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RESEARCH ARTICLE

Nitrogen dioxide exposure and airway responsiveness in individuals with asthma

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Abstract

Controlled human exposure studies evaluating the effect of inhaled nitrogen dioxide (NO_2) on the inherent responsiveness of the airways to challenge by broncho-constricting agents have had mixed results. In general, existing meta-analyses show statistically significant effects of NO_2 on the airway responsiveness of individuals with asthma. However, no meta-analysis has provided a comprehensive assessment of the clinical relevance of changes in airway responsiveness, the potential for methodological biases in the original papers, and the distribution of responses. This paper provides analyses showing that a statistically significant fraction (i.e. 70% of individuals with asthma exposed to NO_2 at rest) experience increases in airway responsiveness following 30-min exposures to NO_2 in the range of 200 to 300 ppb and following 60-min exposures to 100 ppb. The distribution of changes in airway responsiveness is log-normally distributed with a median change of 0.75 (provocative dose following NO_2 divided by provocative dose following filtered air exposure) and geometric standard deviation of 1.88. About a quarter of the exposed individuals experience a clinically relevant reduction in their provocative dose due to NO_2 relative to air exposure. The fraction experiencing an increase in responsiveness was statistically significant and robust to exclusion of individual studies. Results showed minimal change in airway responsiveness for individuals exposed to NO_2 during exercise.

Keywords

Air pollution, airway responsiveness, asthma, nitrogen dioxide

History

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Background

Epidemiologic studies have demonstrated associations between short-term ambient NO_2 exposure and exacerbation of asthma. Evidence of asthma exacerbation is provided by consistent positive associations between short-term NO_2 exposures and hospital admissions and emergency department visits for asthma in children and individuals of all ages (Iskandar et al., 2012; Jalaludin et al., 2008; Son et al., 2013; Strickland et al., 2010; Villeneuve et al., 2007). Ambient NO_2 exposure is also associated with increases in respiratory symptoms in children with asthma (Delfino et al., 2002, 2003; Mann et al., 2010). Affecting the need for medical treatment, transient increases in airway responsiveness following NO_2 have the potential to increase respiratory symptoms and worsen asthma control. The biological plausibility for epidemiologic associations between ambient NO_2 exposure and asthma exacerbation would be supported by controlled human exposure studies demonstrating increases in airway responsiveness in individuals with asthma following exposure to NO_2. However, these controlled human exposure studies have not consistently demonstrated significant effects of NO_2 exposure on airway responsiveness. The primary purpose of this paper is to analyze data from controlled human exposure studies investigating the effect of NO_2 on airway responsiveness in individuals with asthma.

In the general population, airway responsiveness is log-normally distributed with individuals having airway hyper-responsiveness tending to be those with asthma (Cockcroft et al., 1983; Postma & Boezen, 2004). Along with symptoms, variable airway obstruction, and airway inflammation, airway hyper-responsiveness is a primary feature in the clinical definition and characterization of asthma severity (Reddel et al., 2009). However, not all individuals with asthma experience airway hyper-responsiveness. The range in airway responsiveness among individuals with asthma extends into the range of healthy individuals without asthma (Cockcroft, 2010). In asthma, there is a strong relationship between the degree of non-specific airway responsiveness and the intensity of the early airway response to specific allergens to which individuals have become sensitized (Cockcroft & Davis, 2006a). The bronchoconstrictive response to indirect acting agents (especially specific allergens) can be more difficult to predict and control than the bronchoconstrictive response to non-specific agents that act directly on airway smooth muscle receptors (O'Byrne et al., 2009). Consequently, most of the

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available literature relevant to the evaluation of the effects of NO_2 on airway responsiveness has focused primarily on the responses of individuals with asthma to bronchial challenge with ''non-specific'' bronchoconstricting agents (e.g. methacholine, SO_2, cold air).

