UNIVERSITY OF CAPE TOWN

Assessing the health impact of intervention in supermarket bakeries using fractional exhaled nitric oxide (FeNO) and other clinical endpoints for baker's allergy and asthma

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DECLARATION

I, Faisal Al Badri, hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

I empower the university to reproduce for the purpose of research either the whole or any portion of the contents in any manner whatsoever.

Signature: Signed by candidate

Date: 14 March 2018

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DEDICATION

عَلى قَدرِ أَهلِ العَزمِ تَأتي العَزائِمُ وَتَأتي عَلى قَدرِ الكِرامِ المَكارِمُ

وَتَعظُمُ في عَينِ الصَغيرِ صِغارُها وَتَصغُرُ في عَينِ العَظيمِ العَظائِمُ

This thesis work is dedicated to my God, Allah, who has blessed me with strength and health to finish this project.

To my mentor and leader His Majesty Sultan Qaboos bin Said, The Sultan of Oman, for his great and unlimited generosity in granting me this scholarship and honouring me with this invaluable opportunity to study and serve my dear country and his great citizens. Oman and its citizens always pray for you because you have transformed the country to what it is today. May Allah bless you, sir.

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PUBLICATIONS AND PRESENTATIONS

Aspects of this dissertation have been published previously (appendix 1):

Al Badri FM, Jeebhay MF. Factors associated with serial longitudinal changes in exhaled nitric oxide (FeNO)–a review of the literature. Current Allergy and Clinical Immunology. 2017;30(2):98-109.

ABSTRACT

Aim: To assess the health impact of an intervention in supermarket bakeries using fractional exhaled nitric oxide and other clinical endpoints for baker's allergy and asthma after a one-year follow-up period.

Methods: A field randomised controlled trial of 31 bakeries initially assigned to one of two intervention groups (bakery mixer lid and training) (n=244) and a control group (n=93). Health data prior to and after the intervention included a modified ECRHS questionnaire; Phadiatop ® and serum specific IgE to cereal flours (wheat, rye, alpha-amylase); and FeNO performed during the work shift using NIOX MINO®. The data of the two intervention groups was combined into one intervention group for the analysis. Data was analysed using STATA (version 12).

Results: The two groups were comparable with regard to age (32-33 years), proportion of females (55%-57%) and smoking status (38%-40%). The intervention group had a significantly higher prevalence of workers with atopy (42%, p=0.025), work-related chest symptoms (25%, p=0.044) and sensitisation to cereal flour allergens (35%, p=0.042) at baseline than the control group (25%, 15%, 23% respectively). At one year of follow-up, the incidence and level of decline of work-related ocular-nasal and chest symptoms, sensitisation status and elevated FeNO (FeNO >25ppb) was similar in the two groups. The mean difference in FeNO was similar across the two groups (2.2ppb vs 1.7ppb, p=0.860). However, when stratifying according to baseline FeNO >25ppb, the FeNO decline was greater in the intervention group (16.9 ppb) than in the control group (7.7ppb), although not statistically significant (p=0.237). Multivariate logistic regression models (adjusting for smoking, baseline sensitisation to cereal flour, baseline FeNO >25ppb) did not demonstrate an appreciable decline in FeNO (≥10%) in the intervention compared to the control group. However, stratification according to the presence of work-

related ocular-nasal symptoms at baseline demonstrated a significant decline (≥10%) in FeNO in the intervention group compared to the control group (OR=3.73, CI: 1.22-11.42).

Conclusion: This study demonstrates some evidence of an intervention effect on exhaled nitric oxide (FeNO) one year after the intervention, particularly among bakers reporting work-related ocular-nasal symptoms at baseline. The lack of a demonstrably stronger effect on other clinical endpoints can be attributable to the short follow-up period.

ABBREVIATIONS AND ACRONYMS

ACQ:	Asthma Control Questionnaire
AR:	Allergic Rhinitis
ATS:	American Thoracic Society
BA:	Baker's Asthma
ECRHS:	European Community Respiratory Health survey
ERS:	European Respiratory Society
FeNO:	Fractional exhaled nitric oxide
FEV_1:	Forced expiratory volume
FVC:	Forced Vital Capacity
HMW:	High Molecular Weight
IQR	Interquartile Range
LLA:	Laboratory Animal Allergens
LMW	Low Molecular Weight
MCT:	Methacholine Challenge Test
GM:	Geometric Mean
NSBH	Non-Specific Bronchial Hyperresponsiveness
OA:	Occupational Asthma
OR:	Odds Ratio
PFT:	Pulmonary Function Test
SD	Standard Deviation
SIC:	Specific Inhalation Challenge

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PART A: PROTOCOL

Assessing the health impact of intervention in supermarket bakeries using fractional exhaled nitric oxide (FeNO) and other clinical endpoints for baker's allergy and asthma

INTRODUCTION

Background

One of the most common contributors of occupational asthma (OA) is baker's asthma (BA).[1] Its prevalence among bakers was estimated to be between 4-17%.[2-7] Moreover, it contributes up to 20% of all the reported OA cases.[8] Furthermore, the incidence of BA in bakers was found to be between 0.3-2.46 per 1000 person-years.[9 10] However, cross sectional and longitudinal studies have shown a positive exposure-response relationship between BA and bakery dust.[2 11-13] This suggests that reducing allergen exposure would reduce the disease burden.[14-16]

There are various parameters to assess the health impact of intervention including symptoms severity and frequency, lung function such as forced expiratory volume in one second (FEV1) and non-specific bronchial hyperresponsiveness (NSBH) frequency and severity, sensitisation to flour dust allergen and airways inflammatory markers.[17 18] Although the immune mechanism is still unknown, persistent NSBH after exposure cessation was found to be associated with ongoing airway inflammation and higher level of inflammatory cytokines in sputum.[19 20] Furthermore, airway inflammation may be an early marker of allergy.[21 22] Fractional exhaled nitric oxide (FeNO) is a one of the widely used airway inflammatory markers which could assist in respiratory allergic outcomes following the introduction of intervention aimed at reducing allergen exposures. However, FeNO levels can be influenced by various factors, so its role in the clinical management of OA is still controversial.[23]

Justification

The study by Baatjies et al[24] appears to be the only study that evaluated intervention to reduce the exposure levels to flour dust. The investigators evaluated engineering controls and training in dust control measures which demonstrated a significant reduction in flour dust and allergen

exposure levels. However, no studies have been identified that evaluated the health outcomes as a result of introducing intervention in bakeries.

The early detection of airway inflammation through medical surveillance, even prior to the emergence of symptoms, may result in a better prognosis for patients with OA and reduce the financial burden associated with the disease.[15] [25-27] FeNO is a commonly used airway inflammatory marker that has been studied extensively in cross sectional epidemiological studies with very few studies focusing on longitudinal changes in FeNO.

Purpose and benefits

There is limited information on the health outcomes in bakers associated with flour dust reduction and few studies that have evaluated the determinants of longitudinal changes in FeNO. The results of this study would be beneficial in:

a) Providing recommendations to the bakery industry to protect the health of workers.

b) Improving medical surveillance programs in the bakery industry to identify workers who are at risk of developing BA.

Research questions

a) Did the intervention used in the study by Baatjies et al[24] result in improvement of the health outcomes in these bakers?

b) Are the parameters used to evaluate the health outcomes useful in assessing the impact of the intervention?

c) Is FeNO a useful marker to study the long-term impact of allergen exposure reduction in bakers?

d) What are the most important environmental and host determinants of longitudinal changes in FeNO in bakers?

Hypothesis

Does an intervention that results in a significant reduction in flour dust levels improve health outcomes in bakers as measured by a reduction in work-related symptoms, sensitisation and fractional exhaled nitric oxide (FeNO) one year after the intervention?

Aim

To assess the health impact of intervention in supermarket bakeries using fractional exhaled nitric oxide (FeNO) and other clinical endpoints for baker's allergy and asthma.

Objectives

a) To determine the prevalence of work-related symptoms, sensitisation to flour dust allergens and high FeNO in bakers prior to introducing an intervention to reduce flour dust exposure.

b) To determine the incidence of work-related symptoms, sensitisation to flour dust allergens and high FeNO in bakers one year after introducing an intervention to reduce flour dust exposure.

c) To compare the prevalence and incidence of work-related symptoms, sensitisation to flour dust allergens and high FeNO between the intervention and control groups one year after introducing the intervention.

d) To investigate the impact of environmental and host factors on longitudinal changes in FeNO.

METHODOLOGY

Study design

This study was a randomised control field trial that involved the analysis of data that was collected in 2011 as a part of a larger study started in 2003 (figure 1). The original study identified 31 bakeries in the Western Cape Province of South Africa as the potential population for the study that were randomly selected into two intervention groups and one control group. The study was conducted in five phases.

Phase I: Baseline exposure assessment study[28]

During this phase, 18 bakeries from 31 bakeries belonging to a supermarket chain store in the Western Cape province were randomly selected to participate in the study. These bakeries were stratified based on their workforce and the products of these bakeries into small, medium and large size bakeries. Finally, 109 bakers were randomly selected as participants representing different job categories in the bakeries. A total of 211 full-shift personal samples were collected to measure the level of exposure to flour dust in different job categories in the bakeries.

Phase II: Baseline health outcome parameters study[3]

During this phase, a cross sectional survey of 517 workers employed in the 31 bakeries, belonging to the supermarket chain store participated, conducted between June 2003 and June 2004. The study collected baseline data of work-related symptoms using a modified questionnaire from the European Community Respiratory Health Survey (ECRHS),[29] skin prick tests and specific IgE to wheat, rye and fungal amylase, spirometry and MCT.

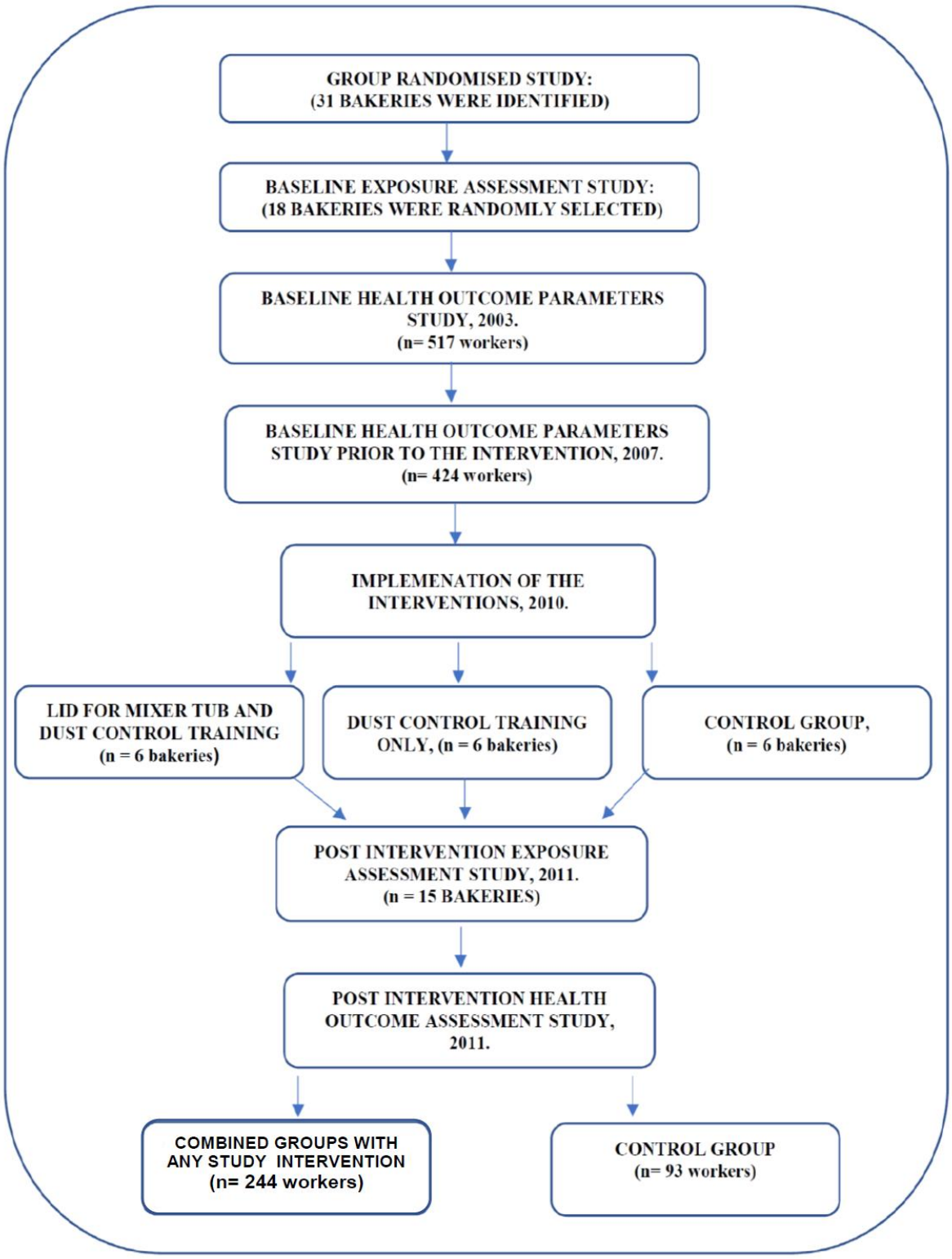

Figure 1: Flow diagram illustrating intervention study design and bakeries assessed pre-and post-intervention.

Phase III: Baseline work-related symptoms, sensitisation and fractional exhaled nitric oxide measurements prior to the intervention[30]

During this phase, cross sectional survey of 424 workers of the previous cohort of 517 workers was conducted in 2007. There were 93 workers from the original cohort that were not traceable and did not participate in this phase. The study collected cross sectional data of chest symptoms, ocular-nasal symptoms and work-related symptoms using a short questionnaire (appendix 2), serum specific IgE to wheat, rye and α-amylase as well as FeNO.

Phase IV: Assessment of the effectiveness of intervention using flour dust levels[24]

During this phase, in 2010, there were 18 bakeries that participated in phase I that were assigned to two intervention groups and a control group, among whom 15 bakeries were evaluated. Full-shift personal samples were again used to measure the flour dust levels in 128 workers representing adequately the different job categories found in these bakeries one year after implementing the intervention.

Phase V: Assessment of the effectiveness of intervention using health outcome parameters

In this phase, in 2011, a cross sectional survey was conducted on 361workers of which 337 were part of the same cohort participated in phases II and III. This study collected data of work-related symptoms using the modified ECRHS questionnaire (appendix 3), specific IgE to wheat, rye and fungal amylase and FeNO (appendices 4 and 5). It is the complete data collected in this phase on 337 workers that was analysed in this study.

Population and sampling

Study population

The study population comprised of 361 bakery workers from 31 supermarket bakeries of a supermarket chain store in the Western Cape province of South Africa. The study population was drawn from the 517 workers evaluated in earlier phases of the original study. These included 318 permanent and 168 casual workers in the bakery as well as 31 asthmatic ex-bakers who had been moved from the bakery section 2 years prior to the original study in June 2003. At follow up, 7 years later, 156 workers were not traceable for further evaluation for various reasons. The current cohort however represented adequately different job categories found in the bakery environment. It included 297 permanent and 40 casual workers.

Sampling strategy

The 31 bakeries were categorized into small, medium and large size based on their workforce volume and the types of products produced. Then, 18 bakeries were randomly selected into 3 different arms; 6 bakeries received lid for mixer and training manual intervention, 6 received training manual intervention only and 6 included as a control group. They were equally selected to represent the 3 different size categories. All the workers of each bakery were included in the study.

In the current study, 3 bakeries from the control arm were not included for various reasons including business reorganization. We were able to approach 361workers among whom 337 (intervention arm n=244, control arm=93) were part of the original cohort that were eligible to participate in the study.

Sample size calculation

According to the previous phases of this study, the prevalence of pre-intervention work-related chest symptoms was 26%.[30] We suspected a decrease in the prevalence of chest symptoms by 50% after the introducing the intervention. Using this information, the minimum sample size for testing a difference between two proportions would be 160 subjects in each arm based on the following formula:

$$n = \frac{n'}{4}\left[1 + \sqrt{1 + \frac{4}{n'|p_2 - p_1|}}\right]^2$$

However, we were able to collect complete data from 337 subjects and, therefore, this entire sample was used for analysis and would, therefore, have sufficient power to assess the changes anticipated. Moreover, the two intervention arms were combined to create one overall intervention group so as to detect differences between those who received the intervention (intervention arm) and those who did not receive any intervention (control arm).

Measurements

Study instrument

Respiratory questionnaire

A standard questionnaire designed based on the protocol for the European Community Respiratory Health Survey (ECRHS)[29] was administered to 361 workers (appendix 3). It gathered information on acute and chronic work-related respiratory and dermatological symptoms and a history of other comorbidities. It, also, covered information related to current and previous employment, levels of exposure to flour dust and tobacco smoke. The modified questionnaire was administered in either English or Afrikaans.

Serum immunological tests

Blood samples were obtained from 355 workers for Serum-specific immunoglobulin IgE. Sera were tested to determine for the presence of atopy using Phadiatop® test (ImmunoCAP 100 System; Phadia, Uppsala, Sweden). The sera were also tested for specific IgE to flour dust allergens such as wheat (f4), rye (f5) and fungal a-amylase (k87) using fluorescence enzyme immunoassay (CAP-FEIA) according to the manufacturer's instructions (Phadia).

Fractional exhaled nitric oxide determination

A hand-held portable nitric oxide sampling device (NIOX MINO) was used to determine FeNO during the work shift in 361 workers. It was performed in a room distant from the bakery area during the work shift throughout the working week according to American Thoracic Society (ATS)/European Respiratory Society (ERS) recommendations.[31 32] The testing of workers had no particular variation with regard to time of testing for the different jobs. The average of three technically adequate FeNO measurements was determined (appendices 4 and 5). Workers were instructed to abstain from smoking, eating or drinking at least one hour before the test. This was confirmed prior to testing, and those who did not follow the instructions were tested at a later stage after ensuring their full compliance with these instructions.

List and definition of variables

Outcome variables

Outcome variables of interest for this study before the intervention

a) Ocular-nasal symptoms related questions:

   i. Presence: "Yes" to at least one of the following 2 questions:
   - "During the past 12 months have you had two or more episodes of: sneezy, itchy or runny nose when you did not have a cold or flu?"

- "During the past 12 months have you had two or more episodes of: red, itchy or watery eyes?"

ii. Work-relatedness: Was assessed in response to one of the two questions:

- "Do your nose or eye symptoms seem better or worse when you are away from work"

- "Does being at work ever cause you to have sneezy/itchy runny nose or red/itchy/watery eyes?"

b) Asthma related questions:

i. Presence of asthma:

- Doctor diagnosed asthma
- Current asthma: "Yes" to at least one of the following 2 questions:

- "Have you had an attack of asthma in the last 12 months?"

- "Are you using any medicines, including inhalers/ pumps, nebulizers, syrups or tablets, for asthma or breathing problems?"

ii. Work-relatedness: Was assessed in response to one of the two questions:

- "Do your chest symptoms seem better or worse when you are away from work"

- "Does being at work ever make your chest tight or wheezy?

c) Serum-specific IgE to wheat, rye and fungal a-amylase: Results was treated as a continuous variable or binary variable (ImmunoCAP $\geq$ 0.35 kU/L).

d) FeNO levels, was treated as a:

i. Continuous variable (log-transformed), or

ii. Binary variable if FeNO > 50ppb and FeNO > 25ppb.

Outcome variables of interest for this study post the intervention

This was assessed by computing the change in the prevalence of ocular-nasal symptoms, asthma symptoms, sensitisation and the level of FeNO (> 25ppb and FeNO > 50ppb) post the intervention. Furthermore, a >10% change in FeNO (decline) was modelled to assess the positive impact of the intervention in causing decreased allergic airway inflammation.

Covariates and confounders

The main covariate of interest was belonging to the intervention or the control arm. Potential confounders considered included smoking (categorical variable), atopy (binary variable based on Phadiotop test), age (continuous variable), corticosteroid use (categorical variable), and baseline FeNO as a continuous variable and a binary variable (> 25ppb).

DATA MANAGEMENT AND ANALYSIS PLAN

STATA statistical package (version 12) was used to analyse the data. Exploratory data analysis was carried to check for the presence of outliers, the extent of missing data as well as the distributions of the key variables and any transformation needed. Descriptive statistics was used to summarize each measured variable. Data is presented as proportions, means (geometric where appropriate) or median and the corresponding standard deviation (SD) or interquartile range (IQR). Chi-square test and an independent t-test (or Fisher's exact test and Wilcoxon sum rank test where appropriate) were used to compare groups at baseline as well as to determine the effect of intervention on the incidence and decline of allergic and respiratory health outcomes at one-year follow-up. Univariate linear regression was used to explore the determinants of longitudinal change in FeNO. Multivariate regression analysis was used to determine predictors of a 10% or more decline in FeNO using the intervention and adjusting

for other confounders such as current smoking, baseline FeNO>25ppb and sensitisation to cereal flour allergens.

LIMITATIONS

a) The post intervention period may not be sufficient to detect an improvement in health outcome parameters. Changes in asthma symptoms and sensitisation may take more than one year to manifest after decreased/cessation of exposure to the offending allergens.[20]

b) Loss to follow-up was approximately 35%, which may impact on the effect estimate.

c) The sample size in the two individual arms may not be sufficient to compare the effect of individual intervention groups separately and would need to be combined to increase the power of the study.

d) The healthy worker effect may introduce probable bias should there be differences between those that were no longer working in the bakeries under study.

e) Some of the bakeries in the control group may have applied changes to their work practices, which may dilute the strength of the effect estimates.

ETHICS AND COMMUNICATION

The original study received ethical approval from the Research Ethics Committee of the University of Cape Town (reference No. 272/2002). This study did not collect any new data and the analysis was conducted on an existing data set from the original study. Therefore, the participants of the original study were not prone to any additional risk. However, the current study received ethical approval from the University of Cape Town, South Africa (appendix 6) The study was conducted in accordance with the World Medical Association Declaration of Helsinki.[33]

Autonomy

All workers signed informed written consent before embarking on the project. Participation was voluntary at no cost to either the worker or the employer.

Confidentiality

Results of the tests with interpretation were only shared with the participants. All data was coded and workers' and the company' confidentiality was maintained. The hard copies data were strictly stored in in cabinets under lock and key at the School of Public Health and Family medicine at University of Cape Town. Furthermore, the data was archived on computer with limited access to researchers only. Only summary data was presented for public presentations. Personal information was only released with the worker's consent, should the need have arisen, to their family doctor or the occupational health clinic.

Benefits

Study participants were informed of their tests results. Those who had abnormal results were offered referral for further evaluation to the Occupational Medicine Clinic at Groote Schuur Hospital. All confirmed cases of occupational diseases have been managed medically, had compensation claims submitted and a medical advice for relocation has been sent to the employer.

Although this study may not add direct benefits for the participants, the findings of the analysis will provide more information about the usefulness of intervention aimed at reducing the flour dust exposure on various health outcomes in bakers. It will also provide information on the usefulness of using FeNO in medical surveillance programs of bakers to identify those at high risk of developing baker's asthma.

Non-maleficence

The original study had negligible risk to the participants except the mild discomfort from the needle prick during blood sample collections. Furthermore, there was no additional risk to the participants since the current study did not require their active physical participation since data had already been collected.

Justice

The reported health problems and disease burden among bakers justified the need for the study as to find ways to reduce the disease burden and improve the quality of life of these workers.

Dissemination of the research results

The results of the study will be disseminated in various forms

a) MMed dissertation to be submitted to the University of Cape Town
b) Academic seminars, research forums and local/international conferences
c) Publications in local and peer reviewed scientific journals
d) Report to the relevant stakeholders

Funding

There was no additional funding required for this sub-study, which was originally funded by research scholarship grants from the Center for Asthma in the Workplace (Montreal, Canada), Medical Research Council (Republic of South Africa), the National Research Foundation FA2006040700028 (Republic of South Africa), the Fogarty International Centre (Bethesda, Maryland, USA), the Allergy Society of South Africa (Cape Town) and University of Cape Town Research Committee (Cape Town) and the baking industry (Cape Town).

REFERENCES

1 Brant A. Baker's asthma. *Curr Opin Allergy Clin Immunol* 2007;7:152–5.

2 Brisman J. Baker's asthma. *Occup Environ Med* 2002;59:498–502.

3 Baatjies R, Lopata A, Sander I, et al. Determinants of asthma phenotypes in supermarket bakery workers. *Eur Respir J* 2009;34:825–33.

4 Cullinan P, Lowson D, Nieuwenhuijsen MJ, et al. Work-related symptoms, sensitisation, and estimated exposure in workers not previously exposed to laboratory rats. *Occup Environ Med* 1994;51:589–92.

5 Pavlovic M, Spasojevic M, Tasic Z, et al. Bronchial hyperactivity in bakers and its relation to atopy and skin reactivity. *Sci Total Environ* 2001;270:71–5.

6 Brant A, Berriman J, Sharp C, et al. The changing distribution of occupational asthma: a survey of supermarket bakery workers. *Eur Respir J* 2005;25:303–8.

7 Quirce S, Diaz-Perales A. Diagnosis and management of grain-induced asthma. Allergy *Asthma Immunol Res* 2013;5:348–56.

8 Ameille J, Pauli G, Calastreng-Crinquand A, et al. Reported incidence of occupational asthma in France, 1996–99: the ONAP programme. *Occup Environ Med* 2003;60:136–41.

9 Leira HL, Bratt U, Slåstad S. Notified cases of occupational asthma in Norway: exposure and consequences for health and income. *Am J Ind Med* 2005;48:359–64.

10 Remen T, Coevoet V, Acouetey D-S, et al. Early incidence of occupational asthma among young bakers, pastry-makers and hairdressers: design of a retrospective cohort study. *BMC Public Health* 2010;10:206.

11 Nieuwenhuijsen MJ, Heederik D, Doekes G, et al. Exposure-response relations of alpha-amylase sensitisation in British bakeries and flour mills. *Occup Environ Med* 1999;56:197–201.

12 Hur G-Y, Koh D-H, Kim H-A, et al. Prevalence of work-related symptoms and serum-specific antibodies to wheat flour in exposed workers in the bakery industry. *Respir Med* 2008;102:548–55.

13 Houba R, Heederik DJ, Doekes G, et al. Exposure-sensitisation relationship for alpha-amylase allergens in the baking industry. *Am J Respir Crit Care Med* 1996;154:130–6.

14 Meijster T, Warren N, Heederik D, et al. What is the best strategy to reduce the burden of occupational asthma and allergy in bakers? *Occup Environ Med* 2011;68:176–82.

15 de Groene GJ, Pal TM, Beach J, et al. Workplace intervention for treatment of occupational asthma: a Cochrane systematic review. *Occup Environ Med* 2012;69:373–4.

16 Heederik D, Henneberger PK, Redlich CA. Primary prevention: exposure reduction, skin exposure and respiratory protection. *Eur Respir Rev* 2012;21:112–24.

17 Tarlo SM, Liss GM. Can medical surveillance measures improve the outcome of occupational asthma? J. *Allergy Clin. Immunol.* 2001;107:583–5.

18 Tarlo SM, Liss GM. Prevention of occupational asthma. *Curr Allergy Asthma Rep* 2010;10:278–86.

19 Piirilä PL, Meuronen A, Majuri M, et al. Inflammation and functional outcome in diisocyanate-induced asthma after cessation of exposure. *Allergy* 2008;63:583–91.

20 Maghni K, Lemière C, Ghezzo H, et al. Airway inflammation after cessation of exposure to agents causing occupational asthma. *Am J Respir Crit Care Med* 2004;169:367–72.

21 Mathias CB, Freyschmidt E-J, Oettgen HC. Immunoglobulin E antibodies enhance pulmonary inflammation induced by inhalation of a chemical hapten. *Clin Exp Allergy* 2009;39:417–25.

