

Original article

Nasal and exhaled nitric oxide in response to occupational latex exposure

Background: Latex sensitivity is an increasing problem among health-care workers. Allergic responses are associated with changes in nitric oxide (NO) generation and the changes secondary to latex have not been described.

Methods: A total of 22 subjects comprising equal numbers of control volunteers and subjects with self-reported latex sensitivity were recruited to undergo latex skin prick testing. Symptom scores, exhaled nitric oxide (eNO), and nasal nitric oxide (nNO) were studied 1) before and after a controlled latex challenge ($n=16$), and 2) at the beginning and end of the working week, during exposure to latex ($n=18$).

Results: Latex challenge caused a significant fall in nNO levels in latex-sensitive subjects, compared to normal control subjects ($P=0.04$). eNO levels also decreased in the latex-sensitive subjects after latex challenge, but to a lesser degree. There were no significant differences between the beginning and end of the working week in terms of eNO or nNO in either group, although symptom scores showed a nonsignificant increase in latex-sensitive subjects.

Conclusions: Fall in nasal NO after latex challenge is associated with reported symptomatic latex sensitivity, and this corresponds to latex skin prick test positivity. Neither nNO nor eNO showed a clear relationship to routine workplace exposure.

**K. Tan, C. Bruce, A. Birkhead,
P. S. Thomas**

Faculty of Medicine, University of New South Wales,
and Department of Respiratory Medicine, Prince of
Wales Hospital, Sydney, Australia

Key words: hypersensitivity; latex; nitric oxide.

Dr Paul S. Thomas
Department of Respiratory Medicine
Prince of Wales Hospital
Randwick
Sydney
NSW 2031
Australia

Accepted for publication 19 February 2001

The frequency and duration of wearing latex gloves by health-care workers has increased since the institution of “universal precautions” reflecting concerns about AIDS and hepatitis. As a result, sensitization to latex gloves and their associated chemicals has become an increasing problem in recent years (1–4). The scale of the problem may also be underreported. Two forms of latex allergy are recognized: 1) immediate or type I and 2) delayed or type IV.

Type I hypersensitivity reactions are thought to be less common than type IV and are due mainly to proteins present in the natural rubber latex which give rise to an IgE-mediated immune response (5). Symptoms include urticaria and oedema of the hands, but if the mucous membranes are affected, asthma, nasal congestion, and conjunctivitis may occur (5). In extreme cases, anaphylaxis may result if the proteins come into contact with damaged skin or mucous membranes (5).

Type IV hypersensitivity is thought to be the more common form, and is mainly caused by chemicals added to the latex during the rubber-manufacturing process. The clinical appearance is that of contact dermatitis or eczema with an itchy erythematous rash, particularly on the back of the hands, and vesicles or blisters may be present in severe cases. It occurs several hours after contact with latex and is maximal after 24–48 h.

It is of importance that these reactions occur more frequently with powdered gloves. It has been suggested that this may be because the starch powder used to prevent latex surfaces adhering can readily bind latex proteins, thus acting as a hapten to enhance allergic potential (6). Furthermore, the powder granules with adsorbed proteins can become airborne (as when gloves are taken off), and this may facilitate transmission to and absorption on mucous membranes (6). Sensitized individuals working in the vicinity may also be exposed to the airborne allergens (6). Testing for latex allergy currently includes skin prick tests using aqueous latex extract, patch testing, or *in vitro* testing (e.g., the radioallergosorbent test [RAST] or enzyme-linked immunosorbent assay [ELISA]) (7, 8). The advantage

Abbreviations. nNO: nasal nitric oxide; eNO: exhaled nitric oxide; iNOS: inducible nitric oxide synthase; RAST: radioallergosorbent test; FEV₁: forced expiratory volume in first second; FVC: forced vital capacity; ppb: parts per billion.

of RAST and ELISA is that they detect specific latex IgE. No testing is absolute, however, and a negative test does not necessarily rule out allergy to a particular allergen.

Nitric oxide (NO) is formed by many cells in the human respiratory tract such as epithelial cells, macrophages, and inflammatory cells, and it may be detected in both exhaled air and the nose (9, 10). There is evidence that respiratory tract NO levels increase in the presence of airway inflammation (11) (as occurs in asthma [12, 13], allergic rhinitis [12, 14], and upper respiratory tract infection [15]). Levels decrease in response to anti-inflammatory treatment, such as glucocorticosteroids (12, 13).