Although not well understood, several mechanisms have been proposed by which NO_2 exposure could lead to increases in airway responsiveness. Non-specific agents can directly act (e.g. histamine, carbachol and methacholine) on airway smooth muscle receptors or indirectly act (e.g. exercise, cold air, mannitol) on smooth muscle through intermediate pathways, especially via inflammatory mediators (Cockcroft & Davis, 2006b). Specific allergen challenges (e.g. house dust mite, cat allergen) also act indirectly via inflammatory mediators to initiate smooth muscle contraction and bronchoconstriction. An increase in inflammatory cells, especially mast cells, could explain an increase in responsiveness to allergens. Increases in inflammatory cells, including mast cells, have been demonstrated in humans following NO_2 exposure (Sandström et al., 1990, 1991). Recently, Ezratty et al. (2014) demonstrated increases in eosinophils and eosinophil cationic protein after repeated NO_2 exposures. Increased responsiveness to methacholine in conjunction with increases in eosinophils and eosinophil cationic protein has been reported (Cockcroft & Davis, 2006b). Recent studies have also shown that certain cytokines (e.g. interleukin-17A) are elevated in individuals with asthma and that these cytokines are associated with increased airway smooth muscle contractility and airway responsiveness (Kudo et al., 2012). Whether neurally mediated or via histamine release from mast cells, a bronchoconstrictive effect of NO_2 has been reported following a 20-min resting exposure to 240 ppb NO_2 (Bylin et al., 1985). Bronchoconstriction shifts the deposition site of challenge agents proximally which increases airway responsiveness to both specific and non-specific agents (Casset et al., 2007; Moss & Oldham, 2006; Wanner et al., 1985). Pre-treatment with ascorbic acid has been shown to prevent NO_2-induced increases in airway responsiveness suggesting that oxidative stress may also play a key role in mediating effects (Mohsenin, 1987a).

Three meta-analyses in the peer-reviewed literature have assessed the effects of NO_2 exposure on airway responsiveness in individuals with asthma (Folinsbee, 1992; Goodman et al., 2009; Kjaergaard & Rasmussen, 1996). Kjaergaard & Rasmussen (1996) reported statistically significant effects of NO_2 exposure on the airway responsiveness of subjects with asthma exposed to less than or equal to 300 ppb NO_2, but not for exposures in excess of 300 ppb NO_2. With consideration given to activity level during exposure, Folinsbee (1992) found statistically significant increases in airway responsiveness of subjects with asthma exposed to NO_2 at rest across all concentration ranges (namely, <200 ppb, 200 to 300 ppb and >300 ppb). However, there was no significant effect of NO_2 exposures on responsiveness during exercise. For instance, following exposures between 200 and 300 ppb NO_2, 76% of subjects exposed at rest had increased responsiveness which was statistically significant, whereas only 52% of subjects exposed with exercise had increased responsiveness which was not a statistically significant change. The analyses of Folinsbee (1992) and Kjaergaard & Rasmussen (1996) effectively assessed non-specific responsiveness since few studies of allergen responsiveness were available.

Goodman et al. (2009) provided meta-analyses and meta-regressions evaluating the effects of NO_2 exposure on airway responsiveness in subjects with asthma. Goodman et al. (2009) evaluated changes in three endpoints following NO_2 exposure relative to a control air exposure: (1) the fraction of subjects with asthma experiencing increases in responsiveness, (2) the provocative dose (PD)[1] of the bronchial challenge agent, and (3) the forced expiratory volume in 1 s (FEV_1) response to the challenge agent. Overall, statistically significant effects of NO_2 exposure on each of these three endpoints were observed. Sixty-four percent (95% CI: 58%, 71%) of subjects with asthma exposed at rest to NO_2 experienced an increase in airway responsiveness, whereas there was no effect of NO_2 exposure during exercise with 52% (95% CI: 43%, 60%) having an increase in responsiveness. Additionally, NO_2 exposure resulted in a reduction in PD and increased the FEV_1 decrement following bronchial challenge.