22 Fernandez-Nieto M, Quirce S, Sastre J. Occupational asthma in industry. *Allergol Immunopathol (Madr)* 2006;34:212–23.

23 Dominguez-Ortega J, Barranco P, Rodríguez-Pérez R, et al. Biomarkers in occupational asthma. *Curr Allergy Asthma Rep* 2016;16:63.

24 Baatjies R, Meijster T, Heederik D, et al. Effectiveness of intervention to reduce flour dust exposures in supermarket bakeries in South Africa. *Occup Environ Med* 2014;71:811–8.

25 Merget R, Pham N, Schmidtke M, et al. Medical surveillance and long-term prognosis of occupational allergy due to platinum salts. *Int Arch Occup Environ Health* 2016;:1–9.

26 Carino M, Romita P, Foti C. Allergy-Related Disorders in the Construction Industry. *ISRN Prev Med* 2013;2013:864679.

27 Labrecque M, Malo J-L, Alaoui KM, et al. Medical surveillance programme for diisocyanate exposure. *Occup Environ Med* 2011;68:302–7.

28 Baatjies R, Meijster T, Lopata A, et al. Exposure to flour dust in South African supermarket bakeries: modeling of baseline measurements of an intervention study. *Ann Occup Hyg* 2010;54:309–18.

29 Burney PG, Luczynska C, Chinn S, et al. The European community respiratory health survey. *Eur Respir J* 1994;7:954–60.

30 Baatjies R, Jeebhay MF. Sensitisation to cereal flour allergens is a major determinant of elevated exhaled nitric oxide in bakers. *Occup Environ Med* 2013;70:310–6.

31 Dweik RA, Boggs PB, Erzurum SC, et al. An official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (FENO) for clinical applications. *Am J Respir Crit Care Med* 2011;184:602–15.

32 ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. *Am J Respir Crit Care Med* 2005;171:912–30.

33 World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA* 2013;310:2191–4.

PART B: LITERATURE REVIEW

FACTORS ASSOCIATED WITH SERIAL LONGITUDINAL CHANGES IN EXHALED NITRIC OXIDE (FeNO)*

* see appendix 1 to view the published article:

Al Badri FM, Jeebhay MF. Factors associated with serial longitudinal changes in exhaled nitric oxide (FeNO)–a review of the literature. Current Allergy and Clinical Immunology. 2017;30(2):98-109.

BACKGROUND

It has been well documented that the major hallmarks of asthma are the presence of airway inflammation, variable airway obstruction and airway hyperresponsiveness (AHR).[1] Among this triad, airway inflammation appears to be increasingly recognised as a major component in the initiation and long-term progression of the disease. There are several methods of measuring airway inflammation in asthma, one being the measurement of fractional exhaled nitric oxide (FeNO) as a marker for asthma in symptomatic individuals.[2] FeNO is a non-invasive marker that has been extensively investigated in several contexts such as diagnosis, initiating treatment, tailoring medication, achieving asthma control and predicting future relapses of asthma. Various studies have demonstrated that FeNO is affected by individual host attributes and environmental factors. FeNO levels at baseline are reported to be higher in men and levels increase with height, atopy, dietary intake of food rich in nitrates, allergen exposures causing sensitisation and viral airway infections.[3-6] FeNO levels can be decreased with bronchoconstriction, inhaled corticosteroid therapy and among both active and passive smokers.[3-6] Most of the studies on the determinants of baseline FeNO have focused on the asthmatic population and fewer studies having evaluated serial changes in FeNO in the general population or in occupational contexts over longer periods of time.

OBJECTIVES OF THE LITERATURE REVIEW

The aim of this review was to

a) Identify the determinants of serial longitudinal changes in FeNO.
b) Determine the usefulness of different interventions in decreasing airway inflammation measured by FeNO.
c) Determine the usefulness of using FeNO in monitoring workers with persistent exposures to respiratory sensitisers.

LITERATURE REVIEW STRATEGY

Due to the paucity of studies on this subject, it included all relevant epidemiological or experimental studies on asthmatic children or adults having a direct or indirect allergen exposure modulation component that were published in English between January 1999 and April 2017. Articles were retrieved from PubMed/Medline and Google Scholar. The key search terms that were used in this review were “asthma”, “FeNO” OR “exhaled nitric oxide” AND “serial” OR “longitudinal”. Furthermore, some articles were retrieved using the functions “similar articles search” in PubMed and “related articles” in Google Scholar as well relevant articles that were cited in the references of these articles. Clinical studies that focused on the effect of medication or inhalation challenge test on FeNO in patients were excluded from this review.

The review identified various factors associated with longitudinal changes in FeNO in 20 studies as outlined in Tables 1-3. The articles were summarized according to the population of interest. Eight of the studies were experimental in design with six being quasi-experimental studies and two being non-randomised controlled trials. Furthermore, there were ten epidemiological studies (five longitudinal, four case-control, and one cross sectional) and two clinical case reports. Most of the studies focused on asthmatic populations but two studies also evaluated FeNO in working populations with exposure to respiratory sensitisers.

ENVIRONMENTAL FACTORS

Allergen exposure

Various studies using specific inhalation challenges (SIC) of suspected allergens in general asthmatic patients have shown that it takes up to 8 hours before FeNO starts to increase and it continues to remain elevated for up to 48 hours[7 8] and in some studies up to 72 hours.[9 10] In

patients suspected of having occupational asthma, most studies have reported changing FeNO levels 20-24 hours post SIC[10-17] and showed a significant increase in FeNO.[2]

Exposure to the relevant allergen in asthmatic populations has been shown to demonstrate positive associations with longitudinal changes in FeNO. Baraldi et al[18] in their case-control study investigating the association between serial FeNO and exposure to grass pollens in atopic asthmatic children sensitised to grass pollen found that exposure in the pollen season led to a two-fold increase in the overall mean FeNO levels, which returned to baseline after the season. This association between exposure to pollen during the pollen season and to other allergens was also reported by Roberts et al[19] Spanier et al[20] and Cutts et al[21]. However, Fowler et al[22] found a negative association ~~between~~ allergen exposure and FeNO (-1.5-fold, 95% CI -1.2 – -1.7-fold). Since exposure was not monitored during the long study period (mean duration was 47 months), there may have been changes in the exposure level over this period that may have introduced bias in the study.

Ongoing exposure to an occupational allergen appears to be one of the determinants of longitudinal change in FeNO. Van der Walt et al evaluated serial FeNO associated with occupational exposure to spices in 150 mill workers after a two days exposure free period during time off work.[11] There was no significant difference in the overall mean FeNO between baseline, after the shift and 24 hours after the baseline pre-shift level. However, there was a >12% increase in FeNO across the 24-hour period in 23% of the workers. The authors concluded that exposure to the spice allergens in this occupational setting was associated with a delayed increase in FeNO from the baseline. Hewitt et al also evaluated serial FeNO among workers exposed to laboratory animal allergens (LAA).[34] The investigators studied 50 animal laboratory workers for five working days after being off work for two days. There was a progressive increase in FeNO (>100 ppb) over the working week in one subject who was seropositive to one or more of the LAA. Furthermore, three additional subjects (one

seropositive with lifelong asthma plus two who were seronegative) experienced >25 ppb fluctuation in FeNO during the working day in the period of observation. However, the mean symptoms score for the four subjects did not differ from the mean symptoms score obtained for the others. Interestingly, the subject that demonstrated progressive increase in FeNO developed typical asthma symptoms six months after the study and was later diagnosed with occupational asthma based on previous positive serology, development of airway hyper-responsiveness and increased FeNO. Both studies were conducted in worker populations comprising both asymptomatic and symptomatic individuals.

Allergen exposure avoidance

Avoidance of exposure to the suspected common aeroallergen has also been studied in asthmatic children. Piacentini et al[23] studied the outcome of allergen avoidance at 1756 m above sea level for three months in 20 asthmatic children who were sensitised to house dust mite (HDM). FeNO levels decreased progressively in the first 2 weeks ($p = 0.014$) and remained unchanged for the next three months. This drop in FeNO was also reported by Peroni et al[25] in HDM sensitised asthmatic children living at high altitudes for 9 months. The mean FeNO levels decreased by 50% in the first 3 months ($p = 0.030$), but there was no further significant change over the remaining period. Huss-Marp et al have argued that avoidance of the allergen is not the only contributing factor in the decline of FeNO observed in these studies, suggesting that physiological changes at high altitude may also contribute to this.[26 37] This study found that FeNO declined significantly in HDM sensitised asthmatic children as a group and in the non-HDM sensitised group or those with intrinsic asthma, but no significant difference in the reduction between the two groups was observed. Karagiannidis et al[38] also reported a similar conclusion. It is however likely that there may have been residual exposure to HDM at this lower altitude compared to the Piacentini and Peroni studies that were done at higher altitudes. Furthermore, it is possible that by combining subjects who were sensitised to

allergens other than HDM (n = 251) and those with intrinsic asthma (n = 22), this may have confounded the findings.

In the occupational context, Merget evaluated the effect of avoidance of exposure to inhalant allergens in two case studies.[35 36] A sensitised farmer to triticale and a mildly sensitised baker to wheat and rye, both demonstrated a decrease in FeNO after avoidance of the potential allergens for two weeks.

Table 1: Experimental studies of serial longitudinal FeNO measurement among asthmatics in the general population (children and adults)

Table 1A. Studies among children

AUTHORS	Piacentini et al, 1999[23]	Piacentini et al, 2001[24]	Peroni et al, 2002[25]
AIM OF STUDY	To evaluate the effect of house dust mite (HDM) avoidance in allergic asthmatic patients on FeNO.	To determine the relationship between exposure level to house dust mite (HDM) allergen and the magnitude of exhaled FeNO after allergen avoidance in a group of allergic asthmatic children.	To study the change in lung volumes and airway inflammatory markers in house dust mite (HDM) sensitised asthmatic children resident in an allergen free environment.
STUDY DESIGN	Quasi-experimental	Quasi-experimental	Quasi-experimental
STUDY POPULATION	20 Italian asthmatic children (6 to 15 years) sensitised to HDM. Thirteen received regular courses of inhaled corticosteroids (ICS).	14 Italian asthmatic children (6 to 15 years) sensitised to HDM.	18 asthmatic children who have moderate to severe asthma and sensitised to HDM

AUTHORS	Piacentini et al, 1999[23]	Piacentini et al, 2001[24]	Peroni et al, 2002[25]
INTERVENTION	Avoidance of HDM by staying at 1756 m above sea level in the Italian Alps for 3 months.	Avoidance of HDM by staying at 1756 m above sea level in the Italian Alps for 3 months.	Avoidance of HDM by staying at 1756 m above sea level in the Italian Alps for 9 months between September and June.
OUTCOME MEASURES	Change in FeNO level.	• The level of HDM group I allergen at home. • Change in FeNO.	FeNO, lung function.
MEASURMENTS	• FeNO was measured daily, always at the same hour starting at the parents' house (T0), for 2 weeks after moving to the residential home until day 15 (T1), after 3 months at the residential	• Children received a regular course of ICS for at least 3 months at the beginning of the study. The ICS course was gradually withdrawn in all the subjects. By T1, none of the	• Measurements taken (i) within 3 days of admission, (ii) after 3 months of stay, (iii) within 2 days before leaving the residential home for the Christmas holiday, (iv) on return to the residential home

	campus (T2), and, in 10 of the 20 patients, 2 weeks after return to their parents at sea level (T3). • FEV1 is measured at T0, T1, T2, and T3. • Serum eosinophil cationic protein (ECP) was measured at T0, T2, and T3.	children received any ICS for 1 month at least. • FeNO was measured at the parent's home (T0) and after 3 months at the residential campus (T1).	after 15 days at home and (v) at the end of the camp after 9 months of staying at residential home. • ICS were withdrawn after few weeks of admission, resumed during the Christmas holiday and withdrawn again during the rest of the study.
RESULTS	• FeNO decreased (p=0.014 at T1) with a significant effect of the time in the first 2 weeks (p=0.026). It didn't change in the period T1 to T2, and returned to T0 level at (p=0.004, T2 vs T3).	Strong correlation between the change in FeNO (T0-T1) and the levels of HDM antigens in the beds of the patients before T0; r=0.618 (p=0.026).	• FeNO decreased from (21.3 ± 3.9 ppb) in September to (11.9 ± 1.7 ppb) in December (p=0.03). No significant change after the holiday in January (12.5 ± 2.6 ppb) nor

	• FeNO did not change significantly from T1 to T2, despite the withdrawal of ICS. • FEV_1 improved gradually.		at the end in June (13.2 ± 2.0 ppb). • The RV to TLC ratio decreased between September and December (p=0.003), increased between December and January (p=0.002) and decreased again between January and June (p=0.002). • No significant correlation between FeNO and lung volumes.

AUTHORS	• Piacentini et al, 1999[23]	• Piacentini et al, 2001[24]	• Peroni et al, 2002[25]
LIMITATIONS	• Absence of a control group at sea level. • Small sample size. • Independent effect of high altitude on FeNO levels. • No mention of important confounders (passive smoking and diet).	• Absence of a control group at sea level. • Small sample size. • Independent effect of high altitude on FeNO levels. • No mention of important confounders (passive smoking and diet).	• Absence of a control group at sea level. • Small sample size. • Independent effect of high altitude on FeNO levels. • No mention of important confounders (passive smoking and diet).
CONCLUSION	FeNO levels decreased over time after the avoidance of the potential allergen in allergic asthmatic children.	FeNO reduction after the avoidance of the potential allergen correlated with the magnitude of the exposure of the allergen prior to avoidance.	Allergen avoidance at high altitudes led to a reduction in FeNO levels in asthmatic children sensitised to HDM.

Table 1A. Studies among children (continued)

AUTHORS	Huss-Marp et al, 2007[26]	Kaminsky et al, 2008[27]	Saito et al, 2013[28]
AIM OF STUDY	To study the effect of rehabilitation of asthmatic children at high altitudes and HDM avoidance on asthma control.	To study the effect of attending an asthma summer camp on airway inflammation as measured by FeNO.	• To examine whether an asthma education program is associated with asthma control. • To compare absolute levels and changes of ACT score, FEV_1, and FeNO over a year after the intervention. • To evaluate FeNO as a marker of asthma control compared to other methods.
STUDY DESIGN	Quasi-experimental	Quasi-experimental	Quasi-experimental

AUTHORS	Huss-Marp et al, 2007[26]	Kaminsky et al, 2008[27]	• Saito et al, 2013[28]
STUDY POPULATION	311 asthmatic children with increased FeNO levels at admission (>17 ppb).	27 asthmatic children	• 12 mild, 21 moderate, and 17 severe persistent asthmatic adult patients (n=50) with poor adherence to medication.
INTERVENTION	Rehabilitation at 1200 m above sea level in the Bavarian Alps.	Participation in a one-week camp during the summer of 2006 in the USA.	Asthma education program providing information about asthma pathogenesis, diagnosis, severity, medications (including side effects), differences between reliever and controller agents, importance of asthma treatment, inhaler device instructions, exacerbation management, peak

			expiratory flow (PEF) monitoring, and a self-management plan.
OUTCOME MEASURES	Change in FeNO level.	FeNO, lung function and ACQ score.	Change of ACT score, FeNO and spirometry levels over time.
MEASURMENTS	• Subjects were grouped according to asthma severity based on the clinical features before therapy from grade I (mild intermittent) to 3 levels of persistent asthma (grades II-IV: mild, moderate, and severe, respectively; grade I: n=67; grade II: n=135; grade III: n=104; grade IV: n=5).	• FeNO, FEV_1 and ACQ score were measured at the beginning and end of the week. ACQ score was also obtained at one month and 6 months after the camp. • The children participated in daily educational activities about asthma.	• Subjects were reviewed four times: at entry and at intervals of 3, 6 and 12 months. • Treatment was not changed in the first 3 months to study the effect of the educational program on asthma control. Thereafter, the treatment could be changed according to the GINA guidelines.

	• FeNO measurement was performed on the day of admission and at discharge (4-6 weeks).		• During the follow-up period, subjects were divided into two groups: - Stable group: treatment had not changed or was decreased over the study period. - Unstable group: increase in ICS dose or other anti-asthmatic drugs added during the study period.
RESULTS	• 47.2 % reduction in the mean FeNO of all 311 children after rehabilitation, with 38.5 ± 22.8 ppb at admission compared to 20.1 ± 13.2 ppb at discharge ($p<0.001$).	• FeNO dropped significantly by 45% ($p <0.001$), but the changes in lung function and ACQ was not statistically significant.	• Decrease in FeNO and increase in ACT scores in the stable asthma group (n=42) compared to the unstable asthma group (n=8); ($p<0.001$) and remained

	• FeNO reduction was significant in all subgroups and most marked in children with mild asthma. • FeNO reduction in asthmatics with isolated HDM sensitisation was no different to the reduction in other children sensitised to other allergens.	• Among the 21 children prescribed with ICS, 18 had decreased FeNO and 3 had increased FeNO. • Among the 4 children not prescribed any ICS, 3 had decreased FeNO, and 1 had an increase in FeNO.	consistent in the stable group over the course of 12 months. • Significant correlations between change in FeNO, ACT score and FEV_1 in all four visits.
LIMITATIONS	• Absence of a control group at sea level. • HDM concentrations in the rehabilitation center not reported. • Independent effect of high altitude on FeNO levels.	• Small sample size. • First day measurements were conducted in the afternoon and the final day measurements in the morning.	• Selection bias. • Hawthorne effect.

	• No mention of important confounders (passive smoking and diet).		
CONCLUSION	FeNO decreased significantly after 4-6 weeks rehabilitation at high altitude, which could be explained by allergen avoidance, absence of air pollution and improved compliance to medication.	FeNO levels declined after attendance of a one-week asthma summer camp, despite no changes in lung function or asthma control, which could be explained by allergen avoidance, absence of exposure to tobacco smoke, and avoidance of psychosocial stressors.	• FeNO levels declined after an asthma education program and remained significant over a year. • Change in FeNO levels correlated significantly with change in ACT score and change in FEV_1 levels.

Table 1B. Studies among adult working population

AUTHORS	Dressel et al, 2007[29]	Dressel et al, 2009[30]
AIM OF STUDY	To assess whether FeNO is useful in detecting a reduction in airway inflammation within a few weeks of an educational intervention in farmers with occupational asthma.	To determine whether long-term changes in FeNO could be detectable a year after an intervention.
STUDY DESIGN	Non-randomised controlled trial	Non-randomised controlled trial
STUDY POPULATION	Cases: 81 animal farmers with occupational asthma sensitised to cow dander and storage mites who participated in a 1-day educational program. Controls: 24 animal farmers with occupational asthma not subjected to an intervention.	• Cases: animal farmers with occupational asthma (n=43) who participated in a 1-day educational program. • Control: animal farmers with occupational asthma (n=15) not subjected to an intervention.
INTERVENTION	A one-day educational program provided general information about the pathogenesis of asthma and allergies, environmental influences, treatment,	A one-day educational program provided general information about the pathogenesis of asthma and allergies, environmental influences, treatment, major

	major occupational allergens causing asthma, particularly cow dander and mites, and prevention methods in the workplace.	occupational allergens causing asthma, particularly cow dander and mites, and prevention methods in the workplace.
OUTCOME MEASURES	FeNO, lung function and a questionnaire.	FeNO, lung function and a questionnaire.
MEASURMENTS	Baseline FeNO measurement and repeated 4–6 weeks after the intervention.	Baseline FeNO measurement and repeated one year after the intervention.
RESULTS	• There was a decline in the proportion of subjects reporting at least one current respiratory symptom at work (p=0.012) in the intervention group. • The geometric mean FeNO decreased from 28.2 to 25.7 ppb (p= 0.042).	• Geometric mean ± SEM of FeNO decreased from (31.5±1.1) to (25±1.1) ppb in the intervention group (p=0.001), while it showed a slight but statistically insignificant increase in the control group (p=0.258) • FEV_1 and FEV_1/FVC did not significantly change over time in either group.

	• Subjects with a baseline FeNO of >35 ppb (n=32), FeNO decreased from 59.7 to 49.2 ppb (p=0.003). • There was no significant change in the spirometry after the intervention.	
LIMITATIONS	• Selection bias. • No mention of important confounders (atopy and diet).	• Selection bias. • No mention of important confounders (atopy and diet).
CONCLUSION	FeNO showed a significant short-term reduction after an educational intervention in animal farmers with workplace-related allergic asthma, which was accompanied by decrease of frequency of symptoms.	FeNO showed a significant long-term reduction after an educational intervention in animal farmers with workplace-related allergic asthma, which may be explained by a reduction in allergen exposure.

Table 2: Epidemiological studies of serial longitudinal FeNO measurement among asthmatic children.

AUTHORS	Barreto et al, 2008[31]	Baraldy et al, 1999[18]
AIM OF STUDY	To assess variations and reproducibility of FeNO in subjects maintained under similar environmental conditions.	• To evaluate the relationship between natural allergen exposure to grass pollen and the changes in FeNO in atopic asthmatic children during and out of the grass pollen season.
STUDY DESIGN	Case-control	Case-control
STUDY POPULATION	• 29 children (12 healthy subjects and 17 asthmatics with good control). • Asthmatics divided into two groups; corticosteroids-naïve (n=9) and corticosteroids-treated (n=8). • All asthmatics were atopic.	• 21 grass pollen sensitised children (age 6 to 16 years) with a seasonal allergic asthma. • 21 non-atopic healthy children age and sex matched with the asthmatics.

AUTHORS	Barreto et al, 2008[31]	Baraldy et al, 1999[18]
INTERVENTION/ EXPOSURE	1-week stay in a countryside sanatorium situated in a wooded area near the Tatra mountains at an altitude of 970 m.	Followed up and monitored before (March), during (May), and after (November) the pollen season.
OUTCOME MEASURES	FeNO, lung function.	FeNO, lung function.
MEASURMENTS	• On arrival, all subjects underwent a clinical examination and a history recorded of symptoms and therapy. Spirometry and FeNO measurements done on all subjects twice a day for the first 2 days and again at 8:00 am on day 7. • All participants avoided food intake and physical exercise for at least 2 hours before testing.	• All patients underwent physical examination, spirometry and measurement of FeNO on three occasions: before (March), during (May) and after the grass pollen season (November). On each occasion, measurements were performed in the afternoon. • The atmospheric pollen counts were condcuted throughout the year using a volumetric spore trap

	• Participants excluded from the study if unsatisfactory lung function maneuvers or respiratory symptoms developed during the weekly follow-up.	and daily mean concentration recorded and expressed as pollen grains per m3 of air per 24 hrs.
RESULTS	• At baseline, the worst lung function observed among corticosteroid treated asthmatics and the highest FeNO values recorded in corticosteroid-naive asthmatics. • The differences in FeNO at baseline between asthmatics versus healthy participants was significant only among corticosteroid-naive patients. • Differences in lung function between the groups remained unchanged at each session. • FeNO levels among corticosteroid-naive as well as treated asthmatics decreased over time and	• ICS therapy was not altered through the pollen season for all subjects. • The mean value of FeNO before the grass pollen season (March) was (12.7 ± 5.1 ppb) and was significantly higher ($p<0.001$) when compared to healthy subjects (7.8 ± 2.7 ppb). • In the pollen season (May) there was a significant ($p<0.001$) two-fold increase in FeNO (21.4 ± 7.6 ppb) with respect to pre-season baseline values. • After the season (November), FeNO returned to pre-seasonal values (12.8 ± 5.8 ppb).

	became statistically non-significant (vs healthy subjects) at the last session (day 7). • No intra-group differences were found between nocturnal and diurnal FeNO in all the groups.	• No relationship was found between the changes in FeNO and changes in symptom scores. • No significant changes in lung function parameters during and after the pollen season.
LIMITATIONS	• Small sample size. • High altitude and climate may affect the results. • No mention of important confounders (passive smoking and diet). • There was no intra-group analysis to study the significance of the mean difference between day 1 and day 7.	• Small sample size. • No FeNO measurements taken for the control group during and after the pollen season. • No mention of important confounders (passive smoking and diet).
CONCLUSION	FeNO decreased a week after allergen avoidance at high altitude.	• Natural allergen exposure during the grass season resulted in an increase in FeNO in asthmatic children which then returned to the level of the pre-seasonal range after the season passed.

Table 2: Epidemiological studies of serial longitudinal FeNO measurement among asthmatic children (continued)

AUTHORS	Adar et al, 2015[32]	Holguin et al, 2015[33]
AIM OF STUDY	To assess variability in FeNO in children in relation to exposures to diesel pollution from school buses.	To assess the effects of traffic emissions on FeNO in children with and without asthma.
STUDY DESIGN	Case-control	Case-control.
STUDY POPULATION	• 275 children riding on diesel school buses, with a mean age of 9.5 years. • 148 were asthmatics, and 127 were healthy subjects.	• 200 children (age 6 to 12 years), • 50% had physician-diagnosed asthma, and 50% were healthy subjects.
INTERVENTION/ EXPOSURE	Children riding on school buses were monitored before, during and after the adoption of clean fuel technology, such as diesel oxidation catalysts (DOCs), closed crankcase ventilation systems (CCVs), ultralow-sulfur diesel (ULSD), and biodiesel.	Children were monitored over a 4-month period to determine the effects of road and traffic densities, carbon and particulate matter on FeNO levels.

AUTHORS	Adar et al, 2015[32]	Holguin et al, 2015[33]
OUTCOME MEASURES	FeNO, lung function, air pollution levels on school buses.	FeNO, traffic-related air pollution levels, traffic densities.
MEASUREMENTS	• Children were assessed an average of 6 times over 4 years. • Lung function and FeNO were measured during monthly data collection sessions at the schools. • Fine and ultra-fine particles (UFP) were measured inside 188 buses during 597 commutes.	• Children were assessed with spirometry, FeNO, and skin allergy testing bi-weekly over 4 months. • Daily respiratory health questionnaires were completed by parents. • Road-density (amount of road length in kilometers in each buffer) and traffic density (vehicle-km/h) within buffer areas around study schools and subject homes were measured. • Air pollution was measured at the schools as follows:

		○ 48-hour average of less than 2.5 um ($PM_{2.5)}$ particulate matter and elemental carbon measurements. ○ Weekly NO_2 measurements.
RESULTS	• Higher FeNO levels were observed among children with asthma, compared to healthy children. • Buses with ULSD were associated with reduction in UFP (-47%; 95% CI, -58 to -34%). • Buses with ULSD were associated with 16% lower FeNO, particularly in asthmatics.	• Higher road density was associated with increased FeNO and reduced FEV_1 in asthmatics. • No association was observed between fine particulate matter ($PM_{2.5}$) or elemental carbon and FeNO.
LIMITATIONS	• Potential for important confounding by time (some technologies only used later in the study). • No mention of important confounders (atopy and diet).	• Differences in measurement frequencies of $PM_{2.5}$ and NO_2 could have affected results. • 48-hour $PM_{2.5}$ measurements may not precisely capture daily pollution fluctuations.

AUTHORS	Adar et al, 2015[32]	Holguin et al, 2015[33]
CONCLUSION	Adoption of cleaner school bus diesel emission technologies reduced childrens' exposure to fine and ultra-fine particles, which was associated with a decrease in FeNO levels, particularly in asthmatics.	Increased traffic exposure leads to increased FeNO particularly in asthmatics.

