood, in exhaled air, and in EBC on the three points of measurements during the week without any exposure other than normal air and during the week with exposure to cooking fumes. When comparing the development in levels of inflammatory markers for all 24 subjects during the 2 weeks, the only difference that reached statistical significance on the 5% level was a slight increase in the concentration of *d*-dimer in blood from the morning before exposure to cooking fumes to the morning after, compared to a slight decrease from the morning before exposure to normal air to the morning after ($P = 0.004$). There also seemed to be a trend of an increase in *d*-dimer and IL-6 in blood and IL-1β in EBC immediately after exposure to cooking fumes. The concentration of ethane in exhaled air showed a trend to increase both immediately after exposure to cooking fumes and on the morning after. The levels of 8-isoprostane and LTB_4 in EBC showed a trend to decrease more after exposure to cooking fumes than after exposure to normal air, whereas the levels of fibrinogen in blood and NO in exhaled air showed only minor variations.

A separate analysis of Groups A and B was performed, but because evidence of heterogeneity was only found for the results from the data of 8-isoprostane, the combined analysis was preferred for the interpretation of the data. The results from the separate analysis may, however, be viewed in the supplementary data (available at *Annals of Occupational Hygiene* online), but the estimates in this analysis should be interpreted with more caution because they seem less robust than those in the combined analysis. Apart from the development of *d*-dimer, post-exposure increases were found only for Group A, which showed an increase in ethane from 2.83 ppb (in the morning before exposure to cooking fumes) to 3.53 ppb (in the morning after; $P = 0.013$) and an increase in levels of IL-1β from 1.04 to 1.39 pg ml^{-1} from the morning before exposure to cooking fumes to immediately after ($P = 0.024$). Group A showed a decrease in levels of 8-isoprostane and LTB_4 (*P*-values = 0.003–0.022) both immediately after exposure to cooking fumes and on the morning of the day after (Time points 1 and 2).

The estimates from the combined analysis on the development of the levels of inflammatory markers are presented in Fig. 1 as 'net changes', which were calculated from the differences in levels from before

Table 2. Mean exposure to particles from cooking fumes and the characteristics for all the 24 volunteers who participated in the study and separate for the two groups A (2-h exposure) and B (4-h exposure).

Group	Personalexposure (mg m–3)	Sex	Age, years	Height, cm	Weight, kg	Known allergy	Current medication
Group A, mean (SD)	19.5 (5.9)	33% female	24.5 (1.8)	177.5 (12.7)	75.3 (16.4)	25%	8.3%
Group B, mean (SD)	42.8 (9.0)	50% female	23.1 (3.0)	172.3 (8.1)	70.7 (13.2)	42%	41.7%
All 24, mean (SD)	31.1 (14.0)	42% female	23.8 (2.5)	174.9 (10.8)	73.0 (14.7)	33%	25.0%

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Table 3. Inflammatory markers before and after the stay in the kitchen with exposure to normal air and cooking fumes for all 24 subjects are given as the geometric mean (95% confidence interval).

		Exposure to normal air			Exposure to cooking fumes		
Timing of sample		0 = Before exposure[a]	1 = Immediately after exposure[b]	2 = 24 h after start of exposure[c]	0 = Before exposure[a]	1 = Immediately after exposure[b]	2 = 24 h after start of exposure[c]
Exhaled air	Ethane (ppb)	2.22 (1.97–2.51)	2.31 (2.03–2.63)	2.32 (2.04–2.63)	2.39 (2.12–2.70)	2.59 (2.30–2.93)	2.77 (2.44–3.14)
	eNO (ppb)	4.96 (4.22–5.83)	5.12 (4.35–6.01)	5.29 (4.50–6.22)	5.11 (4.35–6.01)	5.22 (4.44–6.13)	5.25 (4.47–6.17)
EBC	IL-1β (pg ml^{-1})	0.84 (0.64–1.10)	0.87 (0.66–1.15)	0.80 (0.61–1.06)	0.90 (0.70–1.15)	1.12 (0.87–1.44)	0.95 (0.74–1.23)
	8-isoprostane (pg ml^{-1})	4.54 (3.31–6.24)	3.83 (2.78–5.26)	3.15 (2.29–4.33)	4.87 (3.66–6.47)	3.33 (2.49–4.45)	3.25 (2.45–4.32)
	LTB_4 (pg ml^{-1})	5.60 (4.28–7.33)	5.70 (4.35–7.46)	5.05 (3.86–6.61)	7.20 (5.62–9.24)	5.28 (4.12–6.78)	5.22 (4.07–6.70)
Serum	Fibrinogen (g dl^{-1})	2.66 (2.46–2.88)	2.65 (2.45–2.87)	2.63 (2.43–2.85)	2.68 (2.47–2.90)	2.60 (2.40–2.81)	2.71 (2.50–2.93)
	IL-6 (pg ml^{-1})	0.43 (0.31–0.59)	0.41 (0.30–0.57)	0.33 (0.24–0.46)	0.34 (0.25–0.47)	0.41 (0.30–0.56)	0.33 (0.24–0.45)
	d-dimer (mg ml^{-1})	0.29 (0.24–0.35)	0.28 (0.23–0.33)	0.25 (0.21–0.30)	0.27 (0.23–0.32)	0.28 (0.23–0.33)	0.28 (0.24–0.34)