Goodman et al. (2009) concluded that, ''NO_2 is not associated with clinically relevant effects on AHR [airway hyperresponsiveness] at exposures up to 600 ppb based primarily on the small magnitude of effects and the overall lack of exposure-response associations''. Relative to therapeutic agents used to treat airway responsiveness, which may be considered effective if they are more than double the PD for methacholine, the authors concluded that a −50% change in the PD due to NO_2 exposure would be considered adverse. Using the summary statistics provided in individual studies, the effect of NO_2 exposure was a −27% (95% CI: −37%, −18%) change in the PD. Stratifying by rest and exercise exposure, the NO_2-induced changes in PD were −30% (95% CI: −38%, −22%) and −24% (95% CI: −40%, −7%), respectively. Thus, the authors concluded that the effects of NO_2 exposure on airway responsiveness were sufficiently small so as not to be considered adverse. The appropriateness of weighing the deleterious effects of a generally unavoidable ambient exposure using the criteria for judging the efficacy of beneficial therapeutic agents is not clear. Based on the lack of a monotonic increase in responsiveness with exposure, the authors also suggested that NO_2 is not a causal factor. However, as airway responsiveness data is log-normally distributed (Cockcroft et al., 1983; Postma & Boezen, 2004), use of arithmetic mean PD data may affect the validity of some analyses in the Goodman et al. (2009) study. For example, in the study by Bylin et al. (1988) following exposure to 140 ppb NO_2, there was an arithmetic mean 17% increase in the PD relative to filtered air which was driven by a few individuals; whereas, the median and geometric mean show a 24 and 16% decrease, respectively, in the PD following NO_2 relative to filtered air exposure.

None of the above described meta-analyses provided a comprehensive assessment of the clinical relevance of changes in airway responsiveness, the potential for methodological biases in the original papers, and the distribution of responses. This paper provides such analyses. As done by

[1]Commonly, the provocative dose (PD) is the dose of a challenge agent required to produce a 20% reduction in forced expiratory volume in 1 s (PD20) or a 100% increase in specific airway resistance (PD100).

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Folinsbee (1992), the fraction of individuals having an increase in airway responsiveness following NO_2 exposure was assessed. Due to considerable variability in exposure protocols and the potential for this variability in protocols to affect estimates of PD (see ''Discussion'' section for detailed consideration of factors affecting airway responsiveness and dose-response), the magnitude of NO_2-induced changes in PD was not evaluated in the original work by Folinsbee (1992). Herein, the magnitude of the PD change for non-specific agents was evaluated in studies that presented individual subject data for persons with asthma exposed to NO_2 at rest. The focus on resting exposures and non-specific challenges when assessing the magnitude of change in PD was due to the statistically significant effects of NO_2 exposure on airway responsiveness for these conditions (Folinsbee, 1992). In assessing the magnitude of PD change, additional consideration was given to individuals experiencing a doubling-dose change in PD following exposure to NO_2 relative to filtered air. A doubling dose change in PD is recognized as a potential indicator, although not a validated estimate, of clinically relevant changes in airway responsiveness (Reddel et al., 2009). Additional analyses also evaluate the distribution of PD responses to NO_2 and the dose-response relationship.

Methods

Study and data selection

Studies considered for inclusion into the meta-analyses were identified from the meta-analysis by Goodman et al. (2009), the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008), and from a literature search for controlled human exposure studies of individuals with asthma exposed to NO_2 that were published since the 2008 ISA. Only one new experimental study (Riedl et al., 2012) of NO_2 associated changes in airway responsiveness was published since the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008). For inclusion into the meta-analyses, studies were required to provide data on the number of subjects whose airway responsiveness increased or decreased following exposure to NO_2 and filtered air. The location and type of airway responsiveness data extracted from papers using both resting and exercising exposures is provided in Appendix Table A1.

Tables 1 and 2 present studies included in the present meta-analyses. In general, the subjects recruited for these studies ranged in age from 18 to 50 years with the exception of Avol et al. (1989), who studied children aged 8–16 years. The disease status of subjects was mild asthma in most studies, but ranged from inactive asthma up to severe asthma in a few studies.

For studies that assessed airway responsiveness at multiple time points post-exposure or over repeated days of exposure, the data from the first time point and first day of exposure were selected for inclusion in Tables 1 and 2 in an attempt to reduce the heterogeneity among studies. Selection of the earliest time point assessing airway responsiveness was, in part, due to late phase responses (3–8 h post-allergen challenge) being mechanistically different from early phase responses (<30 min post-allergen challenge) (Cockcroft &

Table 1. Resting exposures to NO_2 and airway responsiveness in individuals with asthma.