Table 2: Epidemiological studies of serial longitudinal FeNO measurement among asthmatic children (continued)

AUTHORS	Roberts et al, 2004[19]	Spanier et al, 2004[20]	Cutts et al. 2013[21]
AIM OF STUDY	To evaluate the effect of pollen on FeNO in children with seasonal asthma.	To evaluate seasonal and environmental effects on FeNO in tobacco-exposed children with asthma.	To evaluate the variability in FeNO over a 10-month period in children with and without asthma.
STUDY DESIGN	Longitudinal study.	Longitudinal study.	Longitudinal study.
STUDY POPULATION	44 children (age 6 to 16 years) with seasonal allergic asthma.	225 children (age 6 to 12 years) with physician-diagnosed asthma.	• 178 primary school children, with a mean age of 9.6 years (SD = 1.3 years). • 47 were asthmatics, and 131 were healthy subjects.
INTERVENTION/ EXPOSURE	Exposure to pollen during the pollen season	Evaluated at baseline, 6, and 12 months and assessed for environmental exposures.	Monitored over a 10-month period and assessed for environmental exposures.

AUTHORS	Roberts et al, 2004[19]	Spanier et al, 2004[20]	Cutts et al. 2013[21]
OUTCOME MEASURES	FeNO, lung function, daily asthma symptoms.	FeNO, settled dust allergens (SDA), indoor airborne particles (IAP), allergen sensitsation, tobacco smoke exposure.	FeNO, lung function, daily asthma symptoms questionnaire.
MEASUREMENTS	• Evaluated before the pollen season; then at regular 4-weekly intervals throughout one pollen season. • All patients underwent physical examination, spirometry and measurement of FeNO. • Atmospheric pollen counts were taken throughout the	• FeNO measured. • Dust mite, dog, cat and cockroach allergens measured with monoclonal ELISA. • Indoor particulate matter measured. • ImmunoCap for allergen sensitisation. • Tobacco smoke exposure measured with using a survey,	FeNO measured at 2-month intervals, on between 4 and 6 different occasions (851 measurements) as follows: • 86 children measured on 6 occasions; • 51 children measured on 4 occasions. • 21 children measured on 4 occasions.

	pollen season with a 7-day volumetric spore trap.	biomarkers (hair and serum cotinine) and a nicotine dosimeter.	
RESULTS	• There was a significant increase in median FeNO during the pollen season (9.2 ppb) compared to before the pollen season (6.2 ppb) ($p=0.002$). • There was a significant relationship between standardized FeNO and pollen counts on the day of measurement ($p<0.01$). • No significant changes were seen in lung-function	• Significant associations were seen between longitudinal change in FeNO and baseline FeNO. • Lowest and highest FeNO levels were seen in winter and autumn (fall), respectively ($p=0.002$). • Being atopic lead to increased FeNO levels. • Cat and dust mite allergen levels significantly increased FeNO.	• Significant associations were seen between initial FeNO concentrations and future values. • FeNO increased during the pollen session (increase in log transformed FeNO 1.34 [95% CI 1.05-1.70]) and elevated also in those with mould at home during September–October.

	parameters before or during the pollen season.	• Airborne nicotine significantly decreased FeNO. • FeNO was not associated with passive smoking exposure when measured by biomarkers and reported smoking by parents.	
LIMITATIONS	• Small sample size. • Absence of a control group of children without seasonal asthma. • No mention of important confounders (daily activities, and diet).	• Absence of a control group of children not exposed to tobacco smoke. • Settled dust allergens were only measured at baseline.	• Uneven numbers of measurements taken on different sample groups. • No mention of important confounders (daily activities, and diet).

AUTHORS	Roberts et al, 2004[19]	Spanier et al, 2004[20]	Cutts et al. 2013[21]
CONCLUSION	Natural allergen exposure during the pollen season increased FeNO in asthmatic children.	Environmental triggers, airborne nicotine, and baseline allergen sensitisation affected FeNO levels.	Both baseline FeNO values and intervals between FeNO measurements were related to individual FeNO values, irrespective of asthma status.

Table 3: Epidemiological studies and case reports of serial longitudinal FeNO measurement in adults

Table 3A. Epidemiological studies among adults and working populations

AUTHORS	Hewitt et al, 2008[34]	Van der Walt et al, 2016[11]	Fowler et al, 2009[22]
AIM OF STUDY	To assess whether serial FeNO measurements may detect exposure-related inflammation in laboratory animal workers	To evaluate the association between FeNO and occupational exposures to spices.	To assess whether exposure to common domestic allergens affects long-term FeNO measurements in adult asthmatics
STUDY DESIGN	Longitudinal study	Cross-sectional study	Longitudinal study
STUDY POPULATION	• Fifty laboratory animal workers in four research centers. • Each subject was exposed to laboratory animals to a minimum of two occasions	• Permanent (n=139) and casual (n=11) workers in a spice mill (total n=150). • Mean employment duration of 6.9 years in the factory and 3.2 years in the current job.	• 165 subjects with asthma, measured at home, 82% of which were atopic, 18% of which were non-atopic. • Each subject was assessed twice, 4 years apart (mean = 47 months).

	per week for six months or longer. • There was no exclusion based on clinical history.		
INTERVENTION/ EXPOSURE	Exposure to laboratory animals in the workplace.	Exposure to spices in the workplace.	Exposure to mite, dog and cat allergens in the household.
OUTCOME MEASURES	Symptom questionnaire, peak expiratory flow measurements, lung function and FeNO.	Immunological tests for common inhalants and occupational agents (garlic, chili pepper and wheat), spirometry and FeNO.	Lung function, FeNO, and immunological tests for sensitisation to mite, dog and cat allergens.
MEASURMENTS	• Subjects were initially assessed on a Friday. • Subjects received instruction on the use of a peak flow meter and a symptom diary.	Serial FeNO measurements were conducted as follows: • Baseline pre-shift on Monday. • Post 8 hour shift.	Allergen exposure was measured using monoclonal ELISA.

	• Having avoided contact with laboratory animals over the weekend, they were assessed at the beginning and at the end of each working day from Monday to Friday of the following week.	• Pre-shift test on the following Tuesday.	Each subject underwent 2 measurements, 4 years apart as follows: • All underwent spirometry and measurement of FeNO. • Allergic sensitisation was measured by skin-prick testing.
RESULTS	• Two of the 50 subjects had a positive specific IgE to one or more laboratory animal allergen (LAA). • Baseline FeNO were highest in two subjects sensitised to	• The geometric mean of FeNO change across shift (15.43 ppb) was very similar to the mean change across the 24-hour period (15.84 ppb).	• There was a mean 1.4-fold decrease in FeNO over the 4-year period ($p<0.001$).

	LAA, having FeNO of 213.0 ppb and 179.0 ppb. • Mean FeNO was (19.8 ± 20.1 ppb) and (21.7 ± 20.8 ppb) in the remaining seronegative symptomatic and non-symptomatic groups, respectively. • Progressive increases in FeNO occurred only in one of the seropositive individuals during the working week.	• FeNO increase (>12%) across a 24-hour period was significantly associated (OR=3.77; CI 1.01-14.24) with exposures to higher spice dust particulate but no effect observed across the 8-hour work shift.	

AUTHORS	• Hewitt et al, 2008[34]	• Van der Walt et al, 2016[11]	• Fowler et al, 2009[22]
LIMITATIONS	• Small sample size. • Subjects were not all asthmatic. • No mention made of important confounders. However, mention was made of the workplace protection not being uniform.	• No comparable unexposed group. • Short follow-up duration	• The level of exposure was not measured at the second visit. • Possibilities for selection bias • No control group of healthy adults. • Possibility of important confounders (high doses of ICS among all participants).
CONCLUSION	Serial FeNO could assist in monitoring patients sensitised to occupational allergens. However, its value decreases if the patient is also sensitised and exposed to	FeNO level increase was more pronounced 24 hours after the exposure to spice dust particulate.	Long-term exposure to dust mite and dog allergens in the home caused significant changes in bronchial hyperresponsiveness, but not necessarily in FeNO levels of asthmatic adults.

	non-occupational allergens which can mask the FeNO changes.		

Table 3B. Case reports of adult working population

AUTHORS	Merget et al, 2014[35]	Merget et al, 2016[36]
AIM OF STUDY	To assess the practicality of serial measurements of FeNO as diagnostic tool for occupational asthma.	A case report of triticale (wheat/rye) allergy in a farmer.
STUDY DESIGN	Case study	Case study
STUDY POPULATION	51-year-old male smoker working as a baker since adolescence who complained of shortness of breath and cough.	29-year-old farmer with hay fever and atopic dermatitis since adolescence diagnosed with work-related asthma.
INTERVENTION	Avoidance of exposure for 2 weeks.	Avoidance of exposure for 2 weeks.
OUTCOME MEASURES	Change of FeNO level over time.	Change of FeNO level over time.
MEASURMENTS	FeNO measurement was performed once daily over a 2 weeks holiday period and thereafter over 3 weeks of working days.	• FeNO measurement was performed once daily during a working week and thereafter over a two-week holiday period.

		• Measurements were done in the evenings, (after work) in the working period.
RESULTS	FeNO decreased to the normal level soon after the start of the holiday period and showed a clear increase after resuming work.	FeNO decreased gradually over time soon after the start of the holiday period.
LIMITATIONS	Case study design	Case study design
CONCLUSION	FeNO decreased over 2 weeks of allergen and irritants avoidance and showed a clear increase over the working day period	FeNO decreased over 2 weeks of allergen avoidance in a patient with occupational asthma to triticale

Exposure to specific sensitising agent

All the studies identified focused mainly on serial FeNO changes with exposure to high molecular weight (HMW) agents. However, it has been previously reported that the increase in FeNO after specific inhalation challenge tests in patients with suspected occupational asthma is more strongly associated with exposure to HMW than low molecular weight (LMW) agents, the former known to be commonly associated with allergic IgE-mediated airway inflammation.[12]

Other exposure characteristics

While it could be hypothesised that exposure duration, exposure concentration or cumulative exposure may play a role in determining serial changes of FeNO in population-based studies, we were unable to identify many studies that evaluated these potential relationships. However, Lemiere et al[12] evaluated this association between serial changes in FeNO and the duration of exposure to the potential allergen during SIC tests and the association was not found to be significant (OR = 0.99, 95% CI 0.9-1.0). Although the overall association between the overall change in FeNO and the concentration of allergens exposure was not statistically significant, Van der Walt et al[11] found a significant association between workers exposed to high concentrations of spices and >12% increase in FeNO in their mill workers (OR = 3.77, 95% CI 1.01-14.24). Furthermore, a positive correlation has also been demonstrated between the degree of FeNO reduction after HDM avoidance and baseline HDM allergen levels measured in the beds of subjects ($r = 0.618$, $p = 0.026$) by Piacentini et al in their asthmatic children.[24]

Air pollution

Exposure to air pollutants could contribute to changing FeNO levels, especially in busy large cities. Adar et al[32] found that reducing ultrafine particles by adoption of clean fuel technology in school buses leaded to a reduction in FeNO among bus riders by 16%. This association was more prominent in asthmatic children. Moreover, Holguin et al[33] found that exposure to higher road density was associated with a significant increase in FeNO in asthmatic children and a non-significant increase in healthy children. Furthermore, both studies found no association between FeNO and exposure to $PM_{2.5}$ particulates. This suggests that $PM_{2.5}$ may not be useful in studying the association between FeNO and air pollution in children.

HOST ASSOCIATED FACTORS

Asthma education

The effect of asthma education programs on asthma control has been evaluated through several studies of children. Kaminsky et al[27] evaluated 25 asthmatic children, 21 on inhaled corticosteroids (ICS), participating in a one-week camp organised with asthma education programs. The overall mean FeNO dropped by 45% ($p<0.001$) at the end of the week. This decline could be attributed to ICS, which was found to reduce FeNO in asthmatics in a dose-dependent manner.[39] [40] However, three of the four children who were not on ICS also demonstrated a decrease in FeNO at the end of the camp. The authors indicate that it is also possible that the changes in FeNO could have been due to other additional factors such as allergen avoidance or absence of anxiety triggers in the home.[41] [42] Furthermore, Kaminsky et al did not specify the asthma control status of the subjects that were evaluated. It is possible that a large proportion were non-compliant or under-treated so that the change in FeNO could have been due to improved asthma control. This was evident in the study by Barreto et al[31] who studied asthmatic children with good asthma control in a sanatorium for one week, which was

accompanied with an asthma education program. The study reported that the mean baseline FeNO in asthmatics on ICS did not differ from the healthy subjects nor did it differ over the entire week. In contrast, asthmatics not on ICS had a higher FeNO baseline ($p < 0.005$) compared to the healthy subjects and showed a progressive decrease over the week until the difference reached non-significance ($p = 0.057$). This is further explained by the study of Jatakanon et al[43] that demonstrated a plateau of exhaled FeNO in response to higher doses of ICS.

Asthma education programs have also been evaluated in adults in domestic and occupational settings. Dressel et al[29] evaluated FeNO after a one-day asthma education program in 81 animal farmers with occupational asthma that were sensitised to cow dander and storage mites. The overall mean FeNO decreased by 9% ($p = 0.042$) after 4-6 weeks after the program and by 18% ($p = 0.003$) among those with a baseline FeNO of >35ppb. There were 43 subjects in this study who were re-evaluated after one year to assess long-term changes in FeNO after the asthma education program.[30] The mean FeNO decreased by 21% ($p = 0.001$) in the intervention group. While measures to reduce exposure to the allergens, such as use of personal protective equipment, changing work clothes before going home and washing hair before going to bed, were major contributors to this reduction in FeNO in both studies, compliance to medication was also cited as an additional contributor to these positive findings. This was evident also in the study by Saito et al[28], which provided asthma education program to 50 asthmatics with poor adherence to medication. The program was aimed at increasing compliance to medications and to improve the inhalation technique. The study reported a significant decrease in the overall mean FeNO after a year of follow-up (visits at 3, 6, 12 months from the baseline) in stable patients ($n = 42$) even though asthma medication was stepped down in 33% of the subjects. However, there was a non-significant reduction in FeNO in 16 subjects who were initially

unstable and required an increase in ICS dosage or additional asthma medication during the course of the study.

Atopy

It is well known that FeNO levels are higher among atopic subjects irrespective of the domestic or occupational exposures.[3-5] These findings were also reported by Van der Walt et al[11] in their study of spice mill workers. However, strong associations between atopic status and longitudinal changes in FeNO have not been studied in detail. Changes in FeNO appear to be independent of the atopic status.[26 34] Spanier et al[20] found that the seasonal variation of FeNO did not differ between the atopic and non-atopic subjects. Moreover, Lemiere et al[12] reported a non-significant positive association between changes in FeNO and atopy status (OR 2.0, 95% CI 0.5-7.5) in their study of FeNO measured after SIC to occupational agents in patients with occupational asthma.

Smoking status

It is well known that smoking is associated with a decreased baseline FeNO in subjects.[3-5] However, strong associations between smoking status and longitudinal changes in FeNO have not been studied in detail. Lemiere et al[12] in their study of patients with occupational asthma found a non-significant positive association between FeNO changes and negative smoking status (OR 1.5, 95% CI 0.5-4.3) after SIC to occupational agents in patients with occupational asthma.

In children, passive smoking may have an effect on longitudinal changes in FeNO. Spanier et al[20] found that exposure to passive smoking measured by a nicotine dosimeter lead to a significant decrease in FeNO. However, this association disappeared when the exposure reported by the parent's survey was used suggesting possible recall bias.

Baseline pulmonary function and fractional exhaled nitric oxide

While the relationship between baseline FeNO and Forced Expiratory Volume (FEV_1) has been evaluated in a number of studies, studies evaluating the association between baseline FEV_1 and the longitudinal change in FeNO are scanty. Peroni et al[25] did not find a correlation between FEV_1 and longitudinal changes in FeNO in their study. Lemiere et al[12] also reported a non-significant association between changes in FeNO post SIC and baseline FEV_1, nor was this association present with a maximum fall in FEV_1 after SIC. However, Saito et al[28] found a significant correlation between change in FeNO and the change in FEV_1 in the first 3 months of their study, which remained significant until the end of the study after 12 months of baseline.

While it could be hypothesised that baseline FeNO may be a good indicator of the magnitude of future change in serial FeNO, studies in this area that evaluated this potential relationship were scant. In children, Spanier et al[20] and Cutts et al[21] found a significant association between baseline FeNO and longitudinal changes in FeNO. However, Lemiere et al[12] were unable to demonstrate an association between baseline FeNO (>25ppb) and change in FeNO post SIC in an adult group.

CONCLUSION

In conclusion, avoiding the exposure of the offending agents has been shown to decrease the airway inflammation as evaluated by FeNO which can be regarded as an important marker of airway inflammation in asthma among both symptomatic and asymptomatic subjects. While the predictors of baseline FeNO levels have been evaluated in several clinical and population-based studies, the evidence for various factors associated with longitudinal changes in FeNO need further investigation. These include smoking, atopy, baseline FeNO, baseline FEV_1 and exposure characteristics (agent and dose), which need further evaluation, especially in

occupational settings. Modulating exposures through the introduction of intervention to reduce allergen exposures can be used to study these changes in epidemiological studies.

REFERENCES

1 Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention. *[Internet]*. 2016. Available from: http://www.ginasthma.org

2 Dominguez-Ortega J, Barranco P, Rodríguez-Pérez R, et al. Biomarkers in occupational asthma. *Curr Allergy Asthma Rep* 2016;16:63.

3 Al-shamkhi N, Alving K, Dahlen SE, et al. Important non-disease-related determinants of exhaled nitric oxide levels in mild asthma–results from the Swedish GA2LEN study. *Clin Exp Allergy* 2016;46:1185–93.

4 Ludviksdottir D, Diamant Z, Alving K, et al. Clinical aspects of using exhaled NO in asthma diagnosis and management. *Clin Respir J* 2012;6:193–207.

5 Alving K, Malinovschi A. Basic aspects of exhaled nitric oxide. *European Respiratory Monograph* 2010;49:1–31.

6 Baatjies R, Jeebhay MF. Sensitisation to cereal flour allergens is a major determinant of elevated exhaled nitric oxide in bakers. *Occup Environ Med* 2013;70:310–6.

7 Ricciardolo FLM, Timmers MC, Sont JK, et al. Effect of bradykinin on allergen induced increase in exhaled nitric oxide in asthma. *Thorax* 2003;58:840–5.

8 Quirce S, Lemière C, De Blay F, et al. Noninvasive methods for assessment of airway inflammation in occupational settings. *Allergy* 2010;65:445–58.

9 Świebocka E, Siergiejko G, Siergiejko Z. Bronchial allergen challenge in allergic children: continuous increase of nitric oxide in exhaled air 72 hours after allergen inhalation independent of bronchial obstruction. *J Aerosol Med Pulm Drug Deliv* 2011;24:17–24.

10 Pedrosa M, Barranco P, López-Carrasco V, et al. Changes in exhaled nitric oxide levels after bronchial allergen challenge. *Lung* 2012;190:209–14.

11 Van der Walt A, Baatjies R, Singh T, et al. Environmental factors associated with baseline and serial changes in fractional exhaled nitric oxide (FeNO) in spice mill workers. *Occup Environ Med* 2016;:1–7.

12 Lemiere C, NGuyen S, Sava F, et al. Occupational asthma phenotypes identified by increased fractional exhaled nitric oxide after exposure to causal agents. *J Allergy Clin Immunol* 2014;134:1063–7.

13 Świerczyńska-Machura D, Krakowiak A, Wiszniewska M, et al. Exhaled nitric oxide levels after specific inahalatory challenge test in subjects with diagnosed occupational asthma. *Int J Occup Med Environ Health* 2008;21:219–25.

14 Baur X, Barbinova L. Latex allergen exposure increases exhaled nitric oxide in symptomatic healthcare workers. *Eur Respir J* 2005;25:309–16.

15 Allmers H, Chen Z, Barbinova L, et al. Challenge from methacholine, natural rubber latex, or 4, 4′-diphenylmethane diisocyanate in workers with suspected sensitisation affects exhaled nitric oxide [change in exhaled NO levels after allergen challenges]. *Int Arch Occup Environ Health* 2000;73:181–6.

16 Piipari R, Piirilä P, Keskinen H, et al. Exhaled nitric oxide in specific challenge tests to assess occupational asthma. *Eur Respir J* 2002;20:1532–7.

17 Barbinova L, Baur X. Increase in exhaled nitric oxide (eNO) after work-related isocyanate exposure. *Int Arch Occup Environ Health* 2006;79:387.

18 Baraldi E, CarrÀ S, Dario C, et al. Effect of natural grass pollen exposure on exhaled nitric oxide in asthmatic children. *Am J Respir Crit Care Med* 1999;159:262–6.

19 Roberts G, Hurley C, Bush A, et al. Longitudinal study of grass pollen exposure, symptoms, and exhaled nitric oxide in childhood seasonal allergic asthma. *Thorax* 2004;59:752–6.

20 Spanier AJ, Hornung RW, Kahn RS, et al. Seasonal Variation and Environmental Predictors of Exhaled Nitric Oxide in Children with Asthma. *Pediatr Pulmonol* 2008;43:576–83.

21 Cutts R, Turner S. Longitudinal measurements of exhaled nitric oxide in children-what is a significant change in FENO? *Pediatr Allergy Immunol* 2013;24:540–8.

22 Fowler SJ, Langley SJ, Truman NJ, et al. Long-term effects of allergen sensitisation and exposure in adult asthma: a prospective study. *World Allergy Organ J* 2009;2:83–90.

23 Piacentini GL, Bodini A, Costella S, et al. Allergen avoidance is associated with a fall in exhaled nitric oxide in asthmatic children. *J Allergy Clin Immunol* 1999;104:1323–4.

24 Piacentini GL, Del Giudice MJM, Bodini A, et al. Exhaled NO reduced on allergen avoidance. *Allergy* 2001;56:251–2.

25 Peroni DG, Piacentini GL, Costella S, et al. Mite avoidance can reduce air trapping and airway inflammation in allergic asthmatic children. *Clin Exp Allergy* 2002;32:850–5.

26 Huss-Marp J, Krämer U, Eberlein B, et al. Reduced exhaled nitric oxide values in children with asthma after inpatient rehabilitation at high altitude. *J Allergy Clin Immunol* 2007;120:471–2.

27 Kaminsky DA, Rice AA, Bissonette M, et al. Exhaled nitric oxide decreases in association with attendance at an asthma summer cAMP. *J Asthma* 2008;45:415–9.

28 Saito J, Sato S, Fukuhara A, et al. Association of asthma education with asthma control evaluated by asthma control test, FEV1, and fractional exhaled nitric oxide. *J Asthma* 2013;50:97–102.

29 Dressel H, Gross C, De la Motte D, et al. Educational intervention decreases exhaled nitric oxide in farmers with occupational asthma. *Eur Respir J* 2007;30:545–8.

30 Dressel H, Gross C, de la Motte D, et al. Educational Intervention in Farmers With Occupational Asthma: Long-Term Effect on Exhaled Nitric Oxide. *J Investig Allergol Clin Immunol* 2009;19:49.

31 Barreto M, Rennerova Z, Montesano M, et al. Variations in exhaled nitric oxide in children with asthma during a 1-week stay in a mountain village sanatorium. *J Asthma* 2008;45:453–8.

32 Adar SD, D'Souza J, Sheppard L, et al. Adopting clean fuels and technologies on school buses: Pollution and health impacts in children. *Am J Respir Crit Care Med* 2015;191:1413–21.

33 Holguin F, Flores S, Ross Z, et al. Traffic-related exposures, airway function, inflammation, and respiratory symptoms in children. *Am J Respir Crit Care Med* 2007;176:1236–42.

34 Hewitt RS, Smith AD, Cowan JO, et al. Serial exhaled nitric oxide measurements in the assessment of laboratory animal allergy. *J Asthma* 2008;45:101–7.

35 Merget R, Sander I, van Kampen V, et al. Serial measurements of exhaled nitric oxide at work and at home: a new tool for the diagnosis of occupational asthma. *Adv Exp Med Biol* 2015;834:49–52.

36 Merget R, Sander I, van Kampen V, et al. Triticale allergy in a farmer. *Am J Ind Med* 2016;59:501–5.

37 Huss-Marp J, Krämer U, Eberlein B, et al. Reply: Exhaled nitric oxide in children with asthma at high altitude. *J. Allergy Clin. Immunol.* 2007;120:1227–8.

38 Karagiannidis C, Hense G, Rueckert B, et al. High-Altitude Climate Therapy Reduces Local Airway Inflammation and Modulates Lymphocyte Activation. *Scand J Immunol* 2006;63:304–10.

39 Kharitonov SA, Yates DH, Barnes PJ. Inhaled glucocorticoids decrease nitric oxide in exhaled air of asthmatic patients. *Am J Respir Crit Care Med* 1996;153:454–7.

40 Jones SL, Herbison P, Cowan JO, et al. Exhaled NO and assessment of anti-inflammatory effects of inhaled steroid: dose-response relationship. *Eur Respir J* 2002;20:601–8.

41 Sandel M, Wright RJ. When home is where the stress is: expanding the dimensions of housing that influence asthma morbidity. *Arch Dis Child* 2006;91:942–8.

42 Chen E, Miller GE. Stress and inflammation in exacerbations of asthma. *Brain Behav Immun* 2007;21:993–9.

43 Jatakanon A, Kharitonov S, Lim S, et al. Effect of differing doses of inhaled budesonide on markers of airway inflammation in patients with mild asthma. *Thorax* 1999;54:108–14.

PART C: JOURNAL ARTICLE MANUSCRIPT

This manuscript has been prepared to be submitted for publication in the journal Occupational and Environmental Medicine. (appendix 7)

KEY MESSAGES AND KEY WORDS

Key messages

What is already known about this subject?
The reduction of workplace exposure to airborne allergen can improve the health outcomes of the exposed workers.

What are the new findings?
Workplace interventions that aim at reducing exposure to occupational sensitisers, resulted in a significant decline (>10%) in fractional exhaled nitric oxide among those with pre-existing upper airway involvement and work-related ocular-nasal symptoms.

How might this impact on policy or clinical practice in the foreseeable future?
Fractional exhaled nitric oxide could be a useful tool to monitor the effectiveness of workplace interventions that aim at reducing exposure to occupational sensitisers, particularly among those with pre-existing upper airway involvement and work-related ocular-nasal symptoms.

Key words

work-related asthma; allergic sensitisation, exposure reduction, workplace interventions.

ABSTRACT

Aim: To assess the health impact of an intervention in supermarket bakeries using fractional exhaled nitric oxide and other clinical endpoints for baker's allergy and asthma after a one-year follow-up period.

Methods: A field randomised controlled trial of 31 bakeries initially assigned to one of two intervention groups (bakery mixer lid and training) (n=244) and a control group (n=93). Health data prior to and after the intervention included a modified ECRHS questionnaire; Phadiatop ® and serum specific IgE to cereal flours (wheat, rye, alpha-amylase); and FeNO performed during the work shift using NIOX MINO®. The data of the two intervention groups was combined into one intervention group for the analysis. Data was analysed using STATA (version 12).

Results: The two groups were comparable with regard to age (32-33 years), proportion of females (55%-57%) and smoking status (38%-40%). The intervention group had a significantly higher prevalence of workers with atopy (42%, p=0.025), work-related chest symptoms (25%, p=0.044) and sensitisation to cereal flour allergens (35%, p=0.042) at baseline than the control group (25%, 15%, 23% respectively). At one year of follow-up, the incidence and level of decline of work-related ocular-nasal and chest symptoms, sensitisation status and elevated FeNO (FeNO >25ppb) was similar in the two groups. The mean difference in FeNO was similar across the two groups (2.2ppb vs 1.7ppb, p=0.860). However, when stratifying according to baseline FeNO >25ppb, the FeNO decline was greater in the intervention group (16.9 ppb) than in the control group (7.7ppb), although not statistically significant (p=0.237). Multivariate logistic regression models (adjusting for smoking, baseline sensitisation to cereal flour, baseline FeNO >25ppb) did not demonstrate an appreciable decline in FeNO (≥10%) in the intervention compared to the control group. However, stratification according to the presence of work-

related ocular-nasal symptoms at baseline demonstrated a significant decline (≥10%) in FeNO in the intervention group compared to the control group (OR=3.73, CI: 1.22-11.42).