We hypothesized that the levels of nasal and exhaled NO in sensitized hospital and research staff would be affected by exposure to airborne latex allergens and would change after both direct challenge and at the end of the working week.

Material and methods

The study protocol was approved by the local ethics committee, and all subjects gave their informed consent.

Subjects

Recruitment was by advertisement in the hospital and university seeking either control subjects or those self-reported as latex sensitive. Eleven self-reported latex-sensitive subjects and 11 normal control subjects were recruited, and classified according to their reported symptoms in response to latex exposure. None of the subjects were using inhaled or oral steroids or antihistamines at the time of the study, and none were suffering from an upper respiratory tract infection. Skin prick tests were performed with a standard panel of antigens including grass pollen and house-dust mite (Bayer, Pymble, NSW, Australia), as well as latex glove preparation (prepared by soaking a powdered latex glove in phosphate-buffered saline) (5). Reactions were classed as positive if the wheal was more than 2 mm in diameter greater than the negative control. Subjects who self-reported sensitivity to latex antigens underwent RAST to latex (K82, Pharmacia, Australian Laboratory Services, Sydney, Australia). Details are shown in Table 1. Two latex-sensitive subjects declined to have a latex challenge, and therefore participated only in study 2.

Protocol

The research was divided into study 1, an active exposure to latex, and study 2, an observational study over a 5-day working week.

Study 1. Sixteen subjects participated in a latex challenge (eight self-reported latex sensitive, eight self-reported normal; subject characteristics are detailed in Table 1).

Study 2. Eighteen subjects participated in the working week exposure arm (nine self-reported latex sensitive, nine self-reported normal).

All subjects completed a symptom questionnaire, which also recorded any relevant medical history. Symptoms assessed subjectively during the past 24 h were given a rating of 0–3 according to degree of severity; they included nasal blockage, sneezing, nasal itching/nose running/post nasal drip, and an overall symptom

assessment, as previously validated (12). Subjects in study 1 (latex challenge) filled in the symptom questionnaire at the beginning and end of the challenge, while those participating in study 2 (working week exposure) filled in the questionnaire at the same time of day at the beginning (Monday) and end (Friday) of the working week.

Measurement of NO

NO was measured by a chemiluminescence analyser (Dasibi Corp, Glendale, CA, USA) sampling with a constant flow of 0.25 l/min. The sensitivity of the analyser was 1 part per billion (ppb). Both exhaled and nasal NO levels were measured in triplicate to determine the mean plateau values. Exhaled NO levels (eNO) were measured by subjects exhaling through a wide-bore Teflon tube connected to the analyser at a rate of 5 l/s for 30 s. This has been shown to generate a positive mouth pressure which closes the soft palate and avoids contamination by nasal NO (16). Nasal NO was measured by introducing the sampling device into one nostril, with subjects holding their breath for 45 s while closing the velum. The sampling device comprised a 0.5-cm diameter tubing connected to the analyser with an attached sponge gasket acting as a seal around the alar nasi. The tubing was placed just inside the nasal cavity such that there was no contact with the mucosa. NO was measured by suction of gas from the nasal cavity at 250 ml/min (12).

Study 1: latex challenge

Latex challenge was based on the method of Baur & Jager (6) in a 7.5 m³ room. Subjects handled up to a maximum of 30 gloves in 30 min by repeatedly gloving/degloving, and using a new glove every minute. The challenge was stopped if the subject developed any one of the

Table 1. Subject characteristics. Subjects 1–8 in each group performed latex challenge, while subjects 3–11 in each group participated in working week study

Latex sensitive	Age/sex (years)	FEV ₁ (% pred)	SPT allergen/latex	RAST (to K82 latex)	Self-reported latex symptoms
1.	22F	3.38 (98)	+/+	0	N
2.	41F	2.97 (101)	+/-	0	N
3.	33M	4.68 (113)	+/+	0	N, E, S
4.	30F	3.28 (106)	+/+	2	N, E, S
5.	24F	2.82 (83)	+/+	2	N
6.	26F	2.65 (75)	+/-	0	N
7.	34F	3.47 (115)	+/+	0	N, E, S
8.	29F	2.00 (56)	+/+	4	N, E, S
9.	43F	–	+/+	nd	N
10.	26F	–	-/-	nd	N, E, R
11.	23F	–	+/+	0	N, E, R