							Change in AR[a]		Average PD ± SE[b]		
Reference	N	NO_2 (ppb)	Exp. (min)	Challenge type	End point	Time post-exp (min)	+	−	Air	NO_2	p Value[c]
Ahmed et al. (1983a)	20	100	60	CARB	sGaw	NA	13	7	6.0 ± 2.4	2.7 ± 0.8	NA
Ahmed et al. (1983b)	20	100	60	RAG	sGaw	IM	10	8	9.0 ± 5.7	11.7 ± 7.6	n.s.
Hazucha et al. (1983)	15	100	60	METH	sRaw	20	6	7	1.9 ± 0.4	2.0 ± 1.0	n.s.
Orehek et al. (1976)	20	100	60	CARB	sRaw	IM	14	3	0.56 ± 0.08	0.36 ± 0.05	<0.01[d]
Tunnicliffe et al. (1994)	8	100	60	HDM	FEV_1	IM	3	5	−14.62 ΔFEV_1	−14.41 ΔFEV_1	n.s.
Bylin et al. (1988)	20	140	30	HIST	sRaw	25	14	6	0.39 ± 0.07	0.28 ± 0.05	n.s.
Orehek et al. (1976)	4	200	60	CARB	sRaw	IM	3	0	0.60 ± 0.10	0.32 ± 0.02	n.s.
Jörres & Magnussen (1990)	14	250	30	SO_2	sRaw	27	11	2	46.5 ± 5.1	37.7 ± 3.5	< 0.01
Barck et al. (2002)	13	260	30	BIR, TIM	FEV_1	240	5	7	−5 ± 2 ΔFEV_1	−4 ± 2 ΔFEV_1	n.s.
Strand et al. (1997)	18	260	30	BIR, TIM	sRaw	240	9	9	860 ± 450	970 ± 450	n.s.
Strand et al. (1998)	16	260	30	BIR	FEV_1	240	11	4	−0.1 ± 0.8 ΔFEV_1	−2.5 ± 1.0 ΔFEV_1	0.03
Bylin et al. (1988)	20	270	30	HIST	sRaw	25	14	6	0.39 ± 0.07	0.24 ± 0.04	<0.01
Tunnicliffe et al. (1994)	8	400	60	HDM	FEV_1	IM	8	0	−14.62 ΔFEV_1	−18.64 ΔFEV_1	0.009
Bylin et al. (1985)	8	480	20	HIST	sRaw	20	5	0	>30	>20	0.04
Mohsenin (1987b)	10	500	60	METH	pEF	IM	7	2	9.2 ± 4.7	4.6 ± 2.6	0.042
Bylin et al. (1988)	20	530	30	HIST	sRaw	25	12	7	0.39 ± 0.07	0.34 ± 0.08	n.s.

BIR, birch; CARB, carbachol; FEV_1, forced expiratory volume in 1 s; HDM, house dust mite allergen; HIST, histamine; IM, immediately after exposure; METH, methacholine; NA, not available; NO_2, nitrogen dioxide; n.s., less than marginal statistical significance, $p > 0.10$; pEF, partial expiratory flow at 40% vital capacity; RAG, ragweed; SO_2, sulfur dioxide; sGaw, specific airway conductance; sRaw, specific airway resistance; TIM, timothy.

[a]Change in AR: number of individuals showing increased (+) or decreased (−) airway responsiveness after NO_2 exposure compared to air.

[b]PD ± SE, Arithmetic or geometric mean provocative dose (PD) ± standard error (SE). See individual papers for PD calculation and dosage units. ΔFEV_1 indicates the change in FEV_1 response at a constant challenge dose.

[c]Statistical significance of increase in airway responsiveness to bronchial challenge following NO_2 exposure compared to filtered air. Statistical tests varied among studies, e.g. sign test, t-test, analysis of variance.

[d]Statistical significance for all asthmatics from analysis by Dawson & Schenker (1979). Orehek et al. (1976) only tested for differences in subsets of individuals classified as ''responders'' and ''non-responders''.

Table 2. Exercising exposures to NO_2 and airway responsiveness in individuals with asthma.