Conclusion: This study demonstrates some evidence of an intervention effect on exhaled nitric oxide (FeNO) one year after the intervention, particularly among bakers reporting work-related ocular-nasal symptoms at baseline. The lack of a demonstrably stronger effect on other clinical endpoints can be attributable to the short follow-up period.

INTRODUCTION

Work-related asthma accounts for at least 15% of adult asthma, resulting in significant morbidity and disability.[1 2] Various strategies have been developed in attempts to reduce the burden of the disease and improve the prognosis of workers with occupational asthma. One of the approaches which has been found to be beneficial is the reduction of exposure to the offending sensitiser.[3 4] However, studies that have evaluated the effectiveness of interventions aimed at reducing exposure to occupational sensitisers are scant.[3 5] Only two studies among these reported objective measurements of the level of exposures encountered.[3 6 7] Furthermore, most of the studies have reported on asthma symptoms, lung function or non-specific bronchial hyperactivity (NSBH) in evaluating the outcome of exposure reduction measures. Few studies have utilised the presence of airway inflammation as an objective outcome measure in assessing the effectiveness of exposure reduction in occupational settings.

Fractional exhaled nitric oxide (FeNO) can be regarded as an important marker of airway inflammation in asthma among both symptomatic and asymptomatic subjects. Whereas the predictors of baseline FeNO levels have been evaluated in several clinical and population-based studies, the evidence for various factors associated with longitudinal changes in FeNO need further investigation.[8] However, ongoing exposure and avoidance of exposure to occupational airborne allergens were the main determinants of longitudinal change in FeNO.[8]

In an earlier phase of this study, the effectiveness of a multi-faceted intervention to reduce exposure to flour dust in supermarket bakeries with high flour dust levels and a population with a high prevalence (13%) of baker's asthma[9] was evaluated.[10] The specially designed intervention strategy was found to be extremely effective in reducing airborne dust and allergen levels by 50%-80%.[11] The aim of the current study was to assess the health impact of these

intervention using FeNO and other clinical endpoints for baker's allergy and asthma one year after the intervention.

METHODS

Study design and population

This study of a group field randomised controlled trial involved the analysis of data that was collected in 2011 as a part of a larger study started in 2003.[10] The original study identified 31 bakeries in the Western Cape province of South Africa as the potential population for the study. Out of these bakeries, 18 were randomly assigned to two intervention groups and one control group, taking into consideration the size of the bakery and number of workers in each bakery (figure 1). Two intervention strategies were developed, using a bakery mixer lid and training, through a focused group discussion that involved bakery workers, managers and engineers.[11] An assessment for baseline environmental exposure was initially performed using full-shift personal samples to measure the level of exposure to flour dust in different job categories in the bakeries.[10] This was followed by a baseline health survey of 424 participants investigating chest symptoms, ocular-nasal symptoms and work-related symptoms using a short questionnaire (appendix 2). Furthermore, serum specific IgE to wheat, rye and α-amylase as well as FeNO were assessed prior to implementing the intervention.[12] One year after implementation, the effectiveness of the intervention was assessed using flour dust levels.[11] Later, a cross sectional survey was administered to 361workers among whom 337 were from the original cohort that participated in earlier phases. In this phase, data was collected on work-related symptoms using the modified the European Community Respiratory Health Survey (ECRHS) questionnaire (appendix 3),[13] specific IgE to wheat, rye and fungal amylase and

FeNO. In the current study, the complete data collected from the 337 workers (244 participants from the two intervention groups and 93 participants from the control group) was analysed.

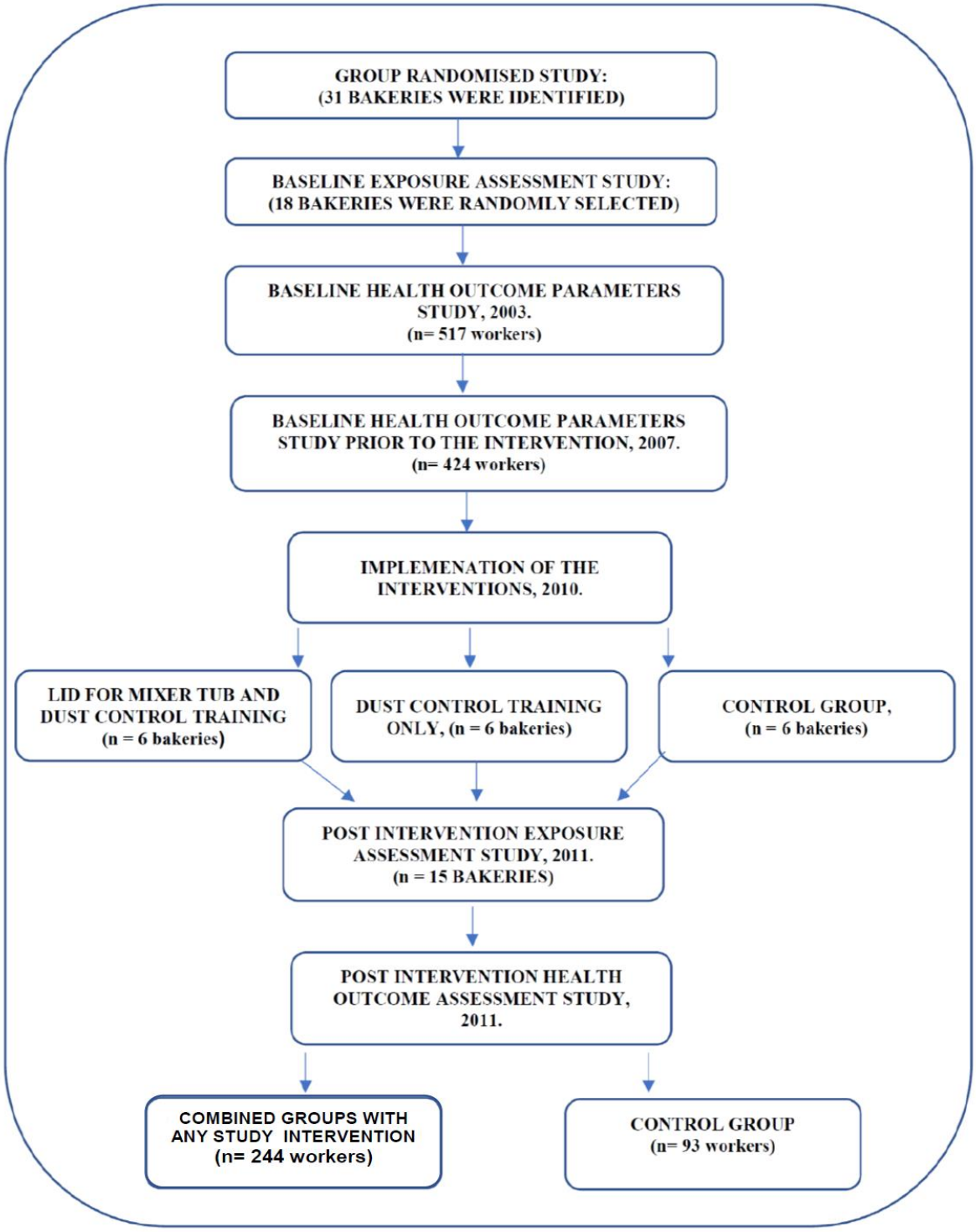

Figure 1: Flow diagram illustrating intervention study design and bakeries assessed pre-and post-intervention.

The data of the two intervention groups (bakery mixer lid group and training group) was combined into one intervention group for the analysis. Eighty-seven workers were not traceable for further evaluation for various reasons (e.g. store closure). However, the current cohort (337 workers) adequately represented different job categories found in the bakery environment. The study received ethical approval from the University of Cape Town, South Africa (appendix 6).

Health outcome assessment

Skin prick tests

The data of skin prick tests (SPTs) was obtained from a previous sub-study.[9] ALK-Abello´ A/S, Horsholm, Denmark was used to test for common local allergens. Reading of the tests was done 15 minutes after testing and areas of the wheal were traced on a clear tape. A wheal of ≥3 mm more than the negative control was considered positive. Atopy was defined as the presence of a positive SPT to one or more common aeroallergens.

Respiratory questionnaire

A standard questionnaire designed based on the protocol for the European Community Respiratory Health Survey (ECRHS)[13] was administered to the participants (appendix 3) in the follow-up study. It gathered information on acute and chronic respiratory and ocular nasal symptoms and work-relatedness of these symptoms. It also covered information related to current and previous employment, levels of exposure to flour dust and tobacco smoke. The modified questionnaire was administered in either English or Afrikaans.

Serum immunological tests

Blood samples were obtained from the participants for serum-specific immunoglobulin IgE. Sera were tested to determine the presence of atopy using Phadiatop® test (ImmunoCAP 100 System; Phadia, Uppsala, Sweden). The sera were also tested for specific IgE to flour dust allergens such as wheat (f4), rye (f5) and fungal a-amylase (k87) using fluorescence enzyme

immunoassay (CAP-FEIA) according to the manufacturer's instructions (Phadia). Results were treated as a continuous or binary variable in which ImmunoCAP ≥ 0.35 kU/L) was considered positive.

Fractional exhaled nitric oxide determination

A hand-held portable nitric oxide sampling device (NIOX MINO) was used to determine FeNO during the work shift. It was performed in a room distant from the bakery area during the work shift throughout the working week, according to American Thoracic Society (ATS)/European Respiratory Society (ERS) recommendations.[14 15] The testing of workers had no particular variation with regard to time of testing for the different jobs. The average of three technically adequate FeNO measurements was determined. Workers were instructed to abstain from smoking, eating or drinking at least one hour before the test. This was confirmed prior to testing, and those who did not follow the instructions were tested at a later stage after ensuring their full compliance with these instructions. Levels of FeNO were treated either continuous variable (log-transformed), or binary variable FeNO >25ppb and FeNO>50ppb. A 10% decline or more in FeNO was considered significant reduction in airway inflammation post intervention.

Statistical analysis

Data was analysed using STATA V.12 computer software (StataCorp, College Station, Texas, USA). Data is presented as proportions, means (geometric where appropriate) or median and the corresponding standard deviation (SD) or interquartile range (IQR). Chi-square test and an independent t-test (or Fisher's exact test and Wilcoxon sum rank test where appropriate) were used to compare groups at baseline as well as to determine the effect of intervention on the incidence and decline of allergic and respiratory health outcomes at one-year follow-up. Univariate linear regression was used to explore the determinants of longitudinal change in FeNO. Multivariate regression analysis was used to determine predictors of a 10% or more

decline in FeNO using the intervention and adjusting for other confounders such as current smoking, baseline FeNO>25ppb and sensitisation to cereal flour allergens. Further subgroup multivariate regression analysis stratified by the presence of work-related ocular nasal symptoms was used to assess effect modification. A two-tailed p-value of <0.05 was considered to be statistically significant.

RESULTS

The general characteristics of the 337 participants are presented in Table 1. The two groups were comparable with regard to age (32-33 years), proportion of females (55%-57%) and smoking status (38%-40%). The intervention group had a significantly higher prevalence of workers with atopy compared to the control group (42%, 25% p=0.025). The intervention group had a significantly higher baseline prevalence of workers with work-related chest symptoms (25%, p=0.044) and sensitisation to cereal flour allergens (35%, p=0.042) at baseline than the control group (15% and 23% respectively). No significant differences were found for other allergic and respiratory health outcomes as shown in Table 1.

Table 1: Baseline characteristics of supermarket bakery workers

Predictor	**Intervention group**			**Control group**			**Chi-square p-value**
	n	**N**	**%**	**n**	**N**	**%**	
Age, median (IQR)	33 (12)	244	-	32 (11)	93	-	0.579
Gender (female)	139	244	57	51	93	55	0.725
Weight, median (IQR)	74.5 (29)	244	-	74 (28)	93	-	0.528
Height, mean±SD[#]	165±8.2	155	-	166±9.3	56	-	0.594
BMI, median (IQR) [#]	27.8 (9.5)	155	-	28.7 (10.6)	56	-	0.583
Current smoker	93	244	38	37	93	40	0.695

Atopy (skin prick test) [#]	65	155	42	14	56	25	0.025
Current steroid use for asthma and/or rhinitis	8	244	3	1	93	1	0.453[*]
Current employment status (Permanent)	214	244	88	83	93	89	0.696
Current job with high exposure	161	222	73	54	83	65	0.203
Duration of employment in the bakery industry, median (IQR)	7 (7)	241	-	7 (10)	89	-	0.869
Ocular-nasal symptoms	83	244	34	31	93	33	0.906
Work-related ocular-nasal symptoms	92	241	38	34	93	37	0.785
Medication use for ocular-nasal symptoms	15	244	6	7	93	8	0.647
Asthma attack in the last 12 months	15	244	6	7	93	8	0.647
Work-related chest symptoms	61	241	25	14	93	15	0.044
Medication use for asthma	12	244	5	3	93	3	0.768[*]
Specific IgE ≥ 0.35 kU/L							
– Wheat	66	200	33	18	80	23	0.083
– Rye	58	200	29	17	80	21	0.186
– α-amylase	2	200	1	1	80	1	1.0[*]
– Any one of the cereal flour allergens	70	200	35	18	80	23	0.042

Baseline FeNO, GM±GSD	17.7±2.3	244		17.8±2.1	93		0.947
FeNO (ppb)							
– <25	179	244	73	67	93	72	0.808
– 25-50	34	244	14	18	93	19	0.218
– >50	31	244	13	8	93	9	0.293

* Fisher's exact test
# Data obtained from a previous sub-study; Baatjies et al.[9]

At one-year of follow-up, the incidence and level of decline of work-related ocular-nasal and chest symptoms, sensitisation status and elevated FeNO was very similar in both groups as shown in Tables 2 and 3. The mean difference in FeNO was similar across the two groups (2.2ppb vs 1.7ppb, p=0.860). However, when stratifying according to baseline FeNO >25ppb, the FeNO decline was greater in the intervention group (16.9 ppb) compared to the control group (7.7ppb), but not statistically significant (p=0.237) (Table 3). This was also evident when stratifying according to baseline FeNO >50ppb. FeNO decline approached borderline significance as the proportional level of decline increased when baseline FeNO>25ppb such that 49% of the intervention group and 28% of the control group experienced a 40% decline (p=0.07).

Table 2: Incidence of allergic and respiratory health outcomes in supermarket bakery workers at one-year follow-up

Predictor	**Intervention group**			**Control group**			**Chi-square**
	n	**N#**	**%**	**n**	**N#**	**%**	**p-value**
Ocular-nasal symptoms	41	161	25	15	62	24	0.844
Work-related ocular-nasal symptoms	41	149	28	15	59	25	0.759
Medication use for ocular-nasal symptoms	38	229	17	18	86	21	0.370
Asthma attack in the last 12 months	4	229	2	1	86	1	1.0*

Work-related chest symptoms	29	180	16	18	79	23	0.199
Medication use for asthma	17	232	7	5	90	6	0.572
Specific IgE ≥ 0.35 kU/L							
– Wheat	11	134	8	2	60	3	0.351
– Rye	12	141	9	3	61	5	0.560
Specific IgE ≥ 0.35 kU/L							
– α-amylase	2	196	1	2	77	3	0.316
– Any one of the cereal flour allergens	14	130	11	3	60	5	0.195
FeNO (ppb)							
– 25-50 ppb	17	179	10	7	66	10	0.796
– >50ppb	10	213	5	1	83	1	0.302*

Population at risk free of the adverse health outcome of interest at baseline

* Fisher's exact test

Table 3: Relative decline in allergic and respiratory health outcomes in supermarket bakery workers assessed at one year follow up

Predictor	**Intervention group**			**Control group**			**Chi-square**
	n	**N#**	**%**	**n**	**N#**	**%**	**p-value**
Work-related ocular-nasal symptoms	37	92	40	17	34	50	0.325
Medication use for ocular-nasal symptoms	4	15	27	3	7	43	0.630*
Asthma attack in the last 12 months	6	10	60	2	5	40	0.608*
Work-related chest symptoms	24	61	39	5	14	36	0.801
Medication use for asthma	3	12	25	0	3	0	1.0*
Specific IgE change, median (IQR)							
– Wheat	0.01 (0.06)	198		0.01 (0.05)	78		0.011

– Rye	0.01 (0.06)	198		0.01 (0.05)	78		0.097
– α-amylase	0 (0.01)	198		0 (0.01)	78		0.311
Specific IgE ≥ 0.35 kU/L							
– Wheat	9	64	14	0	18	0	0.195*
– Rye	8	57	14	1	17	6	0.675*
– α-amylase	0	2	0	0	1	0	-
– Any one of the cereal flour allergens	14	68	21	1	18	6	0.177*
FeNO change, mean±SD#	2.2±23.0	244		1.7±14.5	91		0.857
Proportion decline in FeNO							
– 10% decline	125	244	51	40	91	43	0.177
– 20% decline	100	244	41	33	91	35	0.356
– 30% decline	75	244	31	24	91	26	0.374
– 40% decline	52	244	21	15	91	16	0.287
FeNO change if baseline>25ppb, mean±SD#	16.9±35.2	63		7.7±24.2	25		0.237
Proportion decline in FeNO if baseline >25ppb							
– 10% decline	44	63	70	16	25	64	0.596
– 20% decline	41	63	65	13	25	52	0.256
– 30% decline	36	63	57	9	25	36	0.074
– 40% decline	31	63	49	7	25	28	0.070
FeNO change if baseline >50ppb, mean±SD	26.1±45.5	31		11.5±39.3	8		0.413
Proportion decline in FeNO if baseline>50ppb							
– 10% decline	22	31	71	5	8	63	0.682*
– 20% decline	22	31	71	4	8	50	0.402*
– 30% decline	21	31	68	3	8	38	0.220*
– 40% decline	19	31	61	3	8	38	0.261*

* Fisher's exact test
# Wilcoxon sum rank test

In the univariate linear regression analysis (Table 4), ocular-nasal symptoms and baseline FeNO were significant determinants of the percentage (%) longitudinal change in FeNO.

Current smoking status and work-related ocular nasal symptoms were borderline (p = 0.088 for both predictors). However, belonging to the intervention group, atopy, current use of steroids (for rhinitis/asthma) and duration of employment in the bakery industry were not significant predictors. Multivariate logistic regression models adjusting for smoking, baseline sensitisation to cereal flour and baseline FeNO >25ppb), did not demonstrate an appreciable decline in FeNO (≥10%) experienced by the intervention compared to the control group (Table 5). However, stratification according to the presence of work-related ocular-nasal symptoms at baseline demonstrated a significant decline (≥10%) in FeNO in the intervention group compared to the control group (OR=3.73, CI: 1.22-11.42).

Table 4: Determinants of percentage (%) longitudinal change in fractional exhaled nitric oxide among supermarket bakery workers in univariate linear regression models

Predictor	**Estimate (β1)**	**p-value**
Gender	-0.4159	0.970
Age	0.5874	0.394
Weight	-0.0928	0.734
Height	-12.5942	0.885
BMI	-0.2321	0.803
Current smoker	18.8604	0.088
Current alcohol drinking	-0.7997	0.370
Current steroid use for asthma and/or rhinitis	5.3648	0.866
Ocular-nasal symptoms	22.8486	0.045
Work-related ocular-nasal symptoms	18.9564	0.088
Current job with high exposure	-2.0081	0.871
Duration of employment in the bakery industry	0.8152	0.344
Intervention vs control group	13.2170	0.275
Atopy (skin prick test)	-19.8812	0.192

Specific IgE to wheat	-0.1696	0.751
Specific IgE to wheat ≥ 0.35 kU/L	15.9971	0.232
Specific IgE to rye	-0.0117	0.972
Specific IgE to rye ≥ 0.35 kU/L	-0.4926	0.972
Specific IgE to α-amylase	-7.0740	0.185
Specific IgE to α-amylase ≥ 0.35 kU/L	-81.3346	0.172
Specific IgE to any one of the cereal flour allergens ≥ 0.35kU/L	15.9472	0.227
Baseline FeNO	-0.7098	<0.001
FeNO (ppb) – >25 – >50	 -50.0404 -47.2197	 <0.001 0.005

Table 5: Determinants of 10% or greater decline in FeNO among supermarket bakery workers in multivariate models

Predictor	**Crude odds ratio**			**Bakers with work-related ocular-nasal symptoms**			**Bakers without work-related ocular-nasal symptoms**		
	OR	**95% Confidence Interval (CI)**	**p-value**	**OR**	**95% Confidence Interval (CI)**	**p-value**	**OR**	**95% Confidence Interval (CI)**	**p-value**
Intervention vs Control Group	1.617	0.930 - 2.810	0.088	3.732	1.219 - 11.420	0.021	1.617	0.559 - 2.249	0.747
Current smoker	0.921	0.557 - 1.523	0.749	0.997	0.416 - 2.389	0.995	1.001	0.522 - 1.923	0.996
Specific IgE to any one of the cereal flour allergens ≥ 0.35 kU/L	0.450	0.250 - 0.810	0.008	0.432	0.159 - 1.173	0.099	0.686	0.307 - 1.531	0.357
Baseline FeNO>25ppb	3.571	1.936 - 6.587	<0.001	5.033	1.789 - 14.159	0.002	3.017	1.346 - 6.766	0.007

DISCUSSION

This study is one of the few prospective field randomised control trials, and the first trial in bakers, that evaluated the long-term health outcomes of implementing an intervention at the workplace with the aim of reducing the incidence of occupational allergy and asthma. It is also one of the few studies that evaluated the predictors of longitudinal decline in FeNO. The overall findings of this study demonstrate that workplace interventions that aimed at reducing exposure to flour dust allergens can contribute towards suppression of allergic airway inflammation in exposed workers. This effect was more evident in workers with pre-existing moderate to high airway inflammation (FeNO>25 ppb) and ocular-nasal symptoms.

The study, however, could not demonstrate significant differences in the incidence of asthma symptoms, ocular-nasal symptoms or medication use. This was probably due to the dilution effect on dust exposure as reported previously by Baatjies et al.[11] Managers who were transferred to bakeries in the control group incorporated some elements of the intervention in their new bakeries. This resulted in dust exposures in the intervention group and the control group being very similar post intervention (GM 0.39 vs 0.44 mg/m3).[11]

Aside from the dilution effect, the findings of this study demonstrate that persistent exposure to low level of airborne allergens can result in developing or worsening of asthma and ocular-nasal symptoms as has been reported previously.[16] Also, these findings were similar to the overall findings of previous studies on the health outcomes following removal from exposure to airborne allergens.[3 6 17] A Cochrane systematic review[3] identified only one prospective controlled before-and-after study that evaluated the health outcomes of a workplace intervention aimed at reducing exposure to airborne allergen in farmers.[6] The authors reported a significant decline in respiratory symptoms in the intervention group 4-6 weeks after a one day educational training compared to its baseline. However, the difference between the

intervention group and the control group was not significant (Risk ratio 7.62, 95% CI 0.47-124.22).[3 6] Furthermore, Vandenplas et al[17] reported that only 17.6% of workers recovered fully after reduction of exposure, 60.1% showed improvement but 20.6% of subjects became worse after exposure reduction. By comparison, the current study demonstrated 39% of workers with a decline in work-related chest symptoms and 16% with new onset of work-related chest symptoms. Moreover, de Groene et al[3] reported that only 24.8% of participants reported absence of asthma symptoms after reduction of the exposure. In addition, Munoz et al[18] reported that the asthma control questionnaire (ACQ) scores were very similar among those workers who avoided and those with continued exposure to airborne allergens.

The short duration of follow-up may also have contributed to the marginal findings in sensitisation and symptoms observed in this study. The participants were assessed only one year after implementing the intervention, which may have been insufficient to demonstrate a significant difference between the two groups. Malo and Ghezzo[19] reported that the maximum level of improvement of non-specific bronchial hyperresponsiveness (NSBH) was observed at 2.5-5 years post cessation of exposure. Furthermore, Perfetti et al[20] showed that NSBH improved in 48% of subjects evaluated only after 5 years of exposure cessation, whereas only 19% of workers evaluated at less than 5 years showed improvement. This duration before symptom improvement could be explained by the presence of allergen specific IgE,[21] which can be detected in the sera from a few months to several years after cessation of exposure.[22–25] Previous studies have shown that the half-life of specific IgE to anhydrides, snow crab and detergent enzymes allergens were reported as 12, 20, and 21 months respectively after decreasing the exposure levels of these allergens.[24-26] In this study, the presence of flour specific IgE could explain the marginal findings in sensitisation and symptoms observed in this study. Despite the effectiveness of the intervention in reducing the exposure in the intervention group, the participants in this group may have still been exposed since levels ranging between

0.01–3.27 mg/m^3 of inhalable flour dust were still found despite the overall reduction in dust levels from baseline. This low-level dust exposure may have contributed towards persistence of flour specific IgE and respiratory symptoms. Future studies could investigate the half-life of flour dust allergens and their utility in predicting allergic symptoms.

The results of this study suggest that airway inflammation, measured by FeNO, can be suppressed by reducing exposure to airborne allergens. Although the difference in FeNO between the two groups following the intervention was not significant (p=0.24), the FeNO decline (among those with baseline FeNO >25ppb) was greater in the intervention group (mean=16.9ppb) compared to the control group (mean=7.7ppb). Moreover, apart from both groups having similar allergen exposures, the incidence of sensitisation was also similar. This may explain the non-significant difference in the FeNO decline in both groups since FeNO levels correlate with the level of allergen specific IgE.[12 27] Although the effect of allergen avoidance on FeNO in paediatric studies has been inconsistent,[8] a demonstrable association has been reported in the occupational setting. Dressel et al[6] demonstrated a significant decline in FeNO in the intervention group from its baseline after implementing a workplace intervention. However, the difference between the intervention and control groups was not statistically significant. Moreover, two case reports by Merget et al[28 29] of a baker and a farmer sensitised to wheat, rye and triticale, demonstrated a decrease in FeNO after complete avoidance of the potential allergens for two weeks. However, there was no objective measurement of the exposures before and after the intervention in these studies. In the current study, the effect of occupational intervention on FeNO decline was more prominent in workers with FeNO >25ppb as demonstrated by a strong association observed between a ≥10% decline in FeNO and baseline FeNO >25ppb (OR=3.610, 95%CI: 1.964-6.637).

Various approaches have been used to assess the longitudinal changes in FeNO over time. A decline of 10% in FeNO has been suggested previously for tapering oral corticosteroid in patients with severe asthma.[30] Based on the data and using this as a cutoff to evaluate the effectiveness of the intervention on reducing airway inflammation, various models were explored. Only baseline FeNO>25ppb (β1= -50.04), which is the cutoff level of normal range of FeNO,[31] as well as ocular-nasal symptoms (β1=22.85) were significantly associated with longitudinal change in FeNO while smoking status and work-related ocular-nasal symptoms were of borderline significance in univariate models. Hence the use of the former two variables in the model. Baseline sensitisation to cereal flour allergens was added to the model due to it being strongly associated with FeNO in a previous study.[12] The final model analysis demonstrated only a borderline association (p=0.088) between a ≥10% decline in FeNO and the intervention (OR=1.617, 95%CI: 0.930-2.810), suggesting a marginal intervention effect on the allergen exposure post intervention.[11]

Allergic rhinitis (AR) and asthma frequently coexist in patients,[32] and the presence of upper airway symptoms/AR is a significant predictor of occupational asthma.[33 34] In the occupational setting, there is a strong relationship between occupational asthma and occupational rhinitis,[35] particularly in those workers exposed to HMW agents such as cereal flour dust.[36] Furthermore, it has been demonstrated that FeNO increases progressively in AR patients without lower respiratory symptoms (24.5ppb, CI: 18-31 ppb), those with lower respiratory symptoms without asthma (38 ppb, CI: 27-49ppb), and in patients with asthma (68ppb, CI: 45-92ppb).[37] These data indicates a gradient continuum, which suggests that the total extent of airway inflammation depends on the degree of anatomical involvement of the airways from the nose to the small airways.[38] This relationship between the degree of anatomical involvement and extent of airway inflammation could explain the FeNO decline that was stratified by the presence of work-related ocular-nasal symptoms at baseline. This demonstrated a significant

(p=0.021) intervention effect on ≥10% decline in FeNO (OR=3.73, 95%CI: 1.22-11.42). However, this effect modification by work-related ocular-nasal symptoms could also be explained by the higher proportion of sensitised participants among those with work-related ocular-nasal symptoms compared to those who were asymptomatic (58% vs 42%, p<0.001).