Normal controls	Age/sex (years)	FEV ₁ (% pred)	SPT allergen/latex	RAST (to K82 latex)	Self-reported latex symptoms
1.	22M	5.28 (103)	+/-	nd	–
2.	27M	4.90 (95)	+/-	nd	–
3.	23M	3.71 (88)	+/-	nd	–
4.	42M	4.37 (103)	+/-	nd	–
5.	25F	3.20 (81)	-/-	nd	–
6.	23M	4.91 (102)	+/-	nd	–
7.	40F	2.85 (95)	+/-	nd	–
8.	23F	2.60 (80)	+/-	nd	–
9.	29F	2.60 (83)	-/-	nd	–
10.	37M	3.69 (85)	-/-	nd	–
11.	30M	5.25 (110)	+/-	nd	–

FEV₁: forced expiratory volume in first second; %pred: percent predicted, skin prick test (SPT) allergen: grass, house-dust mite; RAST: radioallergosorbent test, graded 0–6; nd: not done; key to symptoms: N: nasal; E: eyes; R: respiratory; S: skin.

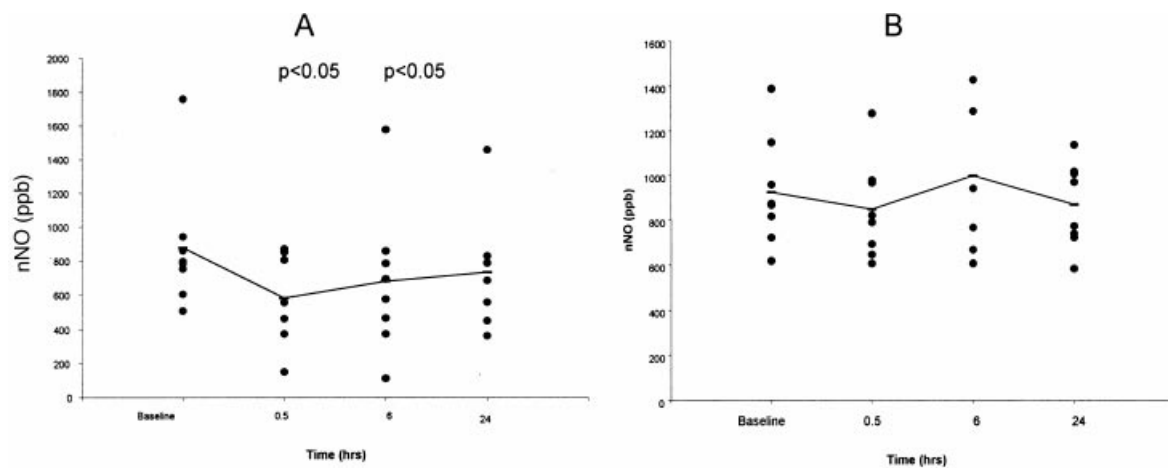


Figure 1. Individual nasal nitric oxide (nNO) values after latex challenge in (A) latex-sensitive and (B) normal subjects. There was significant fall in nNO after latex challenge in sensitive individuals compared to controls ($P < 0.05$, ANOVA; * $P < 0.05$, paired t -test comparing baseline with 0.5- and 6-h time point). Mean values represented by line.

following symptoms: dyspnoea, wheeze, cough, sneezing, hypotension, urticaria, dermatitis, or conjunctivitis; all symptoms were recorded. Spirometry, nNO, and eNO levels were recorded before the challenge, immediately after the challenge, 6 h after the challenge, and approximately 24 h after.

Study 2: working week exposure

Spirometry, nNO, and eNO levels were recorded at the start (Monday morning) and at the end (Friday morning) of the working week. No subject worked during the weekend, and all subjects reported that their working conditions (environment and nature of duties) did not vary significantly from one week to the next. Symptom scores were recorded at each visit.

Statistical analysis

NO data were log transformed to the normal distribution and analysed as percent change from baseline by repeated measures two-way ANOVA, with post hoc Bonferroni corrected t -test. Comparative data were analysed by chi-square test (with Yates' correction), and symptom scores were assessed by Wilcoxon signed rank test. Significance was taken as $P < 0.05$.