Reference	N	NO_2 (ppb)	Exp. (min)	Challenge Type	End point	Time post-exp (min)	Change in AR[a] +	Change in AR[a] −	Average PD ± SE[b] Air	Average PD ± SE[b] NO_2	*p* Value[c]
Roger et al. (1990)	19	150	80	METH	sRaw	120	10[d]	7[d]	3.3 ± 0.7	3.1 ± 0.7	n.s.
Kleinman et al. (1983)	31	200	120	METH	FEV_1	IM	20	7	8.6 ± 2.9	3.0 ± 1.1	<0.05
Jenkins et al. (1999)	11	200	360	HDM	FEV_1	IM	6	5	2.94	2.77	n.s.
Jörres & Magnussen (1991)	11	250	30	METH	sRaw	60	6	5	0.41 ± 1.6	0.41 ± 1.6	n.s.
Strand et al. (1996)	19	260	30	HIST	sRaw	30	13	5	296 ± 76	229 ± 56	0.08
Avol et al. (1988)	37	300	120	COLD	FEV_1	60	11[d]	16[d]	−8.4 ± 1.8 ΔFEV_1	−10.7 ± 2.0 ΔFEV_1	n.s.
Avol et al. (1989)	34	300	180	COLD	FEV_1	60	12[d]	21[d]	−5 ± 2 ΔFEV_1	−4 ± 2 ΔFEV_1	n.s.
Bauer et al. (1986)	15	300	30	COLD	FEV_1	60	9	3	0.83 ± 0.12	0.54 ± 0.10	<0.05
Morrow & Utell (1989a)	20	300	240	CARB	FEV_1	30	7[e]	2[e]	3.31 ± 8.64[e] ΔFEV_1	−6.98 ± 3.35[e] ΔFEV_1	n.s.
Roger et al. (1990)	19	300	80	METH	sRaw	120	8[d]	9[d]	3.3 ± 0.7	3.3 ± 0.8	n.s.
Rubinstein et al. (1990)	9	300	30	SO_2	sRaw	60	4	5	1.25 ± 0.23	1.31 ± 0.25	n.s.
Riedl et al. (2012)	15	350	120	METH	FEV_1	90	6	7	7.5 ± 2.6	7.0 ± 3.8	n.s.
Riedl et al. (2012)	15	350	120	CAT	FEV_1	90	4	11	−6.9 ± 1.7 ΔFEV_1	−0.5 ± 1.7 ΔFEV_1	<0.05[f]
Jenkins et al. (1999)	10	400	180	HDM	FEV_1	IM	7	3	3.0	2.78	0.018
Witten et al. (2005)	15	400	180	HDM	FEV_1	IM	8	7	550 ± 240	160 ± 60	n.s.
Avol et al. (1988)	37	600	120	COLD	FEV_1	60	13[d]	16[d]	−8.4 ± 1.8 ΔFEV_1	−10.4 ± 2.2 ΔFEV_1	n.s.
Roger et al. (1990)	19	600	80	METH	sRaw	120	11[d]	8[d]	3.3 ± 0.7	3.7 ± 1.1	n.s.

CARB, carbachol; CAT, cat allergen; COLD, cold-dry air; FEV_1, forced expiratory volume in 1 s; HDM, house dust mite allergen; HIST, histamine; IM, immediately after exposure; METH, methacholine; NO_2, nitrogen dioxide; n.s., less than marginal statistical significance, $p > 0.10$; SO_2, sulfur dioxide; sRaw, specific airway resistance.

[a]Change in AR: number of individuals showing increased (+) or decreased (−) airway responsiveness after NO_2 exposure compared to air.

[b]PD ± SE, Arithmetic or geometric mean provocative dose (PD) ± standard error (SE). See individual papers for PD calculation and dosage units. ΔFEV_1 indicates the change in FEV_1 response at a constant challenge dose.

[c]Statistical significance of increase in airway responsiveness to bronchial challenge following NO_2 exposure compared to filtered air. Statistical tests varied among studies, e.g. sign test, *t*-test, analysis of variance.

[d]Number of individuals having an increase or decrease in AR is from Folinsbee (1992).

[e]Data for 0.25% carbachol challenge from Appendix H of Morrow & Utell (1989b).

[f]Significantly greater ΔFEV_1 in response to a constant challenge dose following exposure to filtered air than NO_2, i.e. a protective effect of NO_2 exposure.