This study has some important strengths, including being the only prospective trail that measured workplace allergen exposures levels and used FeNO as an outcome measure to evaluate the effectiveness of a workplace intervention. However, there were some limitations. Firstly, some of the bakeries in the control group applied changes to their work practices as a result of rotation of the managers, which diluted the strength of the effect estimates. Secondly, loss to follow-up was approximately 35% of the original cohort, which may have affected the magnitude of the effect estimate. Thirdly, the healthy worker effect may have introduced probable bias if there were differences among bakers that left the bakeries during this period. Fourthly, while the baseline questionnaire was the abbreviated version of the ECRHS questionnaire, the follow up final questionnaire entailed a more detailed questionnaire based on the non-abbreviated ECRHS questionnaire. Although both questionnaires shared the most important symptom domains, this may have introduced some information bias. The use of other objective measures aside from FeNO such as NSBH, could have minimized this, but were not done due to logistic reasons. Finally, the post-intervention period was not sufficiently long enough to detect an appreciable improvement in health outcome parameters such as sensitisation. The effect of intervention on asthma and ocular-nasal symptoms is likely to be more demonstrable if workers were re-evaluated at 2.5-5 years after the intervention.

In conclusion, despite these limitations, the study suggests some evidence of an intervention effect on airway inflammation in bakers even after one-year of follow-up. Furthermore, FeNO could be a useful tool to monitor the effectiveness of workplace interventions, particularly

among those with pre-existing upper airway involvement and work-related ocular-nasal symptoms. Future studies should follow up participants after 2-5 years to demonstrate the effectiveness of workplace interventions on health outcomes. Assembling cohorts with exposure to different occupational allergens such as isocyanates, food processing allergens and animal allergens would be useful. Finally, studies should also consider using other objective measures to evaluate the effectiveness of interventions on allergic respiratory disease outcomes such as non-specific bronchial hyperresponsiveness and spirometry.

REFERENCES

1 Tarlo SM, Malo J-L, de Blay F, *et al.* An Official American Thoracic Society Workshop Report: Presentations and Discussion of the Sixth Jack Pepys Workshop on Asthma in the Workplace. *Ann Am Thorac Soc* 2017;14:1361–72. doi:10.1513/AnnalsATS.201706-508ST

2 Toren K, Blanc PD. Asthma caused by occupational exposures is common - a systematic analysis of estimates of the population-attributable fraction. *BMC Pulm Med* 2009;9:7. doi:10.1186/1471-2466-9-7

3 de Groene GJ, Pal TM, Beach J, *et al.* Workplace interventions for treatment of occupational asthma. *Cochrane database Syst Rev* Published Online First: May 2011. doi:10.1002/14651858.CD006308.pub3

4 Vandenplas O, Dressel H, Nowak D, *et al.* What is the optimal management option for occupational asthma? *Eur Respir Rev* 2012;21:97–104. doi:10.1183/09059180.00004911

5 Rees D, Phillips JI. Investigating the effectiveness of occupational health interventions in the workplace. *Occup Environ Med* 2014;71:809–10. doi:10.1136/oemed-2014-102389

6 Dressel H, Gross C, De la Motte D, *et al.* Educational intervention decreases exhaled nitric oxide in farmers with occupational asthma. *Eur Respir J* 2007;30:545–8.

7 Soyseth V, Kongerud J, Aalen OO, *et al.* Bronchial responsiveness decreases in relocated aluminum potroom workers compared with workers who continue their potroom exposure. *Int Arch Occup Environ Health* 1995;67:53–7.

8 Al Badri FM, Jeebhay MF. Factors associated with serial longitudinal changes in

exhaled nitric oxide (FeNO)–a review of the literature. *Curr Allergy Clin Immunol* 2017;30:98–109.

9 Baatjies R, Lopata A, Sander I, *et al.* Determinants of asthma phenotypes in supermarket bakery workers. *Eur Respir J* 2009;34:825–33. doi:10.1183/09031936.00164408

10 Baatjies R, Meijster T, Lopata A, *et al.* Exposure to flour dust in South African supermarket bakeries: modeling of baseline measurements of an intervention study. *Ann Occup Hyg* 2010;54:309–18.

11 Baatjies R, Meijster T, Heederik D, *et al.* Effectiveness of interventions to reduce flour dust exposures in supermarket bakeries in South Africa. *Occup Environ Med* 2014;71:811–8.

12 Baatjies R, Jeebhay MF. Sensitisation to cereal flour allergens is a major determinant of elevated exhaled nitric oxide in bakers. *Occup Environ Med* 2013;70:310–6.

13 Burney PG, Luczynska C, Chinn S, *et al.* The European community respiratory health survey. *Eur Respir J* 1994;7:954–60.

14 American Thoracic Society. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. *Am J Respir Crit Care Med* 2005;171:912–30. doi:10.1164/rccm.200406-710ST

15 Dweik RA, Boggs PB, Erzurum SC, *et al.* An official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (FENO) for clinical applications. *Am J Respir Crit Care Med* 2011;184:602–15. doi:10.1164/rccm.9120-11ST

16 Jolly Athena T, Klees Julia E, Pacheco Karin A, *et al.* Work-Related Asthma. *J Occup Environ Med* 2015;57:e121–9. doi:10.1097/JOM.0000000000000572

17 Vandenplas O, Dressel H, Wilken D, *et al.* Management of occupational asthma: cessation or reduction of exposure? A systematic review of available evidence. *Eur Respir J* 2011;38:804–11.

18 Munoz X, Viladrich M, Manso L, *et al.* Evolution of occupational asthma: does cessation of exposure really improve prognosis? *Respir Med* 2014;108:1363–70. doi:10.1016/j.rmed.2014.08.001

19 Malo J-L, Ghezzo H. Recovery of methacholine responsiveness after end of exposure in occupational asthma. *Am J Respir Crit Care Med* 2004;169:1304–7. doi:10.1164/rccm.200312-1749OC

20 Perfetti L, Cartier A, Ghezzo H, *et al.* Follow-up of occupational asthma after removal from or diminution of exposure to the responsible agent: relevance of the length of the interval from cessation of exposure. *Chest* 1998;114:398–403.

21 Basketter D, Berg N, Kruszewski FH, *et al.* Relevance of sensitization to occupational allergy and asthma in the detergent industry. *J Immunotoxicol* 2012;9:314–9. doi:10.3109/1547691X.2012.656855

22 Park HS, Lee SK, Lee YM, *et al.* Longitudinal study of specific antibodies to toluene diisocyanate (TDI)-human serum albumin (HSA) conjugate in patients with TDI-induced asthma. *Korean J Intern Med* 2002;17:249–51. doi:10.3904/kjim.2002.17.4.249

23 Piirila PL, Nordman H, Keskinen HM, *et al.* Long-term follow-up of hexamethylene diisocyanate-, diphenylmethane diisocyanate-, and toluene diisocyanate-induced asthma. *Am J Respir Crit Care Med* 2000;162:516–22. doi:10.1164/ajrccm.162.2.9909026

24 Brant A, Zekveld C, Welch J, *et al.* The prognosis of occupational asthma due to

detergent enzymes: clinical, immunological and employment outcomes. *Clin Exp Allergy* 2006;36:483–8.

25 Malo JL, Cartier A, Ghezzo H, *et al.* Patterns of improvement in spirometry, bronchial hyperresponsiveness, and specific IgE antibody levels after cessation of exposure in occupational asthma caused by snow-crab processing. *Am Rev Respir Dis* 1988;138:807–12. doi:10.1164/ajrccm/138.4.807

26 Bernstein DI, Roach DE, McGrath KG, *et al.* The relationship of airborne trimellitic anhydride concentrations to trimellitic anhydride--induced symptoms and immune responses. *J Allergy Clin Immunol* 1983;72:709–13.

27 Tsolakis N, Malinovschi A, Nordvall L, *et al.* Absence of serum IgE antibodies indicates non-type 2 disease in young asthmatics. *Clin Exp allergy* Published Online First: January 2018. doi:10.1111/cea.13103

28 Merget R, Sander I, van Kampen V, *et al.* Serial measurements of exhaled nitric oxide at work and at home: a new tool for the diagnosis of occupational asthma. *Adv Exp Med Biol* 2015;834:49–52. doi:10.1007/5584_2014_67

29 Merget R, Sander I, van Kampen V, *et al.* Triticale allergy in a farmer. *Am J Ind Med* 2016;59:501–5.

30 Hashimoto S, Brinke A Ten, Roldaan AC, *et al.* Internet-based tapering of oral corticosteroids in severe asthma: a pragmatic randomised controlled trial. *Thorax* 2011;66:514–20. doi:10.1136/thx.2010.153411

31 Bjermer L, Alving K, Diamant Z, *et al.* Current evidence and future research needs for FeNO measurement in respiratory diseases. *Respir Med* 2014;108:830–41. doi:10.1016/j.rmed.2014.02.005

32 Bousquet J, Khaltaev N, Cruz AA, *et al.* Allergic Rhinitis and its Impact on Asthma

(ARIA) 2008 update (in collaboration with the World Health Organization, GA(2)LEN and AllerGen). *Allergy* 2008;63 Suppl 8:8–160. doi:10.1111/j.1398-9995.2007.01620.x

33 Foresi A, Leone C, Pelucchi A, *et al.* Eosinophils, mast cells, and basophils in induced sputum from patients with seasonal allergic rhinitis and perennial asthma: relationship to methacholine responsiveness. *J Allergy Clin Immunol* 1997;100:58–64.

34 Alvarez MJ, Olaguibel JM, Garcia BE, *et al.* Airway inflammation in asthma and perennial allergic rhinitis. Relationship with nonspecific bronchial responsiveness and maximal airway narrowing. *Allergy* 2000;55:355–62.

35 Moscato G, Pala G, Barnig C, *et al.* EAACI consensus statement for investigation of work-related asthma in non-specialized centres. *Allergy* 2012;67:491–501. doi:10.1111/j.1398-9995.2011.02784.x

36 Moscato G, Vandenplas O, Gerth Van Wijk R, *et al.* Occupational rhinitis. *Allergy* 2008;63:969–80. doi:10.1111/j.1398-9995.2008.01801.x

37 Rolla G, Guida G, Heffler E, *et al.* Diagnostic classification of persistent rhinitis and its relationship to exhaled nitric oxide and asthma: a clinical study of a consecutive series of patients. *Chest* 2007;131:1345–52. doi:10.1378/chest.06-2618

38 Boulay M-E, Morin A, Laprise C, *et al.* Asthma and rhinitis: what is the relationship? *Curr Opin Allergy Clin Immunol* 2012;12:449–454.

39 Gui W, Wisnewski A V, Neamtiu I, *et al.* Inception cohort study of workers exposed to toluene diisocyanate at a polyurethane foam factory: initial one-year follow-up. *Am J Ind Med* 2014;57:1207–15. doi:10.1002/ajim.22385

APPENDICES

Appendix 1: The published literature review

Allergies in the Workplace

FACTORS ASSOCIATED WITH SERIAL LONGITUDINAL CHANGES IN EXHALED NITRIC OXIDE (FeNO) – A REVIEW OF THE LITERATURE

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ABSTRACT
It has been well documented that the major hallmarks of asthma are the presence of airway inflammation, variable airway obstruction and airway hyperresponsiveness (AHR). Fractional exhaled nitric oxide (FeNO) is a well-known inflammatory marker which has a potential role in the diagnosis and surveillance of those at risk of developing asthma, although its utility in occupational contexts is less clear. This review focuses on identifying the factors associated with longitudinal changes in FeNO in general asthmatic and working populations. The review found that exposure to allergens (common aeroallergens and occupational allergens) or their avoidance, as well as asthma-education programmes (especially those focusing on adherence to inhaled corticosteroid usage) are associated with longitudinal changes in FeNO. Other host-associated factors such as baseline lung function (FEV_1), atopy status and smoking need further evaluation in order to assess their impact on serial longitudinal changes in FeNO.

INTRODUCTION

The presence of airway inflammation, variable airway obstruction and airway hyperresponsiveness (AHR) as major hallmarks of asthma has been well documented.[1] Among this triad, airway inflammation appears to be increasingly recognised as a major component in the initiation and long-term progression of the disease. Airway inflammation in asthma is measured using several methods, one being the measurement of fractional exhaled nitric oxide (FeNO) as a marker for asthma in symptomatic individuals.[2] FeNO is a non-invasive marker that has been extensively investigated in several contexts such as diagnosis, initiating treatment, tailoring medication, achieving asthma control and predicting future relapses of asthma. Various studies have demonstrated that FeNO is affected by individual host attributes and environmental factors. FeNO levels at baseline are reported to be higher in men than in women and levels increase with height, atopy, dietary intake of food rich in nitrates, allergen exposures causing sensitisation, and viral airway infections.[3–6] FeNO levels can be decreased with bronchoconstriction and inhaled corticosteroid therapy, and among both active and passive smokers.[3–6] Most of the studies on the determinants of baseline FeNO have focused on the asthmatic population; fewer studies have evaluated serial changes in FeNO in the general population or in occupational contexts over longer periods of time.

The aim of this review is to identify the determinants of serial longitudinal changes in FeNO and to determine its usefulness in predicting increased asthma risk. Furthermore, this review could also contribute towards developing greater insights into studies of the applicability of FeNO in predicting long-term asthma outcomes and lung function deficits in those with persistent exposures to respiratory sensitisers. It could also contribute developing greater understanding of the clinical improvements in individuals subjected to treatment or exposure interventions. The review includes all the relevant epidemiological or experimental studies on asthmatic children or adults having a direct or indirect allergen exposure modulation component that were published in English between January 1999 and April 2017. Articles were retrieved from PubMed/Medline and Google Scholar. The key search terms that were used in this review were 'asthma', 'FeNO' or 'exhaled nitric oxide' plus either 'serial' or 'longitudinal'. Furthermore, some articles were retrieved using the functions 'similar articles search' in PubMed and 'related articles' in Google Scholar as well relevant articles that were cited in the references of these articles. Clinical studies that focused on the effect of medication or an inhalation challenge test on FeNO in patients were excluded from this review.

The review identifies various factors associated with

longitudinal changes in FeNO in 20 studies as outlined in Tables I–III. The articles were summarised according to the population of interest. Eight of the studies were experimental in design, with six being quasi-experimental studies and two being non-randomised controlled trials. Furthermore, there were ten epidemiological studies (five longitudinal, four case-control and one cross-sectional) and two clinical case reports. Most of the studies focused on asthmatic populations but two studies also evaluated FeNO in working populations with exposure to respiratory sensitisers (Figures 1a–c).

ENVIRONMENTAL FACTORS

ALLERGEN EXPOSURES

Various studies using specific inhalation challenges (SIC) of suspected allergens in general asthmatic patients have shown that it takes up to eight hours before FeNO starts to increase and that it continues to remain elevated for up to 48 hours[27,28] – in some studies up to 72 hours.[29,30] In patients suspected of having occupational asthma, most studies have reported changing FeNO levels 20–24 hours post-SIC[25,30–36] and showed a significant increase in FeNO.[2]

Exposure to the relevant allergen in asthmatic populations has demonstrated positive associations with longitudinal changes in FeNO. Baraldi et al,[16] in their case-control study investigating the association between serial FeNO and exposure to grass pollens in atopic asthmatic children sensitised to grass pollen, found that exposure in the pollen season led to a two-fold increase in the overall mean FeNO levels. These levels returned to baseline after the season. This association between exposure to in-season pollen and to other allergens was also reported by Roberts et al,[19] Spanier et al[20] and Cutts et al.[21] However, Fowler et al[26] found a negative association between allergen exposure and FeNO (1.5-fold mean decrease, 95% CI 1.2-1.7 fold, $p < 0.001$). Since exposure was not monitored during the long study period, changes in the exposure level over this period may have occurred that may have introduced bias in the study.

Figure 1a-b: Measurement of exhaled nitric oxide (FeNO) in a study of spice mill and bakery workers in Cape Town

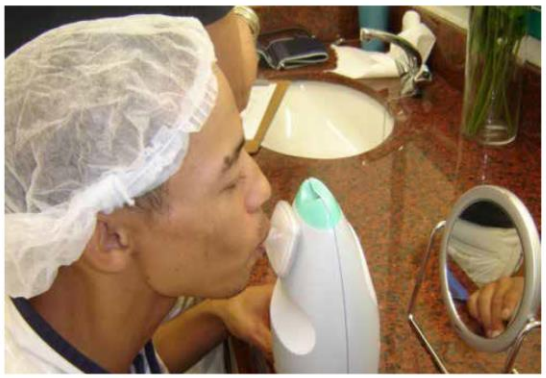

Figure 1b

Ongoing exposure to an occupational allergen appears to be one of the determinants of longitudinal change in FeNO. Van der Walt et al evaluated serial FeNO associated with occupational exposure to spices in 150 mill workers after a two-day exposure-free period during time off work.[25] No significant difference in the overall mean FeNO between baseline, after the shift and 24 hours after the baseline pre-shift level was recorded. However, there was a >12% increase in FeNO across the 24-hour period in 23% of the workers. The authors concluded that exposure to the spice allergens in this occupational setting was associated with a delayed increase in FeNO from the baseline. Hewitt et al also evaluated serial FeNO among workers exposed to laboratory animal allergens (LAA).[24] The investigators studied 50 workers in an animal laboratory for five working days after being off work for two days. There was a progressive increase in FeNO over the working week in one subject who was seropositive to one or more of the LAA. In addition, three subjects experienced >25 ppb fluctuation in FeNO during the working day in the period of observation. Both of these studies were conducted in worker populations comprising both asymptomatic and symptomatic individuals.

ALLERGEN EXPOSURE AVOIDANCE

Avoidance of exposure to the suspected common

Figure 1c: Measurement of exhaled nitric oxide (FeNO) in a study of asthma among school children in Cape Town

TABLE I: EXPERIMENTAL STUDIES OF SERIAL LONGITUDINAL FeNO MEASUREMENT AMONG ASTHMATICS IN THE GENERAL POPULATION (CHILDREN AND ADULTS)

TABLE IA: STUDIES AMONG CHILDREN

AUTHORS	PIACENTINI ET AL, 1999[7]	PIACENTINI ET AL, 2001[8]	PERONI ET AL, 2002[9]
AIM OF STUDY	To evaluate the effect of house-dust mite (HDM) avoidance in allergic asthmatic patients on FeNO.	To determine the relationship between exposure level to house-dust mite (HDM) allergen and the magnitude of exhaled FeNO after allergen avoidance in a group of allergic asthmatic children.	To study the change in lung volumes and airway inflammatory markers in house-dust mite (HDM) sensitised asthmatic children resident in an allergen free environment.
STUDY DESIGN	Quasi-experimental	Quasi-experimental	Quasi-experimental
STUDY POPULATION	20 Italian asthmatic children (6–15 years) sensitised to HDM. Thirteen received regular courses of inhaled corticosteroids (ICS).	14 Italian asthmatic children (6–15 years) sensitised to HDM.	18 asthmatic children who have moderate to severe asthma and sensitised to HDM.
INTERVENTION	Avoidance of HDM by staying at 1 756 m above sea level in the Italian Alps for three months.	Avoidance of HDM by staying at 1 756 m above sea level in the Italian Alps for three months.	Avoidance of HDM by staying at 1 756 m above sea level in the Italian Alps for nine months between September and June.
OUTCOME MEASURES	Change in FeNO level.	• The level of HDM group I allergen at home. • Change in FeNO.	FeNO, lung function.
MEASUREMENTS	• FeNO was measured daily, always at the same hour starting at the parents' house (T0), for two weeks after moving to the residential home until day 15 (T1), after three months at the residential campus (T2), and, in 10 of the 20 patients, two weeks after return to their parents at sea level (T3). • FEV_1 is measured at T0, T1, T2, and T3. • Serum eosinophil cationic protein (ECP) was measured at T0, T2, and T3.	• Children received a regular course of ICS for at least three months at the beginning of the study. The ICS course was gradually withdrawn in all the subjects. By T1, none of the children received any ICS for one month at least. • FeNO was measured at the parent's home (T0) and after three months at the residential campus (T1).	• Measurements taken (i) within three days of admission, (ii) after 3 months of stay, (iii) within two days before leaving the residential home for the Christmas holiday, (iv) on return to the residential home after 15 days at home and (v) at the end of the camp after nine months of staying at residential home. • ICS were withdrawn after few weeks of admission, resumed during the Christmas holiday and withdrawn again during the rest of the study.
RESULTS	• FeNO decreased ($p = 0.014$ at T1) with a significant effect of the time in the first two weeks ($p = 0.026$). It didn't change in the period T1 to T2, and returned to T0 level at ($p = 0.004$, T2 vs T3). • FeNO did not change significantly from T1 to T2, despite the withdrawal of ICS. • FEV_1 improved gradually.	Strong correlation between the change in FeNO (T0–T1) and the levels of HDM antigens in the beds of the patients before T0; $r = 0.618$ ($p = 0.026$).	• FeNO decreased from (21.3 ± 3.9 ppb) in September to (11.9 ± 1.7 ppb) in December ($p = 0.03$). No significant change after the holiday in January (12.5 ± 2.6 ppb) nor at the end in June (13.2 ± 2.0 ppb). • The RV to TLC ratio decreased between September and December ($p = 0.003$), increased between December and January ($p = 0.002$) and decreased again between January and June ($p = 0.002$). • No significant correlation between FeNO and lung volumes.
LIMITATIONS	• Absence of a control group at sea level. • Small sample size. • Independent effect of high altitude on FeNO levels. • No mention of important confounders (passive smoking and diet).	• Absence of a control group at sea level. • Small sample size. • Independent effect of high altitude on FeNO levels. • No mention of important confounders (passive smoking and diet).	• Absence of a control group at sea level. • Small sample size. • Independent effect of high altitude on FeNO levels. • No mention of important confounders (passive smoking and diet).
CONCLUSION	FeNO levels decreased over time after the avoidance of the potential allergen in allergic asthmatic children.	FeNO reduction after the avoidance of the potential allergen correlated with the magnitude of the exposure of the allergen prior to avoidance.	Allergen avoidance at high altitudes led to a reduction in FeNO levels in asthmatic children sensitised to HDM.

TABLE IA: STUDIES AMONG CHILDREN (CONTINUED)

AUTHORS	HUSS-MARP ET AL, 2007[10]	KAMINSKY ET AL, 2008[11]	SAITO ET AL, 2013[12]
AIM OF STUDY	To study effect of rehabilitation of asthmatic children at high altitudes and HDM avoidance on asthma control.	To study effect of attending an asthma summer camp on airway inflammation as measured by FeNO.	• To examine whether an asthma education program is associated with asthma control. • To compare absolute levels and changes of ACT score, FEV_1, and FeNO over a year after the intervention. • To evaluate FeNO as a marker of asthma control compared to other methods.
STUDY DESIGN	Quasi-experimental	Quasi-experimental	• Quasi-experimental
STUDY POPULATION	311 asthmatic children with increased FeNO levels at admission (>17 ppb).	27 asthmatic children.	• 12 mild, 21 moderate, and 17 severe persistent asthmatic adult patients (n = 50) with poor adherence to medication.
INTERVENTION	Rehabilitation at 1 200 m above sea level in the Bavarian Alps.	Participation in a one-week camp during the summer of 2006 in the United States.	Asthma education programme providing information about asthma pathogenesis, diagnosis, severity, medications (including side-effects), differences between reliever and controller agents, importance of asthma treatment, inhaler-device instructions, exacerbation management, peak expiratory flow (PEF) monitoring, and a self-management plan.
OUTCOME MEASURES	Change in FeNO level.	FeNO, lung function and ACQ score.	Change of ACT score, FeNO and spirometry levels over time.
MEASUREMENTS	• Subjects were grouped according to asthma severity based on the clinical features before therapy from grade I (mild intermittent) to three levels of persistent asthma (grades II–IV: mild, moderate, and severe, respectively; grade I: n = 67; grade II: n = 135; grade III: n = 104; grade IV: n = 5). • FeNO measurement was performed on the day of admission and at discharge (4–6 weeks).	• FeNO, FEV_1 and ACQ score were measured at the beginning and end of the week. ACQ score was also obtained at one month and six months after the camp. • The children participated in daily educational activities about asthma.	• Subjects were reviewed four times: at entry and at intervals of three, six and 12 months. • Treatment was not changed in the first three months to study the effect of the educational programme on asthma control. Thereafter, the treatment could be changed according to the GINA guidelines. • During the follow-up period, subjects were divided into two groups: - Stable group: treatment had not changed or was decreased over the study period. - Unstable group: increase in ICS dose or other anti-asthmatic drugs added during the study period.
RESULTS	• 47.2% reduction in the mean FeNO of all 311 children after rehabilitation, with 38.5 ± 22.8 ppb at admission compared to 20.1 ± 13.2 ppb at discharge ($p < 0.001$). • FeNO reduction was significant in all subgroups and most marked in children with mild asthma. • FeNO reduction in asthmatics with isolated HDM sensitisation was no different to the reduction in other children sensitised to other allergens.	• FeNO dropped significantly by 45% ($p < 0.001$), but the changes in lung function and ACQ was not statistically significant. • Among the 21 children prescribed with ICS, 18 had decreased FeNO and three had increased FeNO. • Among the four children not prescribed any ICS, three had decreased FeNO, and one had an increase in FeNO.	• Decrease in FeNO and increase in ACT scores in the stable asthma group (n = 42) compared with the unstable asthma group (n = 8); ($p < 0.001$) and remained consistent in the stable group over the course of 12 months. • Significant correlations between change in FeNO, ACT score and FEV_1 in all four visits.
LIMITATIONS	• Absence of a control group at sea level. • HDM concentrations in the rehabilitation centre not reported. • Independent effect of high altitude on FeNO levels. • No mention of important confounders (passive smoking and diet).	• Small sample size. • First-day measurements were conducted in the afternoon and the final-day measurements in the morning.	• Selection bias. • Hawthorne effect.
CONCLUSION	FeNO decreased significantly after 4–6 weeks rehabilitation at high altitude, which could be explained by allergen avoidance, absence of air pollution and improved compliance to medication.	FeNO levels declined after attendance of a one-week asthma summer camp, despite no changes in lung function or asthma control, which could be explained by allergen avoidance, absence of exposure to tobacco smoke, and avoidance of psychosocial stressors.	• FeNO levels declined after an asthma education program and remained significant over a year. • Change in FeNO levels correlated significantly with change in ACT score and change in FEV_1 levels.