Results

Subjects

Sixteen subjects took part in and completed study 1 (latex challenge) and 18 subjects (nine patients, nine controls) took part in study 2 (working week variation). Six of these eight patients, but none of the controls demonstrated positive skin prick tests to latex (Table 1) ($P < 0.02$, chi-square test with Yates' correction coefficient for $n = 16$). All eight of the patients in study 1 consented to undergo latex RAST, which included seven of the nine cases in study 2.

Study 1: latex challenge

Four of the eight patients developed symptoms during the challenge, and so, in accordance with the protocol,

completed the challenge early. One subject developed itching hands, while the others developed rhinitis. Furthermore, one of the self-reported controls developed conjunctivitis-like symptoms at the end of the allergen challenge. It is of note that all those subjects who developed type I-like reactions had recovered by 6 h after challenge, while the subject who developed a type IV reaction was still complaining of itchy hands 24 h later.

In comparison with control subjects, there was a significant fall in nNO level within the first 6 h after latex challenge in the latex-sensitive patients (Fig. 1) ($P = 0.04$, ANOVA). There was no significant change in eNO after latex challenge ($P = 0.85$, ANOVA; Fig. 2), although the mean fall from baseline to the 0.5- and 6-h time points in latex-sensitive individuals was significant with paired t -tests ($P = 0.031$ and 0.016 , respectively, but one subject was unable to perform this test). There was no statistically significant correlation between log eNO and log nNO (Pearson's correlation coefficient: 0.22 , $P = 0.05$). Spirometry did not change.

Study 2: working week exposure

Latex-sensitive subjects had higher symptom scores than the control group at the beginning of the week (median, range: 3, 0–5), scores which increased slightly by the end of the working week (4, 0–7), but were not significant when compared with the control group (baseline median, range: 1, 0–3; end of week: 1, 0–2; $P = 0.09$, Wilcoxon signed-rank test).

There were no significant differences in nNO levels during the working week ($P = 1$, chi-square test, $n = 18$) (Fig. 3). This was also true of eNO levels during the working week ($P = 1$, chi-square test, $n = 18$) (Fig. 4), nor did spirometry change.

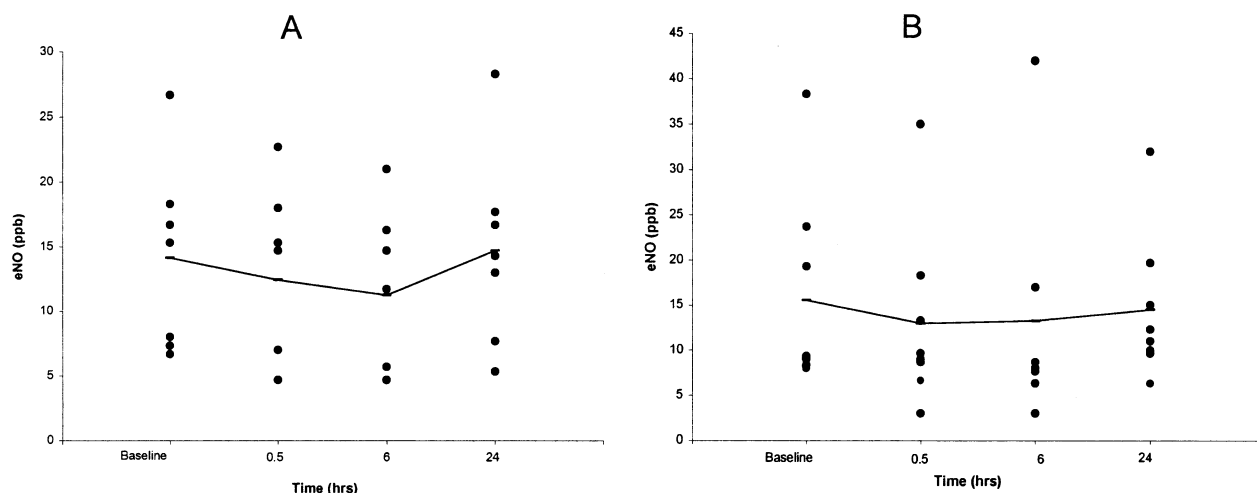


Figure 2. Individual exhaled nitric oxide (eNO) values after latex challenge in (A) latex-sensitive and (B) normal subjects. Fall in eNO was not significant by ANOVA comparing control and latex-sensitive subjects, although analysis by comparison of eNO baseline in latex-sensitive subjects with 0.5- and 6-h time points was significant ($P < 0.05$, paired t -test). Mean values represented by line.