[a]In the morning before entering the kitchen.
[b]When leaving the kitchen after 2 h (Group A) or 4 h (Group B).
[c]Twenty-two hours after leaving the kitchen (Group A) or 20 h after leaving the kitchen (Group B).

the exposure to after the exposure and by subtracting any change that occurred from before staying in the kitchen without exposure to the level after. In addition to the statistically significant development for *d*-dimer, Fig. 1 shows a trend of an increase in levels of ethane, IL-1β, and IL-6 both immediately after exposure to cooking fumes (Time 1) and the morning after (Time 2).

DISCUSSION

The levels of inflammatory markers showed a different development during the period with exposure to cooking fumes compared to the period with exposure to normal air. For some of the markers, the differences reached statistical significance, whereas for most of them, there were only trends. The measured levels of *d*-dimer in plasma developed statistically significantly differently during the two periods of the experiment. In the separate analysis for the Groups A and B, there seemed to be a statistically significant increase in ethane in exhaled air and IL-1β in EBC after exposure to cooking fumes for Group A, but not for Group B. The slight increase in *d*-dimer in blood, IL-1β in EBC, and ethane in exhaled air could reflect an inflammatory response subsequent to the exposure to cooking fumes. It is, however, not entirely clear whether such a response is to be considered pathological or physiological. An increase in *d*-dimer after short-term experimental exposure to UFP has been reported earlier. It has been hypothesized that this increase in *d*-dimer after exposure to UFP reflects a systemic stimulation of fibrin formation, leading to a change in blood coagulability and probably contributing to adverse effects imposed on the arterial endothelium (Samet *et al*. 2009). The lack of a simultaneous increase in the levels of fibrinogen in plasma could be due to different mechanisms as the manner of even normal catabolism is still uncertain, although an endothelial catabolic pathway is suggested as the main pathway (Aliberti *et al*. 2005). Changes in fibrinogen metabolism caused by inflammation are characterized by increased synthesis (Mansoor *et al*. 1997), but this may also be followed by an increased decomposition that can be measured as an increase in levels of *d*-dimer.

IL-1β is one of the most important early-response cytokines, and high levels have been found in fluid obtained by bronchoalveolar lavage of patients with sustained acute respiratory distress syndrome (Park *et al*. 2001). The observed trend towards an increase in IL-1β in EBC immediately after exposure to cooking fumes (although statistically significant only for Group A) seems coherent with the early role of this cytokine in the inflammatory response.

Variations in exhaled ethane have mainly been regarded as an acute and transient reaction in the airway epithelium as a result of oxidative stress