TABLE IB: STUDIES AMONG ADULT WORKING POPULATION		
AUTHORS	DRESSEL ET AL, 2007[13]	DRESSEL ET AL, 2009[14]
AIM OF STUDY	To assess whether FeNO is useful in detecting a reduction in airway inflammation within a few weeks of an educational intervention in farmers with occupational asthma.	To determine whether long-term changes in FeNO could be detectable a year after an intervention.
STUDY DESIGN	Non-randomised controlled trial	Non-randomised controlled trial
STUDY POPULATION	Cases: 81 animal farmers with occupational asthma sensitised to cow dander and storage mites who participated in a one-day educational program. Controls: 24 animal farmers with occupational asthma not subjected to an intervention.	• Cases: animal farmers with occupa-tional asthma (n = 43) who participated in a one-day educational program. • Control: animal farmers with occupa-tional asthma (n = 15) not subjected to an intervention.
INTERVENTION	A one-day educational programme provided general information about the pathogenesis of asthma and allergies, environmental influences, treatment, major occupational allergens causing asthma, particularly cow dander and mites, and prevention methods in the workplace.	A one-day educational programme provided general information about the pathogenesis of asthma and allergies, environmental influences, treatment, major occupational allergens causing asthma, particularly cow dander and mites, and prevention methods in the workplace.
OUTCOME MEASURES	FeNO, lung function and a questionnaire.	FeNO, lung function and a questionnaire.
MEASUREMENTS	Baseline FeNO measurement and repeated 4–6 weeks after the intervention.	Baseline FeNO measurement and repeated one year after the intervention.
RESULTS	• There was a decline in the proportion of subjects reporting at least one current respiratory symptom at work (p = 0.012) in the intervention group. • The geometric mean FeNO decreased from 28.2 to 25.7 ppb (p = 0.042). • Subjects with a baseline FeNO of >35 ppb (n = 32), FeNO decreased from 59.7 to 49.2 ppb (p = 0.003). • There was no significant change in the pulmonary function test after the intervention.	• Geometric mean ± SEM of FeNO decreased from (31.5±1.1) to (25±1.1) ppb in the intervention group (p = 0.001), while it showed a slight but statistically insignificant increase in the control group (p = 0.258) • FEV_1 and FEV_1/FVC did not significantly change over time in either group.
LIMITATIONS	• Selection bias. • No mention of important confounders (atopy and diet).	• Selection bias. • No mention of important confounders (atopy and diet).
CONCLUSION	FeNO showed a significant short-term reduction after an educational intervention in animal farmers with workplace-related allergic asthma, which was accompanied by decrease of frequency of symptoms.	FeNO showed a significant long-term reduction after an educational intervention in animal farmers with workplace-related allergic asthma, which may be explained by a reduction in allergen exposure.

TABLE II: EPIDEMIOLOGICAL STUDIES OF SERIAL LONGITUDINAL FeNO MEASUREMENT AMONG ASTHMATIC CHILDREN		
AUTHORS	BARRETO ET AL, 2008[15]	BARALDY ET AL, 1999[16]
AIM OF STUDY	To assess variations and reproducibility of FeNO in subjects maintained under similar environmental conditions.	• To evaluate the relationship between natural allergen exposure to grass pollen and the changes in FeNO in atopic asthmatic children during and out of the grass pollen season.
STUDY DESIGN	Case-control	Case-control
STUDY POPULATION	• 29 children (12 healthy subjects and 17 asthmatics with good control). • Asthmatics divided into two groups; corticosteroids-naïve (n = 9) and corticosteroids-treated (n = 8). • All asthmatics were atopic.	• 21 grass pollen sensitised children (age 6–16 years) with a seasonal allergic asthma. • 21 non-atopic healthy children age and sex matched with the asthmatics.
INTERVENTION/ EXPOSURE	One-week stay in a countryside sanatorium situated in a wooded area near the Tatra mountains at an altitude of 970 m.	Followed up and monitored before (March), during (May), and after (November) the pollen season.
OUTCOME MEASURES	FeNO, lung function.	FeNO, lung function.
MEASUREMENTS	• On arrival, all subjects underwent a clinical examination and a history recorded of symptoms and therapy. Spirometry and FeNO measurements done on all subjects twice a day for the first two days and again at 8:00 am on day seven. • All participants avoided food intake and physical exercise for at least two hours before testing. • Participants excluded from the study if unsatisfactory lung function maneuvers or respiratory symptoms developed during the weekly follow-up.	• All patients underwent physical examination, spirometry and measurement of FeNO on three occasions: before (March), during (May) and after the grass pollen season (November). On each occasion, measurements were performed in the afternoon. • The atmospheric pollen counts were condcuted throughout the year using a volumetric spore trap and daily mean concentration recorded and expressed as pollen grains per m3 of air per 24 hrs.

AUTHORS	BARRETO ET AL, 2008[15]	BARALDY ET AL, 1999[16]
RESULTS	• At baseline, the worst lung function observed among corticosteroid-treated asthmatics and the highest FeNO values recorded in corticosteroid-naive asthmatics. • The differences in FeNO at baseline between asthmatics versus healthy participants was significant only among corticosteroid-naive patients. • Differences in lung function between the groups remained unchanged at each session. • FeNO levels among corticosteroid-naive as well as treated asthmatics decreased over time and became statistically non-significant (vs healthy subjects) at the last session (day 7). • No intra-group differences were found between nocturnal and diurnal FeNO in all the groups.	• ICS therapy was not altered through the pollen season for all subjects. • The mean value of FeNO before the grass pollen season (March) was (12.7 ± 5.1 ppb) and was significantly higher ($p < 0.001$) when compared to healthy subjects (7.8 ± 2.7 ppb). • In the pollen season (May) there was a significant ($p < 0.001$) two-fold increase in FeNO (21.4 ± 7.6 ppb) with respect to pre-season baseline values. • After the season (November), FeNO returned to pre-seasonal values (12.8 ± 5.8 ppb). • Non-relationship was found between the changes in FeNO and changes in symptom scores. • No significant changes in lung-function parameters during and after the pollen season.
LIMITATIONS	• Small sample size. • High altitude and climate may affect the results. • No mention of important confounders (passive smoking and diet). • There was no intra-group analysis to study the significance of the mean difference between day 1 and day 7.	• Small sample size. • No FeNO measurements taken for the control group during and after the pollen season. • No mention of important confounders (passive smoking and diet).
CONCLUSION	FeNO decreased a week after allergen avoidance at high altitude.	• Natural allergen exposure during the grass season resulted in an increase in FeNO in asthmatic children which then returned to the level of the pre-seasonal range after the season passed.

TABLE II: EPIDEMIOLOGICAL STUDIES OF SERIAL LONGITUDINAL FeNO MEASUREMENT AMONG ASTHMATIC CHILDREN (CONTINUED)

AUTHORS	ADAR ET AL, 2015[17]	HOLGUIN ET AL, 2015[18]
AIM OF STUDY	To assess variability in FeNO in children in relation to exposures to diesel pollution from school buses.	To assess effects of traffic emissions on FeNO in children with and without asthma.
STUDY DESIGN	Case-control.	Case-control.
STUDY POPULATION	• 275 children riding on diesel school buses, with a mean age of 9.5 years. • 148 were asthmatics, and 127 were healthy subjects.	• 200 children (age 6–12 years), • 50% had physician-diagnosed asthma, and 50% were healthy subjects.
INTERVENTION/ EXPOSURE	Children riding on school buses were monitored before, during and after the adoption of clean-fuel technology, such as diesel oxidation catalysts (DOCs), closed crankcase ventilation systems (CCVs), ultralow-sulfur diesel (ULSD), and biodiesel.	Children were monitored over a four-month period to determine the effects of road and traffic densities, carbon and particulate matter on FeNO levels.
OUTCOME MEASURES	FeNO, lung function, air pollution levels on school buses.	FeNO, traffic-related air pollution levels, traffic densities.
MEASUREMENTS	• Children were assessed an average of six times over four years. • Lung function and FeNO were measured during monthly data collection sessions at the schools. • Fine and ultra-fine particles (UFP) were measured inside 188 buses during 597 commutes.	• Children were assessed with spirometry, FeNO, and skin-allergy testing bi-weekly over four months. • Daily respiratory health questionnaires were completed by parents. • Road-density (amount of road length in kilometres in each buffer) and traffic density (vehicle-km/h) within buffer areas around study schools and subject homes were measured. • Air pollution was measured at the schools as follows: - 48-hour average of less than 2.5 um (PM2.5) particulate matter and elemental carbon measurements. - Weekly NO_2 measurements.
RESULTS	• Higher FeNO levels were observed among children with asthma, compared to healthy children. • Buses with ULSD were associated with reduction in UFP (−47%; 95% CI, −58 to −34%). • Buses with ULSD were associated with 16% lower FeNO, particularly in asthmatics.	• Higher road density was associated with increased FeNO and reduced FEV_1 in asthmatics. • No association was observed between fine particulate matter (PM2.5) or elemental carbon and FeNO.
LIMITATIONS	• Potential for important confounding by time (some technologies used only later in the study). • No mention of important confounders (atopy and diet).	• Differences in measurement frequencies of PM2.5 and NO_2 could have affected results. • 48-hour PM2.5 measurements may not precisely capture daily pollution fluctuations.
CONCLUSION	Adoption of cleaner school bus diesel-emission technologies reduced childrens' exposure to fine and ultra-fine particles, which was associated with a decrease in FeNO levels, particularly in asthmatics.	Increased traffic exposure leads to increased FeNO, particularly in asthmatics.

TABLE II: EPIDEMIOLOGICAL STUDIES OF SERIAL LONGITUDINAL FeNO MEASUREMENT AMONG ASTHMATIC CHILDREN (CONTINUED)

AUTHORS	ROBERTS ET AL, 2004[19]	SPANIER ET AL, 2004[20]	CUTTS EL AT, 2013[12]
AIM OF STUDY	To evaluate effect of pollen on FeNO in children with seasonal asthma.	To evaluate seasonal and environmental effects on FeNO in tobacco-exposed children with asthma.	To evaluate variability in FeNO over a ten-month period in children with and without asthma.
STUDY DESIGN	Longitudinal study.	Longitudinal study.	Longitudinal study.
STUDY POPULATION	44 children (age 6–16 years) with seasonal allergic asthma.	225 children (age 6– 12 years) with physician-diagnosed asthma.	• 178 primary school children, with a mean age of 9.6 years (SD = 1.3 years). • 47 were asthmatics, and 131 were healthy subjects.
INTERVENTION/ EXPOSURE	Exposure to pollen during the pollen season.	Evaluated at baseline, six and 12 months and assessed for environmental exposures.	Monitored over a ten-month period and assessed for environmental exposures.
OUTCOME MEASURES	FeNO, lung function, daily asthma symptoms.	FeNO, settled dust allergens (SDA), indoor airborne particles (IAP), allergen sensitsation, tobacco smoke exposure.	FeNO, lung function, daily asthma symptoms questionnaire.
MEASUREMENTS	• Evaluated before the pollen season; then at regular four-weekly intervals throughout one pollen season. • All patients underwent physical examination, spirometry and measurement of FeNO. • Atmospheric pollen counts were taken throughout the pollen season with a seven-day volumetric spore trap.	• FeNO measured. • Dust mite, dog, cat and cockroach allergens measured with monoclonal ELISA. • Indoor particulate matter measured. • ImmunoCap for allergen sensitisation. • Tobacco smoke exposure measured with using a survey, biomarkers (hair and serum cotinine) and a nicotine dosimeter.	FeNO measured at two-month intervals, on between four and six different occasions (851 measurements) as follows: • 86 children measured on six occasions; • 51 children measured on four occasions. • 21 children measured on four occasions.
RESULTS	• There was a significant increase in median FeNO during the pollen season (9.2 ppb) compared to before the pollen season (6.2 ppb) ($p = 0.002$). • There was a significant relationship between standardised FeNO and pollen counts on the day of measurement ($p < 0.01$). • No significant changes were seen in lung-function parameters before or during the pollen season.	• Significant associations were seen between longitudinal change in FeNO and baseline FeNO. • Lowest and highest FeNO levels were seen in winter and autumn (fall), respectively ($p = 0.002$). • Being atopic lead to increased FeNO levels. • Cat and dust mite allergen levels significantly increased FeNO. • Airborne nicotine significantly decreased FeNO. • FeNO was not associated with passive smoking exposure when measured by biomarkers and reported smoking by parents.	• Significant associations were seen between initial FeNO concentrations and future values. • FeNO increased during the pollen session (increase in log transformed FeNO 1.34 [95% CI 1.05-1.70]) and elevated also in those with mould at home during September–October.
LIMITATIONS	• Small sample size. • Absence of a control group of children without seasonal asthma. • No mention of important confounders (daily activities, and diet).	• Absence of a control group of children not exposed to tobacco smoke. • Settled dust allergens were only measured at baseline.	• Uneven numbers of measurements taken on different sample groups. • No mention of important confounders (daily activities and diet).
CONCLUSION	Natural allergen exposure during the pollen season increased FeNO in asthmatic children.	Environmental triggers, airborne nicotine, and baseline allergen sensitisation affected FeNO levels.	Both baseline FeNO values and intervals between FeNO measurements were related to individual FeNO values, irrespective of asthma status.

TABLE III: EPIDEMIOLOGICAL STUDIES AND CASE REPORTS OF SERIAL LONGITUDINAL FeNO MEASUREMENT IN ADULTS

TABLE IIIA: EPIDEMIOLOGICAL STUDIES AMONG ADULTS AND WORKING POPULATIONS

AUTHORS	HEWITT ET AL, 2008[24]	VAN DER WALT ET AL, 2016[25]	FOWLER ET AL, 2009[26]
AIM OF STUDY	To assess whether serial FeNO measurements may detect exposure-related inflammation in laboratory animal workers.	To evaluate association between FeNO and occupational exposures to spices.	To assess whether exposure to common domestic allergens affects long-term FeNO measurements in adult asthmatics.
STUDY DESIGN	Longitudinal study	Cross-sectional study	Longitudinal study
STUDY POPULATION	• 50 laboratory animal workers in four research centres. • Each subject was exposed to a minimum of two occasions per week for six months or longer. • There was no exclusion based on clinical history.	• Permanent (n = 139) and casual (n = 11) workers in a spice mill • (total n = 150). • Mean employment duration of 6.9 years in the factory and 3.2 years in the current job.	• 165 subjects with asthma, measured at home, 82% of which were atopic, 18% of which were non-atopic. • Each subject was exposed to two visits, four years apart (mean = 47 months).
INTERVENTION/ EXPOSURE	Exposure to laboratory animals in the workplace.	Exposure to spices in the workplace.	Exposure to mite, dog and cat allergens in the household.
OUTCOME MEASURES	Symptom questionnaire, peak expiratory flow measurements, lung function and FeNO.	Immunological tests for common inhalants and occupational agents (garlic, chili pepper and wheat), spirometry and FeNO.	Lung function, FeNO, and immunological tests for sensitisation to mite, dog and cat allergens.
MEASUREMENTS	• Subjects were initially assessed on a Friday. • Subjects received instruction on the use of a peak flow meter and a symptom diary. • Having avoided contact with laboratory animals over the weekend, they were assessed at the beginning and at the end of each working day from Monday to Friday of the following week.	Serial FeNO measurements were conducted as follows: • Baseline pre-shift on Monday. • Post-eight-hour shift. • Pre-shift test on the following Tuesday.	Allergen exposure was measured using monoclonal ELISA. Each subject underwent two measurements, four years apart as follows: • All underwent spirometry and measurement of FeNO. • Allergic sensitisation was measured by skin-prick testing.
RESULTS	• Two of the 50 subjects had a positive specific IgE to one or more laboratory animal allergen (LAA). • Baseline FeNO were highest in two subjects sensitised to LAA, having FeNO of 213.0 ppb and 179.0 ppb. • Mean FeNO was (19.8 ± 20.1 ppb) and (21.7 ± 20.8 ppb) in the remaining seronegative symptomatic and non-symptomatic groups, respectively. • Progressive increases in FeNO occurred only in one of the seropositive individuals during the working week.	• The geometric mean of FeNO change across shift (15.43 ppb) was very similar to the mean change across the 24-hour period (15.84 ppb). • FeNO increase (>12%) across a 24-hour period was significantly associated (OR = 3.77; CI 1.01-14.24) with exposures to higher spice dust particulate but no effect observed across the eight-hour work shift.	• There was a mean 1.5-fold decrease in FeNO over the 4-year period ($p < 0.001$).
LIMITATIONS	• Small sample size. • Subjects were not all asthmatic. • No mention made of important con-founders. However, mention was made of the workplace protection not being uniform.	• No comparable unexposed group. • Short follow up duration	• The level of exposure was not measured at the second visit. • Possibilities for selection bias • No control group of healthy adults. • Possibility of important confounders (high doses of ICS among all participants).
CONCLUSION	Serial FeNO could assist in monitoring patients sensitised to occupational allergens. However, its value decreases if the patient is also sensitised and exposed to non-occupational allergens which can mask the FeNO changes.	FeNO level increase was more pronounced 24 hours after the exposure to spice-dust particulate.	Long-term exposure to dust mite and dog allergens in the home caused significant changes in bronchial hyperresponsiveness, but not necessarily in FeNO levels of asthmatic adults.

TABLE IIIB: CASE REPORTS OF ADULT WORKING POPULATIONS		
AUTHORS	MERGET ET AL, 2014[22]	MERGET ET AL, 2016[23]
AIM OF STUDY	To assess the practicality of serial measurements of FeNO as diagnostic tool for occupational asthma.	A case report of triticale (wheat/rye) allergy in a farmer.
STUDY DESIGN	Case study	Case study
STUDY POPULATION	51-year-old male smoker working as a baker since adolescence who complained of shortness of breath and cough.	29-year-old farmer with hay fever and atopic dermatitis since adolescence diagnosed with work-related asthma.
INTERVENTION	Avoidance of exposure for two weeks.	Avoidance of exposure for two weeks.
OUTCOME MEASURES	Change of FeNO level over time.	Change of FeNO level over time.
MEASUREMENTS	FeNO measurement was performed once daily over a two-week holiday period and thereafter over three weeks of working days.	• FeNO measurement was performed once daily during a working week and thereafter over a two-week holiday period. • Measurements were done in the evenings, (after work) in the working period.
RESULTS	FeNO decreased to the normal level soon after the start of the holiday period and showed a clear increase after resuming work.	FeNO decreased gradually over time soon after the start of the holiday period.
LIMITATIONS	Case study design	Case study design
CONCLUSION	FeNO decreased over two weeks of allergen and irritants avoidance and showed a clear increase over the working-day period.	FeNO decreased over two weeks of allergen avoidance in a patient with occupational asthma to triticale.

aeroallergen has also been studied in asthmatic children. Piacentini et al[7] studied the outcome of allergen avoidance at 1 756 m above sea level for three months in 20 asthmatic children who were sensitised to house-dust mite (HDM). FeNO levels decreased progressively in the first two weeks ($p = 0.014$) and remained unchanged for the next three months. This drop in FeNO was also reported by Peroni et al[9] in HDM-sensitised asthmatic children living at high altitudes for nine months. The mean FeNO levels decreased by 50% in the first three months ($p = 0.030$); however, no further significant change was recorded over the remaining period. Huss-Marp et al have argued that avoidance of the allergen is not the only contributing factor in the decline of FeNO observed in these studies. This suggests that physiological changes at high altitude may also be a contributing factor.[10,37] This study found that FeNO declined significantly in HDM-sensitised asthmatic children as a group and in the non-HDM-sensitised group or those with intrinsic asthma; however, no significant difference in the reduction between the two groups was observed. Karagiannidis et al[38] also reported a similar conclusion. It is, however, likely that there may have been residual exposure to HDM at this lower altitude compared to the Piacentini and Peroni studies that were done at higher altitudes. Furthermore, it is possible that by combining subjects who were sensitised to allergens other than HDM ($n = 251$) and those with intrinsic asthma ($n = 22$), this may have confounded the findings.

In an occupational context, Merget evaluated the effect of avoidance of exposure to inhalant allergens in two case studies: a farmer sensitised to triticale and a baker mildly sensitised to wheat and rye both demonstrated a decrease in FeNO after avoidance of the potential allergens for two weeks.[23,39]

EXPOSURE TO SPECIFIC SENSITISING AGENTS

All the studies identified focused mainly on serial FeNO changes with exposure to high molecular weight (HMW) agents. However, it has been previously reported that the increase in FeNO after specific inhalation-challenge tests in patients with suspected occupational asthma is more strongly associated with exposure to HMW than to low molecular weight (LMW) agents, the former known to be commonly associated with allergic IgE-mediated airway inflammation.[31]

OTHER EXPOSURE CHARACTERISTICS

Finally, while it could be hypothesised that exposure duration, exposure concentration or cumulative exposure may play a role in determining serial changes of FeNO in population-based studies, we were unable to identify many studies that evaluated these potential relationships. However, Lemiere et al[31] evaluated this association between serial changes in FeNO and the duration of exposure to the potential allergen during SIC tests and the association was found not to be significant (OR = 0.99, 95% CI 0.9–1.0). Although the overall association between the overall change in FeNO and the concentration of allergen exposure was not statistically significant, Van der Walt et al[25] found a significant association between workers exposed to high concentrations of spices and a >12% increase in FeNO in their mill workers (OR = 3.77, 95% CI 1.01–14.24). Furthermore, a positive correlation has also been demonstrated between the degree of FeNO reduction after HDM avoidance and baseline HDM allergen levels measured in the beds of subjects ($r = 0.618$, $p = 0.026$) by Piacentini et al in their study of asthmatic children.[8]

AIR POLLUTION

Exposure to air pollutants could contribute to changing

FeNO levels, especially in busy large cities. Adar et al[17] found that reducing ultrafine particles by the adoption of clean-fuel technology in school buses led to a 16% reduction in FeNO among bus passengers. This association was more prominent in asthmatic children. Moreover, Holguin et al[18] found that exposure to higher road traffic density was associated with a significant increase in FeNO in asthmatic children and a non-significant increase in healthy children. Furthermore, both studies found no association between FeNO and exposure to PM2.5 particulates. This suggests that PM2.5 may not be useful in studying the association between FeNO and air pollution in children.

HOST-ASSOCIATED FACTORS

ASTHMA EDUCATION TO IMPROVE ASTHMA CONTROL

The effect of asthma education programmes on asthma control has been evaluated through several studies of children. Kaminsky et al[11] evaluated 25 asthmatic children, 21 of them on inhaled corticosteroids (ICS), participating in a one-week camp organised with asthma education programmes. The overall mean FeNO dropped by 45% ($p < 0.001$) at the end of the week. This decline could be attributed to the increase in ICS compliance, which was found to reduce FeNO in asthmatics in a dose-dependent manner.[40,41] However, three of the four children who were not on ICS also demonstrated a decrease in FeNO at the end of the camp. The authors indicate that it is also possible that the changes in FeNO could have been due to other additional factors such as allergen avoidance or absence of anxiety triggers in the home.[42,43] Furthermore, Kaminsky et al did not specify the asthma control status of the subjects that were evaluated. It is possible that a large proportion were non-compliant or under-treated so that the change in FeNO could have been due to improved asthma control. This was evident in the study by Barreto et al,[15] who studied asthmatic children with good asthma control in a sanatorium for one week, a study that was accompanied by an asthma education programme. The study reported that the mean baseline FeNO in asthmatics on ICS neither differed from that of the healthy subjects nor did it differ over the entire week. In contrast, asthmatics not on ICS had a higher FeNO baseline ($p < 0.005$) compared to the healthy subjects and showed a progressive decrease over the week until the difference reached non-significance ($p = 0.057$). This is further explained by the study of Jatakanon et al[44] that demonstrated a plateau of exhaled FeNO in response to higher doses of ICS.

Asthma education programmes have also been evaluated in adults in domestic and occupational settings. Dressel et al[13] evaluated FeNO after a one-day asthma education programme in 81 animal farmers with occupational asthma who were sensitised to cow dander and storage mites. The overall mean FeNO decreased by 9% ($p = 0.042$) after 4–6 weeks after the programme and by 18% ($p = 0.003$) among those with a baseline FeNO of >35ppb. The 43 subjects in this study were re-evaluated after one year to assess the long-term changes in FeNO after the asthma education programme.[14] The mean FeNO decreased by 21% ($p = 0.001$) in the intervention group. While measures to reduce exposure to the allergens – such as the use of personal protective equipment, changing work clothes before going home and washing hair before going to bed – were major contributors to this reduction in FeNO in both studies, compliance with medication was also cited as an additional contributor to these positive findings. This was evident also in the study by Saito et al,[12] which provided an asthma education programme to 50 asthmatics with poor adherence to medication. The programme was aimed at increasing compliance with medications and at improving the inhalation technique. The study reported a significant decrease in the overall mean FeNO after a year of follow-up visits (at 3, 6 and 12 months from the baseline) in stable patients ($n = 42$), even though asthma medication was stepped down in 33% of the subjects. However, a non-significant reduction in FeNO was recorded in 16 subjects who were initially unstable and required an increase in ICS dosage or additional asthma medication during the course of the study.

ATOPY

It is well known that FeNO levels are higher among atopic subjects, irrespective of the domestic or occupational exposures.[3–5] These findings were also reported by Van der Walt et al[25] in their study of spice-mill workers. However, strong associations between atopic status and longitudinal changes in FeNO have not been studied in detail. Changes in FeNO appear to be independent of atopic status.[10,24] Spanier et al[20] found that the seasonal variation of FeNO did not differ between atopic and non-atopic subjects. Moreover, Lemiere et al[31] reported a non-significant positive association between changes in FeNO and atopy status (OR 2.0, 95% CI 0.5–7.5) in their study of FeNO measured after SIC to occupational agents in patients with occupational asthma.

SMOKING STATUS

It is well known that smoking is associated with a decreased baseline FeNO in subjects.[3–5] However, strong associations between smoking status and longitudinal changes in FeNO have not been studied in detail. Lemiere et al,[31] in their study of patients with occupational asthma, found a non-significant positive association between FeNO changes and negative smoking status (OR 1.5, 95% CI 0.5–4.3) after SIC to occupational agents in patients with occupational asthma.

In children, passive smoking may have an effect on longitudinal changes in FeNO. Spanier et al[20] found that exposure to passive smoking measured by a nicotine dosimeter led to a significant decrease in FeNO. However, this association disappeared when the exposure reported by the parent's survey was used – suggesting possible recall bias.

BASELINE LUNG FUNCTION AND FeNO

Whereas the relationship between baseline FeNO and forced expiratory volume (FEV_1) has been evaluated in a number of studies, there are few studies evaluating the association between baseline FEV_1 and the longitudinal change in FeNO. Peroni et al[9] did not find a correlation between FEV_1 and longitudinal changes in FeNO in their study. Lemiere et al[31] also reported a non-significant association between changes in FeNO post-SIC and baseline FEV_1. This association was also not present with a maximum fall in FEV_1 after SIC. However, Saito et al[12] found a significant correlation between a change in FeNO and the change in FEV_1 in the first three months of their study. This change remained significant until the end of the study after 12 months of baseline.

While it could be hypothesised that baseline FeNO may be a good indicator of the magnitude of future change in serial FeNO, studies in this area that evaluated this potential relationship were scant. In children, Spanier et al[20] and Cutts et al[21] found a significant association between baseline FeNO and longitudinal changes in FeNO. However, Lemiere et al[31] were unable to demonstrate an association between baseline FeNO (>25ppb) and a change in FeNO post-SIC in an adult group.

CONCLUSION

To conclude, FeNO can be regarded as an important marker of airway inflammation in asthma among both symptomatic and asymptomatic subjects. Whereas the predictors of baseline FeNO levels have been evaluated in several clinical and population-based studies, the evidence for various factors associated with longitudinal changes in FeNO need further investigation. These include smoking, atopy, baseline FeNO, baseline FEV_1 and exposure characteristics (agent and dose), which need further evaluation, especially in occupational settings.

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

This article has been peer reviewed.