Discussion

Type I latex glove allergy among health-care workers is now well recognized, and there have been several studies to determine its prevalence (1–4). There may be some variation in prevalence between Caucasian and Oriental populations (e.g., 3.3% in Leung et al., 7% in Sinha et al., and 17% in Yassin et al.). Some individuals with demonstrated latex sensitivity (high specific IgE levels and/or positive skin prick test to latex) may also be asymptomatic (2, 3). It has also been shown that individuals with type IV latex allergy (by definition, unlikely to have type I hypersensitivity) may eventually develop type I reactions after continued exposure to latex (5). Those who report only skin reactions suggestive of type IV hypersensitivity to latex may have a positive skin prick test and RAST to latex, which was noted in this study also. Thus, it appears that both types of hypersensitivity may coexist.

The changes in nNO after latex challenge may be explained by the fact that most of the allergen which reaches the respiratory tract would accumulate in the nose, and that any consequent inflammatory response would be most marked in that area. Most NO in the nose is produced in the paranasal sinuses by the inducible form of NO synthase (iNOS), which may be always expressed in human sinus epithelium. NO is an inflammatory marker, and it may be initially surprising that after allergen challenge, levels of nasal NO actually decrease, at least in the short term. Although respiratory tract NO is increased in various inflammatory conditions affecting the respiratory tract, including asthma, allergic rhinitis, and upper respiratory tract infections (11–14), nNO was measured shortly after latex challenge, thus focusing on an acute inflammatory response.

Kharitonov et al. also noted very similar findings, with a fall in nNO in subjects with allergic rhinitis who

were challenged with allergen, despite the fact that it was increased in unchallenged symptomatic rhinitic subjects during the pollen season (12). In addition, Silkoff et al. found a decrease in nasal NO, associated with nasal congestion, after allergen challenge (17). Thus, in an acute inflammatory reaction, nNO levels appear to decrease, whereas in more chronic inflammatory conditions, an increase may be expected. Similar changes have also been noted in eNO in allergic asthmatic subjects after allergen challenge, in that eNO decreased initially and later showed a significant rise (18). Kharitonov et al. suggest two possible explanations (12) for the difference between acute and chronic responses: either inflammatory swelling of the nasal

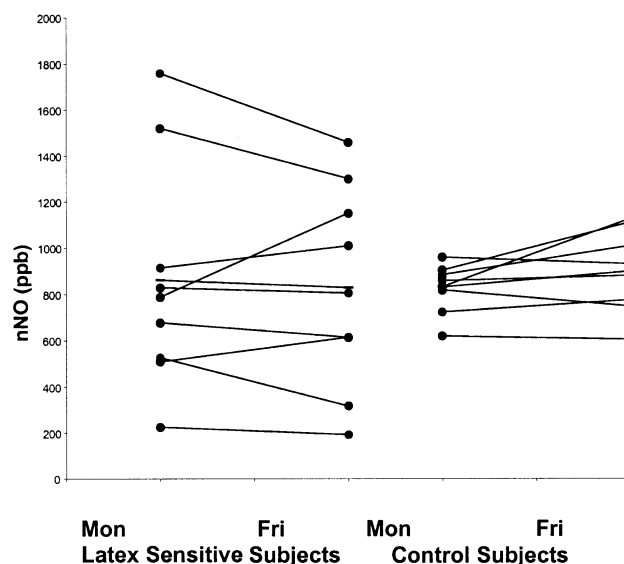


Figure 3. Individual nasal nitric oxide (nNO) levels and mean (—) measured at beginning (Mon) and end (Fri) of working week in latex-sensitive and normal subjects. There were no statistically significant differences.