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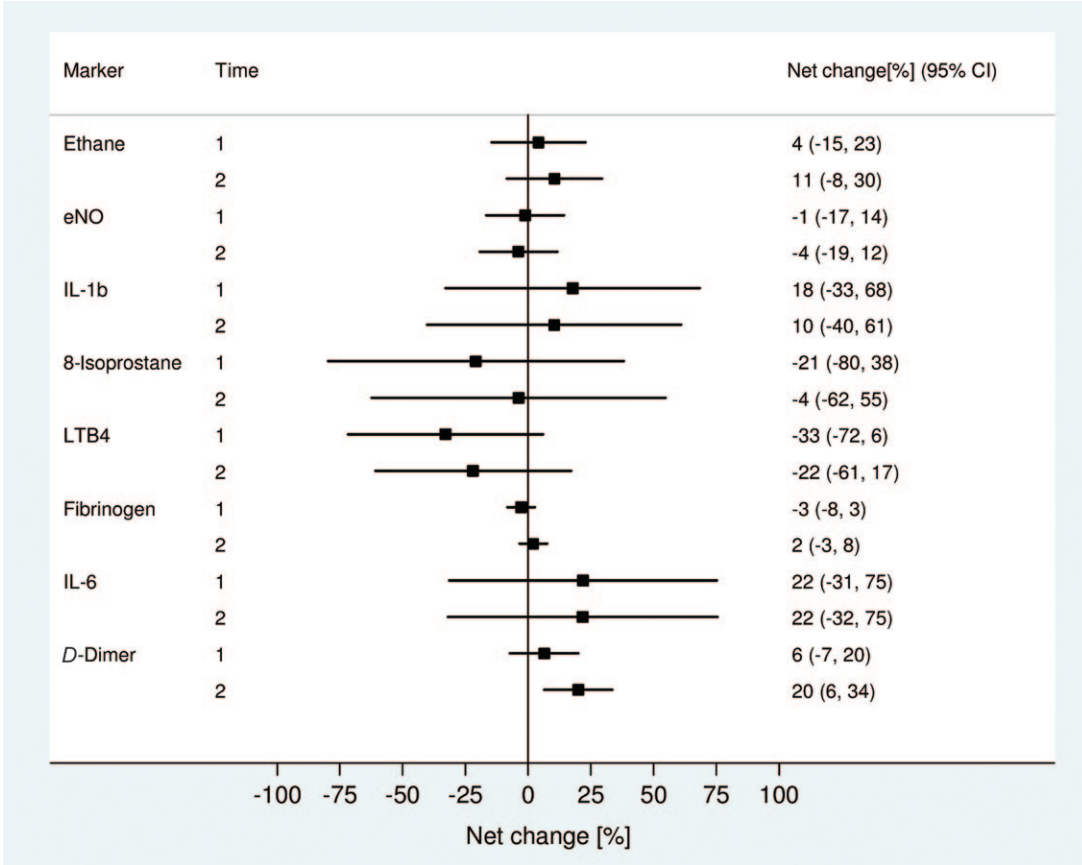

Fig. 1. Net change in levels of inflammatory markers from exposure to cooking fumes for all 24 subjects, according to time since exposure. The estimates are presented as geometric mean changes in percent (95% CI). Time 1 is the net change in levels of inflammatory markers from before exposure to immediately after the exposure. Time 2 is the net change in levels from before entering the kitchen to 24 h after entering the kitchen.

(Kneepkens, Lepage, and Roy 1994; Kanoh, Kobayashi, and Motoyoshi 2005; Gorham *et al.* 2009). However, because the possible increase in our study was found 24 h after the exposure, one could perhaps also regard this as a sign of a more persistent reaction.

In the separate analysis, 8-isoprostane showed a somewhat unexpected development for Group A, with a relatively high starting level the morning before exposure to cooking fumes, followed by a decrease both immediately after the exposure to cooking fumes and the morning after. A similar pattern can also be seen for LTB_4. When looking at the three morning samples taken without any prior experimental exposure, which are presented in Table 3 (Samples 0 and 2 in the week without exposure, and Sample 0 in the week with exposure), it turned out that the day-to-day variation in measured levels of the inflammatory markers in EBC was high. When viewed in this context, the slightly paradoxical post-exposure decrease in LTB_4 and 8-isoprostane seems more likely to be a result of unexplained variability in the estimates rather than an effect related to the exposure. This shows the importance of including situations without exposure in similar studies in order to explore and account for diurnal variability and other systemic effects. In the present study, all the subjects went through a session with exposure to normal air first and then a session with exposure to cooking fumes a week later. As has also been argued in other studies (Barregard *et al.* 2008), we chose not to randomize the order of these sessions because possible long-lasting inflammatory effects from the exposure to cooking fumes could then have affected a control session 1 week later, whereas the opposite seemed less likely.