REFERENCES

1. Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention [Internet]. 2016. Available from: http://www.ginasthma.org.
2. Dominguez-Ortega J, Barranco P, Rodríguez-Pérez R, Quirce S. Biomarkers in occupational asthma. Curr Allergy Asthma Rep. 2016;16(9):63.
3. Alshamkhi N, Alving K, Dahlen SE, Hedlin G, Middelveld R, Bjerg A, et al. Important nondiseaserelated determinants of exhaled nitric oxide levels in mild asthma–results from the Swedish GA2LEN study. Clin Exp Allergy. 2016;46(9):1185–1193.
4. Ludviksdottir D, Diamant Z, Alving K, Bjermer L, Malinovschi A. Clinical aspects of using exhaled NO in asthma diagnosis and management. Clin Respir J. 2012;6(4):193–207.
5. Alving K, Malinovschi A. Basic aspects of exhaled nitric oxide. Exhaled biomarkers. 2010;49:1–31.
6. Baatjies R, Jeebhay MF. Sensitisation to cereal flour allergens is a major determinant of elevated exhaled nitric oxide in bakers. Occup Environ Med. 2013;70(5):310–316.
7. Piacentini GL, Bodini A, Costella S, Vicentini L, Peroni D, Zanolla L, et al. Allergen avoidance is associated with a fall in exhaled nitric oxide in asthmatic children. J Allergy Clin Immunol. 1999;104(6):1323–1324.
8. Piacentini GL, Del Giudice MJM, Bodini A, Costella S, Vicentini L, Peroni D, et al. Exhaled NO reduced on allergen avoidance. Allergy. 2001;56(3):251–252.
9. Peroni DG, Piacentini GL, Costella S, Pietrobelli A, Bodini A, Loiacono A, et al. Mite avoidance can reduce air trapping and airway inflammation in allergic asthmatic children. Clin Exp Allergy. 2002;32(6):850–855.
10. Huss-Marp J, Krämer U, Eberlein B, Pfab F, Ring J, Behrendt H, et al. Reduced exhaled nitric oxide values in children with asthma after inpatient rehabilitation at high altitude. J Allergy Clin Immunol. 2007;120(2):471–472.
11. Kaminsky DA, Rice AA, Bissonette M, LaRose T, Phillips L, Cohen L, et al. Exhaled nitric oxide decreases in association with attendance at an asthma summer cAMP. J Asthma. 2008;45(5):415–419.
12. Saito J, Sato S, Fukuhara A, Sato Y, Nikaido T, Inokoshi Y, et al. Association of asthma education with asthma control evaluated by asthma control test, FEV_1, and fractional exhaled nitric oxide. J Asthma. 2013;50(1):97–102.
13. Dressel H, Gross C, De la Motte D, Sültz J, Jörres RA, Nowak D. Educational intervention decreases exhaled nitric oxide in farmers with occupational asthma. Eur Respir J. 2007;30(3):545–548.
14. Dressel H, Gross C, de la Motte D, Sultz J, Jorres RA, Nowak D. Educational Intervention in Farmers With Occupational Asthma: Long-Term Effect on Exhaled Nitric Oxide. J Investig Allergol Clin Immunol. 2009;19(1):49.
15. Barreto M, Rennerova Z, Montesano M, Alterio A, Trubacova D, Ronchetti R, et al. Variations in exhaled nitric oxide in children with asthma during a 1-week stay in a mountain village sanatorium. J Asthma. 2008;45(6):453–458.
16. Baraldi E, CarrÀ S, Dario C, Azzolin N, Ongaro R, Marcer G, et al. Effect of natural grass pollen exposure on exhaled nitric oxide in asthmatic children. Am J Respir Crit Care Med. 1999;159(1):262–266.
17. Adar SD, D'Souza J, Sheppard L, Kaufman JD, Hallstrand TS, Davey ME, et al. Adopting clean fuels and technologies on school buses: Pollution and health impacts in children. Am J Respir Crit Care Med. 2015;191(12):1413–1421.
18. Holguin F, Flores S, Ross Z, Cortez M, Molina M, Molina L, et al. Traffic-related exposures, airway function, inflammation, and respiratory symptoms in children. Am J Respir Crit Care Med. 2007;176(12):1236–1242.
19. Roberts G, Hurley C, Bush A, Lack G. Longitudinal study of grass pollen exposure, symptoms, and exhaled nitric oxide in childhood seasonal allergic asthma. Thorax. 2004;59(9):752–756.
20. Spanier AJ, Hornung RW, Kahn RS, Lierl MB, Lanphear BP. Seasonal Variation and Environmental Predictors of Exhaled Nitric Oxide in Children with Asthma. Pediatr Pulmonol. 2008;43(6):576–583.
21. Cutts R, Turner S. Longitudinal measurements of exhaled nitric oxide in children-what is a significant change in FeNO? Pediatr Allergy Immunol. 2013;24(6):540–548.
22. Merget R, Sander I, van Kampen V, Raulf-Heimsoth M, Hagemeyer O, Marek E, et al. Serial measurements of exhaled nitric oxide at work and at home: a new tool for the diagnosis of occupational asthma. Adv Exp Med Biol. 2015;834:49–52.
23. Merget R, Sander I, van Kampen V, Raulf M, Brüning T. Triticale allergy in a farmer. Am J Ind Med. 2016;59(6):501–505.
24. Hewitt RS, Smith AD, Cowan JO, Schofield JC, Herbison GP, Taylor DR. Serial exhaled nitric oxide measurements in the assessment of laboratory animal allergy. J Asthma. 2008;45(2):101–107.
25. Van der Walt A, Baatjies R, Singh T, Jeebhay MF. Environmental factors associated with baseline and serial changes in fractional exhaled nitric oxide (FeNO) in spice mill workers. Occup Environ Med. 2016;(0):1–7.
26. Fowler SJ, Langley SJ, Truman NJ, Woodcock A, Simpson A, Custovic A. Long-term effects of allergen sensitization and exposure in adult asthma: a prospective study. World Allergy Organ J. 2009;2(5):83–90.
27. Ricciardolo FLM, Timmers MC, Sont JK, Folkerts G, Sterk PJ. Effect of bradykinin on allergen induced increase in exhaled nitric oxide in

asthma. Thorax. 2003;58(10):840–845.
28. Quirce S, Lemière C, De Blay F, Del Pozo V, Gerth Van Wijk R, Maestrelli P, et al. Noninvasive methods for assessment of airway inflammation in occupational settings. Allergy. 2010;65(4):445–458.
29. Świebocka E, Siergiejko G, Siergiejko Z. Bronchial allergen challenge in allergic children: continuous increase of nitric oxide in exhaled air 72 hours after allergen inhalation independent of bronchial obstruction. J Aerosol Med Pulm Drug Deliv. 2011;24(1):17–24.
30. Pedrosa M, Barranco P, López-Carrasco V, Quirce S. Changes in exhaled nitric oxide levels after bronchial allergen challenge. Lung. 2012;190(2):209–214.
31. Lemiere C, NGuyen S, Sava F, D'alpaos V, Huaux F, Vandenplas O. Occupational asthma phenotypes identified by increased fractional exhaled nitric oxide after exposure to causal agents. J Allergy Clin Immunol. 2014;134(5):1063–1070.
32. Świerczyńska-Machura D, Krakowiak A, Wiszniewska M, Dudek W, Walusiak J, Pałczyński C. Exhaled nitric oxide levels after specific inhalatory challenge test in subjects with diagnosed occupational asthma. Int J Occup Med Environ Health. 2008;21(3):219–225.
33. Baur X, Barbinova L. Latex allergen exposure increases exhaled nitric oxide in symptomatic healthcare workers. Eur Respir J. 2005;25(2):309–316.
34. Allmers H, Chen Z, Barbinova L, Marczynski B, Kirschmann V, Baur X. Challenge from methacholine, natural rubber latex, or 4, 4′-diphenylmethane diisocyanate in workers with suspected sensitization affects exhaled nitric oxide [change in exhaled NO levels after allergen challenges]. Int Arch Occup Environ Health. 2000;73(3):181–186.
35. Piipari R, Piirilä P, Keskinen H, Tuppurainen M, Sovijärvi A, Nordman H. Exhaled nitric oxide in specific challenge tests to assess occupational asthma. Eur Respir J. 2002;20(6):1532–1537.
36. Barbinova L, Baur X. Increase in exhaled nitric oxide (eNO) after work-related isocyanate exposure. Int Arch Occup Environ Health. 2006;79(5):387.
37. Huss-Marp J, Krämer U, Eberlein B, Pfab F, Ring J, Behrendt H, et al. Reply: Exhaled nitric oxide in children with asthma at high altitude. Vol. 120, Journal of Allergy and Clinical Immunology. 2007. p. 1227–1228.
38. Karagiannidis C, Hense G, Rueckert B, Mantel PY, Ichters B, Blaser K, et al. High Altitude Climate Therapy Reduces Local Airway Inflammation and Modulates Lymphocyte Activation. Scand J Immunol. 2006;63(4):304–310.
39. Merget R, Sander I, van Kampen V, Raulf-Heimsoth M, Hagemeyer O, Marek E, et al. Serial measurements of exhaled nitric oxide at work and at home: a new tool for the diagnosis of occupational asthma. In: Environment Exposure to Pollutants. Springer; 2014. p. 49–52.
40. Kharitonov SA, Yates DH, Barnes PJ. Inhaled glucocorticoids decrease nitric oxide in exhaled air of asthmatic patients. Am J Respir Crit Care Med. 1996;153(1):454–457.
41. Jones SL, Herbison P, Cowan JO, Flannery EM, Hancox RJ, McLachlan CR, et al. Exhaled NO and assessment of anti-inflammatory effects of inhaled steroid: dose-response relationship. Eur Respir J. 2002;20(3):601–608.
42. Sandel M, Wright RJ. When home is where the stress is: expanding the dimensions of housing that influence asthma morbidity. Arch Dis Child. 2006;91(11):942–948.
43. Chen E, Miller GE. Stress and inflammation in exacerbations of asthma. Brain Behav Immun. 2007;21(8):993–999.
44. Jatakanon A, Kharitonov S, Lim S, Barnes PJ. Effect of differing doses of inhaled budesonide on markers of airway inflammation in patients with mild asthma. Thorax. 1999;54(2):108–114.

Appendix 2: English questionnaire at baseline

UCT OCCUPATIONAL ALLERGY AND ASTHMA STUDY AMONG

BAKERY WORKERS IN THE WESTERN CAPE PROVINCE

OF SOUTH AFRICA - 2006

ENGLISH QUESTIONNAIRE

Survey Number _____

A. IDENTIFICATION DATA

1.Surname _____

2. First name/s _____

3. Work number _____

4. Date of birth: Day_____Month_____Year____

5. Gender: Male (1)

Female (2)

8. Interviewer's initials _____

9. Date of interview:

Day____Month_____Year_____

10. Bakery: _____

11. Did you change your job since the last interview?

Yes (1)

No (2)

Not applicable (3)

11.1 If Yes or NA, what is your new job?

_____

12. Which shift have you been working today?

04:00 - 12:00 (1)

07:00 - 16:00 (2)

08:00 - 17:00 (3)

09:00 - 18:00 (4)

12:00 - 21:00 (5)

B.HEALTH PROBLEMS

Recent chest infections

1. Have you had the flu or sinusitis in the past 3 weeks?

Yes (1)

No (2)

2. Have you had any of the following symptoms in the past **12 months** (at night, with exercise, exposure to cold air, work exposures)?

2.1 chest tightness Yes (1)

No (2)

2.2 shortness of breath Yes (1)

No (2)

2.3 wheezing or whistling in your chest Yes (1)

No (2)

2.4 dry cough Yes (1)

No (2)

2.5 Asthma Yes (1)

No (2)

3. Are you being treated for Tuberculosis (TB)?

Yes (1)

No (2)

3.1 If yes, for how long? _____months _____weeks

If YES, to question no 3, indicate to person that the tests will not be done today. Schedule another appointment in three months time since the start of TB medication.

Nose and eye symptoms

4. Have you ever had any nose or eye problems due to allergies and/or hay fever?

Yes (1)

No (2)

C. SMOKING HISTORY

1. Do you smoke?

Yes (1)

No (2)

1.1 If yes, have you smoked tobacco (cigarettes or pipe) for as long as a year?

Yes (1)

No (2)

1.2 If yes, how many cigarettes per day do you smoke or did you smoke?

_____

1.3 Have you smoked (cigarettes/tobacco) in the last hour?

Yes (1)

No (2)

D. ALCOHOL CONSUMPTION

1. Do you drink alcohol?

Yes (1)

No (2)

1.1 If yes, when have you last consumed alcohol?

1-2 hours ago (1)

1 day ago (2)

1 week ago (3)

1.2 How much alcohol did you consume?

_____

E. MEDICATION USAGE (show booklet)

1. Are you taking any medicine/s from a doctor or clinic at the moment for asthma, and or hayfever?

Yes (1)

No (2)

1.1 If yes, what are you taking and when last did you take them?

Names No. of hours since last dose

_____ _____

_____ _____ _____

_____ _____ _____

F. GREEN VEGETABLE CONSUMPTION

1. How often do you eat the following vegetable products?

Type of product	**Daily**	**1 to 3 times a week**	**1 to 3 times per month**	**Never**
1.1 Green salad	1	2	3	4
1.2 Spinach & other green leafy vegetables	1	2	3	4

2. When did you last consume green salad and/or spinach/other green leafy vegetables?

1-2 hours ago (1)

1 day ago (2)

1 a week ago (3)

G. PHYSICAL ACTIVITY

1. Do you exercise?

Yes	(1)
No	(2)

2. When was the last time you exercised?

1-2 hours ago	(1)
1 day ago	(2)
1 week ago	(3)

H. SPIROMETRY/LUNG FUNCTION TEST

1. Have you ever had a spirometry/lung function test?

Yes	(1)
No	(2)

2. If yes, when last did you blow into a lung function machine?

1-2 hours ago	(1)
1 day ago	(2)
1 week ago	(3)
> a week ago	(4)

I. RECENT FOOD INTAKE

1. Did you have anything to eat or drink in the last hour?

Yes	(1)
No	(2)

If YES to above question, reschedule test for at least 1 hour later

the same day or another date.

J. WORK-RELATED SYMPTOMS

1. Does being at work ever make your chest tight

or wheezy?

Yes (1)

No (2)

2. Does being at work ever cause you to have sneezy/

itchy/runny nose or red/itchy/watery eyes?

Yes (1)

No (2)

Appendix 3: English questionnaire at follow-up

UCT OCCUPATIONAL ALLERGY AND ASTHMA STUDY AMONG

BAKERY WORKERS IN THE WESTERN CAPE PROVINCE

OF SOUTH AFRICA - 2011

ENGLISH QUESTIONNAIRE

Survey Number _____

A. IDENTIFICATION DATA

1. Surname _____

2. First name/s _____

3. Address _____

_____

_____

_____

4. Work number _____

5. Date of birth: Day_____Month_____Year____

6. Gender: Male (1)

Female (2)

7. Home Language: English (1)

Afrikaans (2)

Xhosa (3)

Other (4)

8. Interviewer's initials _____

9. Date of interview:

Day____Month_____Year_____

10. Bakery: _____

11. Are you a casual or permanent worker?

Casual (VTE) (1)

Permanent (2)

12. Were you part of the previous surveys?

Yes (1)

No (2)

12.1 If yes, which year:

2003 Yes (1)

No (2)

2007 Yes (1)

No (2)

B.HEALTH PROBLEMS

Wheeze and tightness in the chest

1. Have you ever had wheezing or whistling in your chest in the past?

Yes (1)

No (2)

If YES, go on to Question 1.1

If NO, skip to Question 2

1.1 If yes, when was the first time you had these symptoms.

Date: Month _____ Year _____

1.2 Have you had wheezing or whistling in your chest at any time in the **last 12 months?**

Yes (1)

No (2)

If YES, go on to Question 1.2.1

If NO, skip to Question 2

1.2.1 Have you been short of breath when the wheezing noise was present?

Yes (1)

No (2)

1.2.2 Have you had this wheezing or whistling when you **did not have a cold or flu?**

Yes (1)

No (2)

2. Have you been woken up with a feeling of tightness in your chest at any time in the **last 12 months?**

Yes (1)

No (2)

Shortness of breath

3. Have you had an attack of shortness of breath that came on during the daytime when you were at rest at any time in the **last 12 months?**

Yes (1)

No (2)

4. Have you had an attack of shortness of breath that came on following running or exercise at any time in the

last 12 months?

Yes (1)

No (2)

5. Have you been woken by an attack of shortness of breath at any time in the **last 12 months?**

Yes (1)

No (2)

Cough and phlegm from the chest

6. Have you been woken by an attack of coughing at any time in the **last 12 months?**

Yes (1)

No (2)

7. Do you usually cough first thing in the morning?

Yes (1)

No (2)

8. Do you usually cough during the rest of the day, or at night?

Yes (1)

No (2)

If YES, go on to Question 8.1

If NO, skip to Question 9

8.1 Do you cough like this on most days/nights for as much as three or more months in each of the last two years?

Yes (1)

No (2)

9. Do you usually bring up any phlegm from your chest first thing in the morning?

Yes (1)

No (2)

10. Do you usually bring up any phlegm from your chest during the day, or at night?

Yes (1)

No (2)

If YES, go on to Question 10.1

If NO, skip to Question 11

10.1 Do you bring up phlegm like this on most days/ nights for as much as three or more months in each of the last two years?

Yes (1)

No (2)

Breathing

11. Do you ever have trouble with your breathing?

Yes (1)

No (2)

If YES, go on to Question 11.1

If NO, skip to Question 12

11.1 Do you have this trouble:

Give all options at once

Insert a cross (X) next to one answer only

a) continuously so that your breathing is never quite right? _____

b) repeatedly, but it goes away completely between the times when it troubles you? _____

c) only rarely? _____

12. Are you disabled from walking by a condition other than heart or lung disease?

Yes (1)

No (2)

If YES, state the condition _____

and go on to Question 13

If NO, go to Question 12.1

12.1 Are you troubled by shortness of breath when hurrying on level ground or walking up a slight hill?

Yes (1)

No (2)

If YES, go on to Question 12.1.1

If NO, skip to Question 13

12.1.1 Do you get short of breath walking with other people of your own age on level ground?

Yes (1)

No (2)

12.1.1.1 Do you have to stop for breath when walking at your own pace on level ground?

Yes (1)

No (2)

Asthma

13. Have you ever had asthma?

Yes (1)

No (2)

If YES, go on to Question 13.1

If NO, skip to Question 13.8

13.1 If yes, was this confirmed by a doctor?

Yes (1)

No (2)

13.2 How old were you when you were told you have asthma?

Give all options at once

Insert a cross (X) next to one answer only

a) Only before you were 17 years old ____

b) Only at the age of 17 years or older ____

c) Both ____

The following references to "attack" of asthma refers to episodes of wheezing, shortness of breath, chest tightness or cough attributed to asthma

13.3.1 How old were you when you had your first attack of asthma?

_____ years old

13.3.2 How old were you when you had your most recent attack of asthma?

_____ years old

13.4.1-6 Which months of the year do you usually have attacks of asthma?

13.4.1 January/February

Yes (1)

No (2)

13.4.2 March/April

Yes (1)

No (2)

13.4.3 May/June

Yes (1)

No (2)

13.4.4 July/August

Yes (1)

No (2)

13.4.5 September/October

Yes (1)

No (2)

13.4.6 November/December

Yes (1)

No (2)

13.5 Have you had an attack of asthma in the last **12 months?**

Yes (1)

No (2)

If YES, go on to Question 13.5.1

If NO, skip to Question 13.6

13.5.1 How often have you had an attack of asthma in the **last 12 months?**

Give all options at once

Insert a cross (X) next to one answer only

a) Every day _____

b) More than 2 times a week _____

c) More than 1 time per month _____

d) 3 to 12 times in the whole year _____

e) 1 to 2 times in the whole year _____

13.6 Are your chest symptoms caused by, or made worse by any of the following:

Answer all questions

13.6.1 Contact with animals/pets

Yes (1)

No (2)

13.6.2 Grass or flowers

Yes (1)

No (2)

13.6.3 Heavy exercise

Yes (1)

No (2)

13.6.4 Breathing cold air

Yes (1)

No (2)

13.6.5 Dusts or sprays at work

Yes (1)

No (2)

13.6.6 Tobacco smoke

Yes (1)

No (2)

13.6.7 Change in the weather

Yes (1)

No (2)

13.7 Do your chest symptoms seem better or worse when you are away from work (for example, on weekends, off-shift and vacations)?

Give all options at once

Insert a cross (X) next to one answer only

a) Stay the same _____

b) Get better _____

c) Get worse _____

13.8 Does being at work ever make your chest tight or wheezy?

Yes (1)

No (2)

If YES, go on to Question 13.8.1

If NO, skip to Question 13.9

13.8.1 When did you first notice having problems with chest tightness or wheeze at work?

Date: Month _____ Year _____

13.8.2 Is there anything that you work with that causes you to have these chest symptoms?

Yes (1)

No (2)

If YES, go on to Question 13.8.3 (**specify wheat, rye &/or premix**) or any other substance

If NO, skip to Question 13.9

13.8.3 What do you think is causing these symptoms?

_____

13.9 Have you ever had to change or leave your work area, either temporarily or permanently, in this bakery or any other bakery because of any chest symptoms?

Yes (1)

No (2)

If YES, go on to Question 13.9.1

If NO, skip to Question 13.10

13.9.1 What type of job were you doing when this happened?

_____

13.9.2 Was this a job in this bakery?

Yes (1)

No (2)

If YES, go on to Question 13.9.2.1

If NO, skip to Question 13.10

13.9.2.1 What area/section did you move to?

_____

13.9.2.2 What job did you do there?

_____

13.9.2.3 Did your symptoms improve when you changed jobs?

Yes (1)

No (2)

13.10 Have you ever worked in a job or jobs that exposed you to vapours, gas, dust or fumes?

Yes (1)

No (2)

If YES, go on to Question 13.10.1.

List the jobs beginning with the most recent

If NO, skip to Question 13.11

13.10.1 What was or is this job? _____

(if current job write 'current job')

13.10.2 Before that? _____

13.10.3 Before that? _____

13.11 Has there ever been an instance when you inhaled a large amount of vapour, gas, dust or fumes in any of these jobs that resulted in you developing a tight chest, wheeze or cough?

Yes (1)

No (2)

If YES, go on to Question 13.11.1.

If NO, skip to Question 13.12

13.11.1 What was or is this job? _____

(if current job write 'current job')

13.12 Are you using any medicines, including inhalers/ pumps, nebulizers, syrups or tablets, for asthma or breathing problems?

Yes (1)

No (2)

If YES, go on to Question 13.12.1, showing examples of each

If NO, skip to question 13.13

13.12.1 Which medicines?

_____

_____

_____

13.12.2 Do you take these medicines every day even when you do not have any trouble breathing?

Yes (1)

No (2)

13.13 Have you ever been treated for any of the following:

Answer all questions

13.13.1 Repeated chest infections as a child

Yes (1)

No (2)

UNK (3)

13.13.2 Tuberculosis (TB)

Yes (1)

No (2)

UNK (3)

13.13.3 Chronic bronchitis

Yes (1)

No (2)

UNK (3)

Nose and eye symptoms

14. Have you ever had any nose or eye problems or allergies such as hay fever?

Yes (1)

No (2)

If YES, go on to Question 14.1 Answer all questions

If NO, skip to Question 14.4

14.1 How old were you when you first noticed these symptoms?

_____ years old

14.2 During the past 12 months have you had two or more episodes of:

14.2.1 sneezy, itchy or runny nose when you did not have a cold or flu?

Yes (1)

No (2)

14.2.2 red, itchy or watery eyes

Yes (1)

No (2)

14.2.3 Do you usually have the nose or eye symptoms at any particular time of the year?

Yes (1)

No (2)

14.2.3.1 If YES, which is the worst season?

Give all options at once

Insert a cross (X) next to one answer only

a) Winter _____

b)

Spring _____

c) Summer _____

d) Autumn _____

If YES to any of the above in question 14.2, go on to Question 14.3

If NO, skip to Question 14.4

14.3 Do your nose or eye symptoms seem better or worse when you are away from work (for example, on weekends, off-shift and vacations)?

Give all options at once

Insert a cross (X) next to one answer only

a) Stay the same _____

b) Get better _____

c) Get worse _____

14.4 Does being at work ever cause you to have sneezy/ itchy/runny nose or red/itchy/watery eyes?

Yes (1)

No (2)

If YES to any one of the above, go on to Question 14.4.1

If NO, skip to Question 14.5

14.4.1 Since when have you been having these symptoms at work?

Date: Month ____ Year ____

14.4.2 Is there anything that you work with that causes you to have these symptoms?

Yes (1)

No (2)

If YES, go on to Question 14.4.3 (**specify wheat, rye &/or premix**) or any other substance

If NO, skip to Question 14.5

14.4.3 What do you think is causing these symptoms?

_____

14.5 Are you using any medicines, including nose sprays, drops, tablets or injections, for any nose or eye symptoms at present?

Yes (1)

No (2)

If YES, go on to Question 14.5.1

If NO, go on to Question 14.6

Present a chart with different samples of allergy medicines

(N.B. a worker might show you his/her medicines).

14.5.1 Which medicines?

_____

_____

14.6 Did you have hay fever (itchy or watery eyes/nose) as a child?

Yes (1)

No (2)

Other allergic conditions

15. Did you have eczema as a child?

Yes (1)

No (2)

16. Are you allergic to insect stings or bites?

Yes (1)

No (2)

If YES, go on to Question 16.1

If NO, skip to Question 17

16.1.1-3 What kind of reactions do you have?

16.1.1 Breathing difficulty, feeling faint, fever?

Yes (1)

No (2)

16.1.2 Redness, itching or swelling at the sting site

Yes (1)

No (2)

16.1.3 Other: _____

17. Have you ever had any difficulty with your breathing after taking medications or injections that you did not have before?

Yes (1)

No (2)

If YES, go on to Question 17.1

If NO, skip to 18.1

17.1 Which medicines?

_____ _____

18.1-6 When you are near animals (such as cats, dogs or horses), near feathers (including pillows, quilts or duvets), near grass and flowers, or in a dusty part of the house, do you ***ever***

18.1 Start to cough?

Yes (1)

No (2)

18.2 Start to wheeze?

Yes (1)

No (2)

18.3 Get a tight chest?

Yes (1)

No (2)

18.4 Start to feel short of breath?

Yes (1)

No (2)

18.5 Get a runny/stuffy nose or sneeze?

Yes (1)

No (2)

18.6 Get itchy or watery eyes?

Yes (1)

No (2)

18.7 Get itchy skin/rash?

Yes (1)

No (2)

19. Have you ever had an illness or trouble caused by eating a particular type of food/fruit?

Yes (1)

No (2)

If YES, go on to Question 19.1

If NO, skip to 20

19.1 What type of food/fruit was this?

_____

19.1.1-6 Did this illness or trouble include:

19.1.1 Itchy skin or rash

Yes (1)

No (2)

19.1.2 Diarhoea or vomiting

Yes (1)

No (2)

19.1.3 Runny or stuffy nose

Yes (1)

No (2)

19.1.4 Severe headaches

Yes (1)

No (2)

19.1.5 Breathlessness/tight chest/wheeze

Yes (1)

No (2)

19.1.6 Other:_____

19.2 Was the food canned or preserved?

Yes (1)

No (2)

UNK (3)

19.3 Do you experience these problems when you drink

fizzy drinks also?

Yes (1)

No (2)

C. FAMILY HISTORY

1. Do/did any members of your family (blood relatives) ever have any kind of allergies?

Do not include relatives by marriage

If family history is completely unknown (subject is adopted, etc.), mark UNK and do not complete table. Move to next section

Yes (1)

No (2)

UNK (3)

If YES, complete table below. Insert a cross (X) in the appropriate block for each option

Type of Allergy	**NO ONE** in family	**YES**, present in the family			**Do Not Know**
		Parent	Brother/ Sister	Child	
1.1 Hay fever	1	2	3	4	5
1.2 Eczema	1	2	3	4	5
1.3 Asthma	1	2	3	4	5

1.4 Wheat	1	2	3	4	5
related allergy					
1.5 Other	1	2	3	4	5
allergy					
Specify:					
_____					
_____					

D. SMOKING HISTORY

1. Have you ever smoked tobacco (cigarettes or pipe) for as long as a year?

'YES' means at least 20 packs of cigarettes or 360 grams of tobacco in a lifetime or at least one cigarette per day for one year

Yes (1)

No (2)

If YES, go on to Question 1.1

If NO, skip to Question 2

1.1 How old were you when you started smoking?

_____ years old

1.2 Do you now smoke?

'YES' means smoking tobacco in the last month or more

Yes (1)

No (2)

If YES, go on to Question 1.2.1

If NO, skip to Question 1.3.1

1.2.1-2. How much do you now smoke on average?