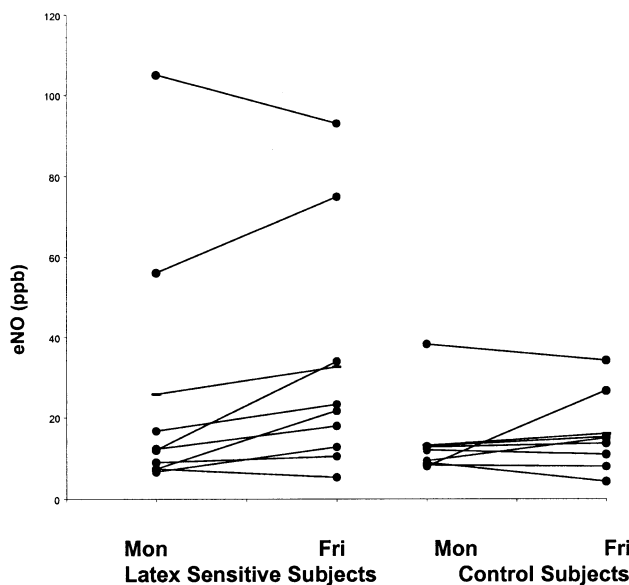


Figure 4. Individual exhaled nitric oxide (eNO) levels and mean (—) measured at beginning (Mon) and end (Fri) of working week in latex-sensitive and normal subjects. There were no statistically significant differences.

mucosa may result in blockage of the orifices, decreasing the contribution from the nasal sinuses, or vasodilatation associated with the acute inflammatory response may increase the removal of NO from the nasal mucosa, because hemoglobin is a powerful ligand for NO.

In this study, allergen challenge caused a fall in nNO in 7/8 cases, but not all noted symptoms. Rhinomanometry might have been a method of objectively assessing the response. It is of interest that Silkoff et al. found that the NO synthase inhibitor L-NAME failed to increase nasal patency despite decreasing nasal NO levels by >30%, suggesting that NO does not mediate nasal congestion (17). However, they did not administer L-NAME after allergen challenge, and it is also possible that the decrease in nasal NO could actually be from “consumption” of NO to cause vasodilatation of the nose. Indeed, in our study, nasal NO levels had to fall by about 50% after latex challenge for nasal symptoms to be noted, a finding which is consistent with the findings of Silkoff et al. (17). Symptoms, and nasal and exhaled NO levels did not appear to vary in a consistent fashion during the working week. This may be related to exposure, since it was difficult to standardize the levels of exposure between subjects.

The latex-allergen preparation for the skin prick test has similar sensitivity values for latex-glove extract to those of Blanco et al. (8). Comparing different modes of

diagnosing latex sensitivity was not the purpose of this small study, but RAST identified fewer sensitive subjects than skin prick tests or a 20% fall in nasal NO levels after allergen challenge (3/8 cases vs 6/8 cases vs 7/8 cases, respectively). This may be because the type and intensity of reactions are not closely related to levels of latex-specific IgE (6), a fact which may explain the low number of RAST positive responses.

An alternative and more controversial explanation for the observed discrepancy is that the hypersensitivity to latex is not exclusively mediated by IgE. Indeed, mast cells, the principal effector cell in type I reactions, degranulate in response to various agents in addition to IgE, namely, the complement components C3a and C5a, Ca^{2+} ionophores, and even tissue damage independent of immune responses. In support of this, Oettgen et al. (19) have shown that IgE-deficient mice can develop acute anaphylactic reactions, and Vage et al. (20) have shown that elutable factors from latex can activate complement.

Another class of agents that may be of relevance are lectins, which have been postulated to underlie autoimmune diseases such as rheumatoid arthritis and coeliac disease (21). Lectins may activate complement in an antibody-independent fashion, and as essentially carbohydrate-complexed structures, it is unlikely that they would induce a humoral immune response, since this relies on peptide presentation in conjunction with class 2 MHC to T cells. Hence, lectins should neither promote immunoglobulin class switching nor induce immunologic memory, both of which are central to IgE-mediated type I reactions. It may thus be important that one of the principal allergens in *Hevea brasiliensis* latex is a lectin.

In conclusion, we have demonstrated that nasal NO decreases after airborne allergen challenge in subjects who are allergic to latex. This suggests that reported latex sensitivity and nasal NO are related. Nasal NO may be a way of monitoring a response to acute latex exposure, but does not appear to be useful for demonstrating variation over the course of a week of exposure at work.

Acknowledgments

We thank our coworkers in the hospital and the University of New South Wales for volunteering and for their time, particularly Cynthia Choi and other members of the Department of Physiotherapy, and also Prof. D. Wakefield and A. Eigenstetter for performing the RAST. This work was supported in part by NH & MRC, the Ramaciotti Foundation, and the Viertel Foundation.