The low number of participants and the low statistical power of this study might have led to bias with

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more non-positive than negative results. *A priori* power estimates were not attempted because there were scarce data on how large the effects could be expected to be from the chosen short-term exposure to cooking fumes on the levels of the inflammatory markers. There are, however, some quite similar studies of EBC in different occupational settings, which have unveiled effects from specific exposures to airway irritants on exhaled markers (Barreto *et al*. 2006; Boyce *et al*. 2006; Caglieri *et al*. 2006; Barregard *et al*. 2008). Changes in markers in blood have been shown in other studies with a comparable sample size (Sjögren *et al*. 1999; Corradi *et al*. 2002; Hilt *et al*. 2002; Barregard *et al*. 2006; Barregard *et al*. 2008; Samet *et al*. 2009). The higher share of non-positive results in our study could indicate that short-term exposure to cooking fumes has less or a different effect than the exposures to other airway irritants, such as ozone, welding fumes, swine dust, ship engine work, tunnel work, and chromium, which have been investigated in similar studies previously. Alternative explanations could be that the methodology applied in our study was less sensitive or that controlling for variation during a period without exposure made our study more conservative than some of the previous studies. Some of the studies mentioned herein were performed in workers at their worksites and some on voluntary subjects as in our study. A possible difference in reaction between subjects with previous exposure and subjects without, due to sensitization, would be interesting to elucidate in future studies.

The two groups A and B had some differences in the exposure, with Group B having both a longer exposure time and higher concentrations of cooking fumes. Nevertheless, the difference in exposure for Groups A and B should probably be regarded as minor and low compared to the levels of exposures that might be needed to induce easily detectable inflammatory changes. Thus, clear differences in the measured effects on inflammatory markers between the two groups may not have been expected. This may explain why the test of heterogeneity shows mostly no difference in the results between the two groups and supports the decision of interpreting the data for all 24 subjects together. However, when exploring the data separately for the two groups, one might get the impression that the exposure to cooking fumes had more effect on the subjects in Group A than the subjects in Group B, even though the subjects in Group B experienced a somewhat higher exposure. There might be unknown factors within the subjects making some of them more susceptible to being affected by the exposure. In the present study, with few subjects, there is a chance that such factors may be distributed unequally between the two groups A and B, contributing to such slightly paradoxical observations.

Apart from being non-smokers and free of respiratory disease, we applied no other inclusion criteria for the study subjects. It turned out that eight of the 24 subjects reported having had allergy. We lacked information about what kind of allergies, but none of the subjects had allergic symptoms when the investigations were done. However, two of them used antihistamine medication at the time of the experiment. When looking separately at the groups with reported allergy and the use of medication, they did not differ from the whole group with regard to any outcome, neither did excluding them from the statistical analysis change the results. At the time of the experiments, we tried to be careful to instruct the participants to act normally and without excesses of any kind during the 2 weeks. Even so, it cannot be ruled out that some of them have had variations in their day-to-day physical activities that may have influenced the results. Variation in outdoor temperatures during the days of the experiments is another factor that is beyond control. On the days of the present study, the mean temperatures ranged from –7.3 to + 16.8 °C for Group A and from +5.4 to + 19.4 °C for Group B. As this might have influenced the results, we would with hindsight, recommend performing such experimental studies under more stable climatic conditions.

It is conceivable that the levels of exposures to cooking fumes applied in our study were representative for conditions that can be found in both professional and domestic settings (Svendsen *et al*. 2002; Sjaastad and Svendsen 2009). In any case, the applied exposures must be regarded as moderate. As the exposures for both groups, and in particular for Group B, were at levels that led to some subjective discomfort, we did not find it appropriate to go any higher. Yet, the exposures may still have been too low and/or too short to cause the expected effect. However, in a similar study with exposure to UFP for 2 h, cardiac effects and mild inflammatory and prothrombic responses were shown (Samet *et al*. 2009).

Most previous studies on the effects from exposure to cooking fumes have looked at manifest diseases and chronic respiratory effects following chronic exposures (Coggon *et al*. 1986; Ng, Hui, and Tan 1993; Ng and Tan 1994; Zhong *et al*. 1999; Zhou *et al*. 2000; Svendsen, Sjaastad, and Sivertsen 2003). In the present study, we focused on the possible acute responses to short-term exposure to cooking fumes. To our knowledge, this is the first study to look at inflammatory markers in that context.

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CONCLUSION

In our experimental setting, we were able to unveil only small changes in the levels of inflammatory markers in exhaled air and in blood after short-term exposure to moderate concentrations of cooking fumes. The applied methods can be of relevance for identifying reactions in the airway mucosa and for the prevention of chronic respiratory diseases.

SUPPLEMENTARY DATA

Supplementary data can be found at http://annhyg.oxfordjournals.org/.

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