1.2.1 Number of cigarettes per day _____

1.2.2 Pipe tobacco in grams/week _____

1.3. Have you stopped smoking completely?

Yes (1)

No (2)

If YES, go on to Question 1.3.1

If NO, skip to Question 1.4

1.3.1. How old were you when you stopped smoking completely?

_____ years old

1.3.1.1 How many years in total did you smoke cigarettes? (Do not include the years you stopped before you started again)

_____ years

1.3.2.1-2 On average of the entire time you smoked, how much did you smoke?

1.3.2.1 Number of cigarettes per day _____

1.3.2.2 Pipe tobacco in grams/week _____

1.4 Do you or did you inhale the smoke?

Yes (1)

No (2)

2. Have you been regularly exposed to tobacco smoke from other people smoking cigarettes or pipe in the last 12 months?

'Regularly' means on most days or nights

Yes (1)

No (2)

E. DIETARY HISTORY/DOMESTIC ACTIVITIES

1. How often have you eaten the following grain products in the last 12 months?

Go through each wheat product option and insert a cross (X) in the block for each option

Type of wheat product	**Daily**	**1 to 3 times a week**	**1 to 3 times per month**	**Never**
1.1 White	1	2	3	4
bread/Rolls				
1.2 Brown	1	2	3	4
bread/Rolls				
1.3 Whole				
wheat bread/	1	2	3	4
rolls				
1.4 Rye bread/	1	2	3	4
rolls				
2. Pastries	1	2	3	4
3. Cereals	1	2	3	4
4. Biscuits	1	2	3	4
containing				
wheat				
5.Pasta	1	2	3	4
containing				
wheat				
6. Other	1	2	3	4
Specify:				
_____				
_____				

2.Have you changed your diet or avoided certain grain (eg. wheat/rye/soya) products because they do not agree with you when you eat them?

Yes (1)

No (2)

If YES, go on to Question 2.1

If NO, skip to Question 3

2.1 What grain products have you avoided?

_____

_____

3. Do you bake at home?

Yes (1)

No (2)

If YES, go on to Question 3.1

If NO, go to Question 4

3.1 How often do you do baking at home?

a) once a month _____

b) 2-3 times a month _____

c) 2-3 times per week _____

d) once a week _____

e) everyday _____

3.2 What do you bake?

a) bread/rolls _____

b) cakes/biscuits _____

c) tarts/pastries _____

d)

Other: _____

Specify:_____

4. Does any one else bake at home?

Yes (1)

No (2)

F. WORK HISTORY IN THE BAKERY INDUSTRY

1. How long have you been working at this bakery?

_____ years

_____ months

Present job

2. How long have you been working in your current job?

_____ years

_____ months

3. In which area/section are you currently working?

_____

3.1 What is your job in this area/section?

Job Title: _____

3.2 What products do you produce:

a)		
doughs	Yes	(1)
	No	(2)
b)		
pastry	Yes	(1)
	No	(2)
c) croissants	Yes	(1)
	No	(2)
d) bread,rolls	Yes	(1)
	No	(2)
e) cakes/tarts	Yes	(1)
	No	(2)

f)

biscuits	Yes	(1)
	No	(2)
g) confectionary	Yes	(1)
	No	(2)
h) other	Yes	(1)
	No	(2)

Specify:_____

3.3 What ingredients do you work with?

a) Flour (wheat, rye)

Yes	(1)
No	(2)

b) Baking additives (premix)

Yes	(1)
No	(2)

c) Icing sugar

Yes	(1)
No	(2)

d) Nuts (peanuts, hazelnuts)

Yes (1)

No (2)

e) Seeds (sesame, lupine)

Yes (1)

No (2)

f) Other Yes (1)

No (2)

Specify:_____

3.4 Do you ever do other jobs during your shift on a regular basis (almost every day)?

Yes (1)

No (2)

If **Yes**, which jobs? _____

_____

3.5 How much dust would you say your current job produces:

Give all options at once

Insert a cross (X) next to one answer only

a) None _____

b) A little _____

c) An average amount _____

d) A lot _____

3.5.1 What aspect of your work would you say is very dusty?

a) Tipping/Dispensing

Yes (1)

No (2)

N/A (3)

b) Weighing

Yes (1)

No (2)

N/A (3)

c) Sifting

Yes (1)

No (2)

N/A (3)

d)

Mixing

Yes (1)

No (2)

N/A (3)

e) brushing table

Yes (1)

No (2)

N/A (3)

f) dough handling

Yes (1)

No (2)

N/A (3)

g) other

Yes (1)

No (2)

N/A (3)

Specify:_____

3.5.1.1 What type of cleaning activities in your daily work are very dusty.

3.5.1.1.1 Cleaning work table surfaces?

Yes (1)

No (2)

N/A (3)

3.5.1.1.2 Sweeping floors?

Yes (1)

No (2)

N/A (3)

3.5.1.1.3 Cleaning equipment (mixers, cutters)

Yes (1)

No (2)

N/A (3)

3.5.2 How far do you work from the source of the dust?

Give all options at once

Insert a cross (X) next to one answer only

a) Right next to the source _____

b) About 1-2 metres away _____

c) More than 3 metres away _____

d) Does not apply _____

3.6 Do you use any respiratory protective equipment/

mask on a regular basis (almost every day) while doing your job?

Yes (1)

No (2)

If NO, skip to Question 4

If YES, continue with Question 3.6.1

3.6.1 Which type of mask (show respirators)?

3.6.1.1 FFP2 Yes (1)

No (2)

3.6.1.2 Paper Yes (1)

No (2)

3.6.1.3 Other:_____

3.6.2 For which tasks do you use the mask?

3.6.2.1 Pouring flour into mixer

Yes (1)

No (2)

3.6.2.2 Cleaning Yes (1)

No (2)

3.6.2.3 Maintenance of machinery

Yes (1)

No (2)

3.6.2.4 Other: _____

3.7 Which of the following methods of **dust control** in the bakery have you **found useful** in your daily work activities?

3.7.1 Rubbing table with flour?

Yes (1)

No (2)

N/A (3)

3.7.2 Using a sieve to dust table?

Yes (1)

No (2)

N/A (3)

3.7.3 Using oil instead of flour on dough table?

Yes (1)

No (2)

N/A (3)

3.7.4 Cleaning floors with a bristle broom?

Yes (1)

No (2)

N/A (3)

3.7.5 Cleaning floors with a bristle broom and water?

Yes (1)

No (2)

N/A (3)

3.7.6 Cleaning floors using a rubber scraper?

Yes (1)

No (2)

N/A (3)

3.7.7 Cleaning floors using a microfibre mop?

Yes (1)

No (2)

N/A (3)

3.7.8 Cleaning floors with a "spaghetti" mop?

Yes (1)

No (2)

N/A (3)

3.7.9 Cleaning with a vacuum cleaner?

Yes (1)

No (2)

N/A (3)

3.7.10 Using a mixer with a **lid and flap**?

Yes (1)

No (2)

N/A (3)

3.7.11 Starting the mixer on a slow speed?

Yes (1)

No (2)

N/A (3)

Previous jobs in current bakery

4.Have you changed your job since your last interview (2003/2007)?

Yes (1)

No (2)

If NO, skip to question 5

If YES, continue with question 4.1

4.1 What other jobs did you do here since then?

Start with the first job that you changed to and work forward.

No	Job	From	To	Years	Months
1					
2					
3					
4					
5					

Previous work in other bakeries

5. Have you worked in any other bakeries beside Pick 'n Pay?

Yes (1)

No (2)

If NO, skip to Section G

If YES, continue with question 5.1

5.1 What is the total amount of time you have worked in the bakery industry before you started working in this bakery?

Years_____ Months _____

5.2 Why did you change jobs?

_____

_____

G. HEALTH AND SAFETY EDUCATION AND TRAINING

1. Have you had any health and safety education and training on how to protect yourself when working with flour dust?

Yes (1)

No (2)

2.1 If yes, what form of training?

2.1.1 On the job training?

Yes (1)

No (2)

2.1.2 Dust control video presentation

Yes (1)

No (2)

2.1.3 Dust control handbook?

Yes (1)

No (2)

2.1.4 Posters in your bakery

Yes (1)

No (2)

2.1.5 Manager's workshops conducted by UCT

	Yes	(1)
	No	(2)
	N/A	(3)

2.1.6	Other?	Yes	(1)
		No	(2)

Specify:_____

THANK YOU FOR ANSWERING THE QUESTIONNAIRE

Appendix 4: Exhaled nitric oxide /PFT pre-test data collection sheet

UCT OCCUPATIONAL ALLERGY AND ASTHMA STUDY AMONG

BAKERY WORKERS IN THE WESTERN CAPE PROVINCE

OF SOUTH AFRICA - 2011

EXHALED NITRIC OXIDE /PFT PRE-TEST DATA COLLECTION SHEET

Survey Number _____

A. IDENTIFICATION DATA

1.Surname _____

2. First name/s _____

3. Work number _____

4. Date of birth: Day_____Month_____Year____

5. Interviewer's initials _____

6. Date of interview:

Day____Month_____Year_____

7. Bakery: _____

B.HEALTH PROBLEMS

Recent chest infections

1. Have you had the flu or sinusitis in the past 3 weeks?

Yes (1)

No (2)

2. Are you being treated for Tuberculosis (TB)?

Yes (1)

No (2)

2.1 If yes, for how long? _____months _____weeks

If YES, to question no 2, indicate to person that the tests will not be done today. Schedule another appointment in three months time since the start of TB medication.

3. Have you had a heart attack or stroke in the last 3 months?

Yes (1)

No (2)

4. Do you have epilepsy?

Yes (1)

No (2)

5. Have you had any recent operation (in the last 12 months)?

If ***Yes***, what type and how many months ago?

_____ _____months

If **YES** to any of the above **Q3-5**, indicate to the person that the lung function tests will not be done. If NO, proceed with the rest of the screening questions

6.For women:

6.1 Are you Pregnant?	Yes	(1)
	No	(2)
6.2 Are you Breastfeeding?	Yes	(1)
	No	(2)

If **Pregnant**, indicate to the person that the **Lung Function Test will not be done** today, but proceed with NIOX

If **Breastfeeding**, proceed with Lung Function Test with Post-Bronchodilator. Proceed with the rest of the screening questions.

C. ALCOHOL CONSUMPTION

1. Do you drink alcohol?

Yes	(1)
No	(2)

1.1 If yes, when have you last consumed alcohol?

1-2 hours ago	(1)
1 day ago	(2)
1 week ago	(3)

1.2 How much alcohol did you consume?

_____

D. MEDICATION USAGE (show booklet)

1. Are you taking any medicine/s from a doctor or clinic at the moment for asthma, and or hayfever?

Yes (1)

No (2)

1.1 If yes, what are you taking and when last did you take them?

Names No. of hours since last dose

_____ _____

_____ _____ _____

_____ _____ _____

2. Are you taking any medicine/s from a doctor or clinic at the moment for any heart condition, or your eyes?

Yes (1)

No (2)

If short-acting beta-2-agonist or anti-cholinergic inhalers used in the **last 4** hours or long-acting MDI or theophylline used in **last 8** hours, reschedule spirometry and counsel accordingly.

E. GREEN VEGETABLE CONSUMPTION

1. How often do you eat the following vegetable products?

Type of product	**Daily**	**1 to 3 times a week**	**1 to 3 times per month**	**Never**
1.1 Green salad	1	2	3	4
1.2 Spinach & other green leafy vegetables	1	2	3	4

2. When did you last consume green salad and/or spinach/other green leafy vegetables?

1-2 hours ago	(1)
1 day ago	(2)
1 a week ago	(3)

F. PHYSICAL ACTIVITY

1. Do you exercise?

Yes	(1)
No	(2)

2. When was the last time you exercised?

1-2 hours ago	(1)
1 day ago	(2)
1 week ago	(3)

G. SPIROMETRY/LUNG FUNCTION TEST

1. Have you ever had a spirometry/lung function test?

Yes	(1)
No	(2)

2. If yes, when last did you blow into a lung function machine?

1-2 hours ago	(1)
1 day ago	(2)
1 week ago	(3)
> a week ago	(4)

H. RECENT FOOD INTAKE

1. Did you have anything to eat or drink in the last hour?

Yes (1)

No (2)

2. Have you smoked in the last hour?

Yes (1)

No (2)

If YES to above questions, reschedule NIOX test for at least 1 hour later the same day or another date.

Appendix 5: Exhaled nitric oxide data collection sheet

UCT OCCUPATIONAL ALLERGY AND ASTHMA STUDY AMONG BAKERY WORKERS IN THE WESTERN CAPE PROVINCE OF SOUTH AFRICA - 2011

EXHALED NITRIC OXIDE DATA COLLECTION SHEET

Survey Number _____

Date: _____

Time _____

Ambient NO concentration (ppb) _____

Ambient temperature (degrees celcius) _____

1. Subject's blood pressure systolic _____

diastolic _____

2. Subject's age (in years) _____

3.1 Subject's height (in centimetres) _____

3.2 Subject's weight (in kilograms) _____

4. Gender: Male (1)

Female (2)

5. Effort number (start) _____

6.1 FENo measurement (ppb) 1st effort _____

NIOX machine number of efforts completed _____

6.2 FENo measurement (ppb) 2nd effort _____

NIOX machine number of efforts completed _____

7. FENO printout appended		
	Yes	(1)
	No	(2)

Appendix 6: Ethics approval letter

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room E53-46
Groo

Teleph
Email: sumayah
Website: www.health.uct.ac.za/fhs/research

07 September 2017

HREC REF:633/2017

Prof M Jeebhay
Division of Occupational Medicine
School of Public Health & Family Medicine
FHS

Dear Prof Jeebhay

PROJECT TITLE: ASSESSING THE HEALTH IMPACT OF INTERVENTIONS IN SUI BAKERIES USING FRACTIONAL EXHALED NITRIC OXIDE (FeNO) AND OTHER ENDPOINTS FOR BAKER'S ALLERGY AND ASTHMA (MMED CANDIDATE - DR F I STUDY LINKED TO 272/2002

Thank you for submitting your study to the Faculty of Health Sciences Human Researc Committee (HREC) for review.

It is a pleasure to inform you that the HREC has **formally approved** the above-menti

Approval is granted for one year until the 30 September 2018.

Please submit a progress form, using the standardised Annual Report Form if the beyond the approval period. Please submit a Standard Closure form if the study is comp approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/

We acknowledge that the student: - Dr F Al Badri will also be involved in this

Please quote the HREC REF in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** ob Institutional approval, where necessary, before the research may occur.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE

Federal Wide Assurance Number: FWA00001637.

HREC 633/2017

Institutional Review Board (IRB) number: IRB00001938
This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP), South African Good Clinical Practice Guidelines (DoH 2006), based on the Association of the British Pharmaceutical Industry Guidelines (ABPI),and Declaration of Helsinki (2013) guidelines.
The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

Appendix 7: Occupational and Environmental Medicine journal guidelines

BMJ Journals

Occupational & Environmental Medicine

GENERAL INSTRUCTIONS[1]

Occupational and Environmental Medicine is an international peer reviewed journal covering current developments in occupational and environmental health worldwide. Occupational and Environmental Medicine publishes high-quality research relating to the full range of chemical, physical, ergonomic, biological and psychosocial hazards in the workplace and to environmental contaminants and their health effects. The journal welcomes research aimed at improving the evidence-based policy and practice of occupational and environmental research; including the development and application of novel biological and statistical techniques in addition to evaluation of interventions in controlling occupational and environmental risks.

Editorial policy

Occupational and Environmental Medicine adheres to the highest standards concerning its editorial policies on publication ethics, scientific misconduct, consent and peer review criteria. To view all BMJ Journal policies please refer to the BMJ Author Hub policies page.

Articles are published under an exclusive licence (or non-exclusive licence for UK Crown and US Federal Government employees) and authors retain copyright. Articles can also be published under a Creative Commons licence to facilitate reuse of the content; please refer to the Occupational and Environmental Medicine Copyright Author Licence Statement.

Presentation of statistical data

We strongly encourage authors to observe the following guidelines:

- Only essential tables and graphs should be included. Large tables should be kept to a minimum.
- Epidemiological measures of association (e.g. ratios or differences of rates, risks, odds, or prevalences) are preferred for contrasts of disease occurrence.

- Confidence intervals should be reported for measures of association.
- P-values may be reported if necessary for tests such as trend tests or non-parametric tests etc but should be given as quantitative values e.g. p=0.032 rather than relative to a cut point e.g. p<0.05.
- Generally numerical findings should not be reported to more than 1 or 2 decimal places.
- The approach to carrying out any statistical modelling should be described, including strategies for selection of explanatory variables and goodness of fit. The models presented in the paper should be clearly described and justified, with appropriate references given.
- Results from observational studies (cohort, case-control, or cross-sectional designs) should be reported following the guidelines in the STROBE statement, results of randomised trials should be reported following the CONSORT guidelines, and systematic reviews and meta-analyses should follow the PRISMA guidelines.

Article publishing charges

During submission, authors can choose to have their article published open access for 1950 GBP (exclusive of VAT for UK and EU authors). Authors can also choose to publish their article in colour for the print edition – instead of the default option of black and white – for 250 GBP. There are no submission, page or online-only colour figure charges.

For more information on open access, funder compliance and institutional programmes please refer to the BMJ Author Hub open access page.

Submission guidelines

Please review the below article type specifications including the required article lengths, illustrations, table limits and reference counts. The word count excludes the title page, abstract, tables, acknowledgements, contributions and references. Manuscripts should be as succinct as possible.

For further support when making your submission please refer to the resources available on the BMJ Author Hub. Here you can also find general formatting guidelines across BMJ and a formatting checklist.

Original research

Authors should also provide key messages with original research submissions under the following headings:

1. What is already known about this subject?
2. What are the new findings?
3. How might this impact on policy or clinical practice in the foreseeable future?

Word count: up to 3,500

Structured abstract: up to 250 words; ‘Objectives’, ‘Methods’, ‘Results’, ‘Conclusions’

Tables/Illustrations: up to 5

References: up to 40

MANUSCRIPT FORMAT[2]

General instructions

The manuscript must be submitted as a Word document (BMJ Case Reports and Veterinary Record Case Reports request that authors submit using a template which should also be in Word format). PDF is not accepted.

The manuscript should be presented in the following order:

- Title page.
- Abstract, or a summary for case reports (Note: references should not be included in abstracts or summaries).
- Main text separated under appropriate headings and subheadings using the following hierarchy: BOLD CAPS, bold lower case, Plain text, Italics.
- Tables should be in Word format and placed in the main text where the table is first cited. Tables should also be cited in numerical order.
- Acknowledgments, Competing Interests, Funding and all other required statements.
- References. All references should be cited in the main text in numerical order.
  Figures must be uploaded as separate files (view further details under the Figures/illustrations section). All figures must be cited within the main text in numerical order and legends should be provided at the end of the manuscript.
  Online Supplementary materials should be uploaded using the File Designation "Supplementary File" on the submission site and cited in the main text.

Style

Acronyms and abbreviations should be used sparingly and fully explained when first used. Abbreviations and symbols must be standard. SI units should be used throughout, except for blood pressure values which should be reported in mm Hg.
Whenever possible, drugs should be given their approved generic name. Where a proprietary (brand) name is used, it should begin with a capital letter.

Figures/illustrations

Images must be uploaded as separate files. All images must be cited within the main text in numerical order and legends must be provided (ideally at the end of the manuscript).
Video: How to improve your graphs and tables

Colour images and charges

For certain journals, authors of unsolicited manuscripts that wish to publish colour figures in print will be charged a fee to cover the cost of printing. Refer to the specific journal's instructions for authors for more information.
Alternatively, authors are encouraged to supply colour illustrations for online publication and black and white versions for print publication. Colour publication online is offered at no charge, but the figure legend must not refer to the use of colours.
Detailed guidance on figure preparation

File types

Figures should be submitted in TIFF or EPS format. JPEG files are acceptable in some cases. A minimum resolution of 300 dpi is required, except for line art which should be 1200 dpi. Histograms should be presented in a simple, two-dimensional format, with no background grid. For figures consisting of multiple images/parts, please ensure these are submitted as a single composite file for processing. We are unable to accept figures that are submitted as multiple files.
During submission, ensure that the figure files are labelled with the correct File Designation of "Mono Image" for black and white figures and "Colour Image" for colour figures.
Figures are checked using automated quality control and if they are below the minimum standard you will be alerted and asked to resupply them.
Please ensure that any specific patient/hospital details are removed or blacked out (e.g. X-rays, MRI scans, etc). Figures that use a black bar to obscure a patient's identity are NOT accepted

Tables

Tables should be in Word format and placed in the main text where the table is first cited. Tables must be cited in the main text in numerical order. Please note that tables embedded as Excel files within the manuscript are NOT accepted. Tables in Excel should be copied and pasted into the manuscript Word file.
Tables should be self-explanatory and the data they contain must not be duplicated in the text or figures. Any tables submitted that are longer/larger than 2 pages will be published as online only supplementary material.

References

Authors are responsible for the accuracy of cited references and these should be checked before the manuscript is submitted.

Citing in the text

References must be numbered sequentially as they appear in the text. References cited in figures or tables (or in their legends and footnotes) should appear at the end of the reference list to avoid re-numbering if tables and figures are moved around at peer review/proof stage. Reference numbers in the text should be inserted immediately after punctuation (with no word spacing)—for example,[6] not [6].
Where more than one reference is cited, these should be separated by a comma, for example,[1, 4, 39]. For sequences of consecutive numbers, give the first and last number of the sequence separated by a hyphen, for example,[22-25]. References provided in this format are translated during the production process to superscript type, and act as hyperlinks from the text to the quoted references in electronic forms of the article.
Please note that if references are not cited in order the manuscript may be returned for amendment before it is passed on to the Editor for review.

Preparing the reference list

References must be numbered consecutively in the order in which they are mentioned in the text.
Only papers published or in press should be included in the reference list. Personal communications or unpublished data must be cited in parentheses in the text with the name(s) of the source(s) and the year. Authors should request permission from the source to cite unpublished data.

Journals from BMJ use a slightly modified version of Vancouver referencing style (see example below, or download here). Note that The BMJ uses a different style (please see below).

BMJ reference style

List the names and initials of all authors if there are 3 or fewer; otherwise list the first 3 and add ‘et al.’ (The exception is the Journal of Medical Genetics, which lists all authors). Use one space only between words up to the year and then no spaces. The journal title should be in italic and abbreviated according to the style of Medline. If the journal is not listed in Medline then it should be written out in full.
Check journal abbreviations using PubMed
Check citation information using PubMed

Example references

Journal article

13 Koziol-Mclain J, Brand D, Morgan D, et al. Measuring injury risk factors: question reliability in a statewide sample. *Inj Prev* 2000;6:148–50.

Chapter in book

14 Nagin D. General deterrence: a review of the empirical evidence. In: Blumstein A, Cohen J, Nagin D, eds. Deterrence and Incapacitation: Estimating the Effects of Criminal Sanctions on Crime Rates. Washington, DC: National Academy of Sciences 1978:95–139.

Book

15 Howland J. Preventing Automobile Injury: New Findings From Evaluative Research. Dover, MA: Auburn House Publishing Company 1988:163–96.

Abstract/supplement

16 Roxburgh J, Cooke RA, Deverall P, et al. Haemodynamic function of the carbomedics bileaflet prosthesis [abstract]. *Br Heart J* 1995;73(Suppl 2):P37.

Electronic citations

Websites are referenced with their URL and access date, and as much other information as is available. Access date is important as websites can be updated and URLs change. The “date accessed” can be later than the acceptance date of the paper, and it can be just the month accessed.

Electronic journal articles

Morse SS. Factors in the emergency of infectious diseases. *Emerg Infect Dis* 1995 Jan-Mar;1(1). www.cdc.gov/nciod/EID/vol1no1/morse.htm (accessed 5 Jun 1998).

Electronic letters

Bloggs J. Title of letter. *Journal name* Online [eLetter] Date of publication. url eg: Krishnamoorthy KM, Dash PK. Novel approach to transseptal puncture. *Heart* Online [eLetter] 18 September 2001. http://heart.bmj.com/cgi/eletters/86/5/e11#EL1

Legal material

Toxic substances Contro Act: Hearing on S776 Before the Subcommittee of the Environment of the Senate Comm. on Commerce, 94th Congress 1st September (1975).

Washington v Glucksberg 521 US 702 (1997)

Law references

The two main series of law reports, Weekly Law Reports (WLR) and All England Law Reports (All ER) have three volumes a year.
For example:
Robertson v Post Office [1974] 1 WLR 1176
Ashcroft v Mersey Regional Health Authority [1983] 2 All ER 245
R v Clarence [1868] 22 QBD 23
Wimpey Construction UK Ltd v Poole (1984) Times, 3 May

There are good historical precedents for the use of square and round brackets. Since 1891, round ones have referred to the date of the report, square ones to the date of publication of the report. Apart from not italicising the name of the case, we use the lawyers’ style; be careful with punctuation. Here are some more examples:

Caparo Industries plc v Dickman and others [1990] 1 All ER 568-608.
R v Clarence [1888] 22 QBD 23.
Finlayson v HMAdv 1978 SLT (Notes) 60
Block v Martin (1951) 4 DLR 121

Official Journal of the European Communities: at the top of the page it gives the No, vol, and page and, at the other side of the header, the date.
The abbreviation for the title is given in parentheses under the title. Jiggle these elements around to get, eg:

Council Directive of 14 June 1989. Offical Journal of the European Communities No L 1989 June 28:181/44-6. (89/831/EEC.

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Find a DOI

How to cite articles with a DOI before they have appeared in print

1. Alwick K, Vronken M, de Mos T, et al. Cardiac risk factors: prospective cohort study. *Ann Rheum Dis*Published Online First: 5 February 2004. doi:10.1136/ard.2003.001234

How to cite articles with a DOI once they have appeared in print

1. Vole P, Smith H, Brown N, et al. Treatments for malaria: randomised controlled trial. *Ann Rheum Dis*2003;327:765–8 doi:10.1136/ard.2003.001234 [published Online First: 5 February 2002].

PLEASE NOTE: RESPONSIBILITY FOR THE ACCURACY AND COMPLETENESS OF REFERENCES RESTS ENTIRELY WITH THE AUTHOR.

Online only supplementary material

Additional figures and tables, methodology, raw data, etc may be published online only as supplementary material. If your paper exceeds the word count you should consider if any parts of the article could be published online only. Please note that these files will not be copyedited or typeset and will be published as supplied, therefore PDF files are preferred.
All supplementary files should be uploaded using the File Designation "Supplementary File". Please ensure that any supplementary files are cited within the main text of the article.
Some journals also encourage authors to submit translated versions of their abstracts in their local language, which are published online only alongside the English version. These should be uploaded using the File Designation "Abstract in local language".

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REFERENCES

1 Occupational and Environmental Medicine, BMJ Publishing Group. Occupational and environmental medicine authors guidlines. http://oem.bmj.com/pages/authors/#article_publishing_charges (accessed 19 Mar 2018).

2 Occupational and Environmental Medicine BMJ Publishing Group. Formatting paper - BMJ Author Hub. http://authors.bmj.com/writing-and-formatting/formatting-your-paper/ (accessed 19 Mar 2018).

3 EndNote Clarivate Analytics. BMJ modified Vancouver style. http://www.endnote.com/downloads/style/bmj-british-medical-journal (accessed 19 Mar 2018).