References

1. SINHA A, HARRISON PV. Latex glove allergy among hospital employees: a study in the north-west of England. *Occup Med* 1998;**48**:405–410.
2. YASSIN MS, LIERL MB, FISCHER TJ, O'BRIEN K, CROSS J, STEINMETZ C. Latex allergy in hospital employees. *Ann Allergy* 1994;**72**:245–249.
3. KIBBY T, AKL M. Prevalence of latex sensitization in a hospital employee population. *Ann Allergy Asthma Immunol* 1997;**78**:41–44.
4. LEUNG R, HO A, CHAN J, CHOY D, LAI CK. Prevalence of latex allergy in hospital staff in Hong Kong. *Clin Exp Allergy* 1997;**27**:167–174.
5. AXELSSON JG, JOHANSSON SG, WRANGSJÖ K. IgE-mediated anaphylactoid reactions to rubber. *Allergy* 1987;**42**:46–50.
6. BAUR X, JAGER D. Airborne antigens from latex gloves. *Lancet* 1990;**335**:912.
7. KIM KT, SAFADI GS, SHEIKH KM. Diagnostic evaluation of type I latex allergy. *Ann Allergy Asthma Immunol* 1998;**80**:66–70.
8. BLANCO C, CARILLO T, ORTEGA N, ALVAREZI M, DOMINGUEZ C, CASTILLO R. Comparison of skin prick test and specific serum IgE determination for the diagnosis of latex allergy. *Clin Exp Allergy* 1998;**24**:971–976.
9. DUBOIS AB, DOUGLAS JS, STITT JT, MOHSEENIN V. Production and absorption of nitric oxide gas in the nose. *J Appl Physiol* 1998;**84**:1217–1224.
10. LUNDBERG JO, FARKAS-SZALLASI T, WEITZBERG E, et al. High nitric oxide production in human paranasal sinuses. *Nat Med* 1995;**1**:370–373.
11. BARNES PJ. Nitric oxide and airway disease. *Ann Med* 1995;**27**:389–393.
12. KHARITONOV SA, RAJAKULASINGAM K, O'CONNOR B, DURHAM SR, BARNES PJ. Nasal nitric oxide is increased in patients with asthma and allergic rhinitis and may be modulated by nasal glucocorticoids. *J Allergy Clin Immunol* 1997;**99**:58–64.
13. YATES DH, KHARITONOV SA, THOMAS PS, BARNES PJ. Endogenous nitric oxide is decreased in asthmatics by a specific inhibitor of nitric oxide synthase. *Am J Respir Crit Care Med* 1996;**154**:247–250.
14. ARNAL J-F, DIDIER A, RAMI J, et al. Nasal nitric oxide is increased in allergic rhinitis. *Clin. Exp. Allergy* 1997;**27**:358–262.
15. KHARITONOV SA, YATES DH, BARNES PJ. Increased nitric oxide in exhaled air of normal human subjects with upper respiratory tract infections. *Eur Respir J* 1995;**8**:295–297.
16. KHARITONOV SA, BARNES PJ. Nasal contribution to exhaled nitric oxide during exhalation against resistance or during breath holding. *Thorax* 1997;**52**:540–544.
17. SILKOFF PE, ROTH Y, MCCLEAN P, COLE P, CHAPNIK J, ZAMEL N. Nasal nitric oxide does not control basal nasal patency or acute congestion following allergen challenge in allergic rhinitis. *Ann Otol Rhinol Laryngol* 1999;**108**:368–372.
18. KHARITONOV SA, O'CONNOR BJ, EVANS DJ, BARNES PJ. Allergen-induced late asthmatic reactions are associated with elevation of exhaled nitric oxide. *Am J Respir Crit Care Med* 1995;**151**:1894–1899.
19. OETTGEN HC, MARTIN TR, WYNshaw-BORIS A, DENG C, DRAZEN JM, LEDER P. Active anaphylaxis in IgE-deficient mice. *Nature* 1994;**370**:367–370.
20. VAGE DI, GARRED P, LEA T, MOLLNES TE. Elutable factors from latex-containing materials activate complement and inhibit cell proliferation. An *in vitro* biocompatibility study of medical devices. *Complement Inflamm* 1990;**7**:63–70.
21. FREED DL. Do dietary lectins cause disease? *BMJ* 1999;**318**:1023–1024.