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Davis, 2006b; O'Byrne et al., 2009). It should be noted that Tables 1 and 2 are sorted by NO_2 exposure concentration and, as such, studies that evaluated multiple NO_2 exposure concentrations appear in multiple rows.

Fraction of individuals with NO_2-induced increase in airway responsiveness

Based on the summary data in Tables 1 and 2, the fraction of individuals experiencing a NO_2-induced increase in airway responsiveness was assessed in a manner consistent with the analysis conducted by Folinsbee (1992). Specifically, a two-tailed sign test was used to assess the statistical significance of directional changes in airway responsiveness between the NO_2 and filtered air exposure days. The non-parametric sign test, which assumes only that the responses of each subject are independent and makes no assumptions about the distribution of the response data, is appropriate to test the null hypothesis that observed values have the same probability of being positive or negative. This test allows estimation of whether a significant fraction of individuals experience an increase or decrease in airway responsiveness, but does not provide information on the magnitude of the change in that endpoint. The significance of a two-tailed sign test was calculated in Microsoft® Office Excel® (Redmond, WA) 2013 (subsequently, Excel®) as described by Currell & Dowman (2014).

Magnitude and distribution of NO_2-induced increase in airway responsiveness

Individual subject airway responsiveness data for non-specific challenges following resting exposures to filtered air and NO_2 were available for extraction from five studies (Bylin et al., 1985, 1988; Jörres & Magnussen, 1990; Mohsenin, 1987b; Orehek et al., 1976). Data were obtained for 72 individuals and 116 NO_2 exposures. Twenty individuals in the Bylin et al. (1988) study were exposed to three NO_2 concentrations and four individuals in the Orehek et al. (1976) study were exposed to two NO_2 concentrations. The change in PD (dPD) due to NO_2 for each individual was assessed as:

$$\mathrm{dPD} = \frac{\mathrm{PD}_{\mathrm{NO_2}}}{\mathrm{PD}_{\mathrm{air}}}$$

where, PD_{NO2} and PD_{air} are the PD following NO_2 and air exposures, respectively. Given that airway responsiveness is recognized as being log-normally distributed (Cockcroft et al., 1983; Postma & Boezen, 2004), this method of assessing dPD provides non-negative values for log transformation and plotting.

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The distribution of dPD data (median and geometric standard deviation, GSD) was determined for each study and overall for all subjects. To assess the distribution of dPD, the cumulative percentile for each datum was determined using the Excel® PERCENTRANK function. The lowest and highest dPD were assigned the cumulative probabilities of 0.1 and 99.9% rather than the 0 and 1 assigned by Excel®. Next, the standard normal deviate (z) for each cumulative percentile was determined using the Excel® NORMSINV function. The natural logarithms of the dPD were subsequently regressed against their corresponding z-values using the Excel® INTERCEPT and SLOPE functions. The median equals e (base of natural logarithms, 2.71828) raised to the power of the intercept and the GSD equals e raised to the power of in the slope.

To assess the potential ''adversity'' or clinical relevance of changes in dPD, a sign test was utilized to determine whether there were a statistically greater number of individuals experiencing a doubling dose reduction in dPD (<0.5) versus those having a doubling dose increase in dPD (>2). Sensitivity analyses were performed to ensure that no single study or group of exposures affected the distribution of dPD or the assessment of a doubling dose change. The sensitivity analyses were performed by removing entire studies or repeated subject exposures to multiple concentrations in two studies. Additionally, broad ranges of NO_2 exposure concentrations (e.g. the upper or lower half of the data) were excluded for the sensitivity analyses to see if specific exposure concentrations were driving results. Finally, dose-response was assessed by regressing the logarithms of dPD against NO_2 exposure concentration and against the product of NO_2 exposure concentration and duration using the Excel® Regression Data Analysis Tool.

Results

Fraction of individuals with NO_2-induced increase in airway responsiveness

Tables 1 and 2 provide all studies having data on the fraction of individuals experiencing a change (increase or decrease) in airway responsiveness following both NO_2 and filtered air exposures. The statistical significance reported in the original publications for changes in airway responsiveness following NO_2 exposure compared to filtered air is also provided in these tables. Based on all listed studies, the general tendency of most studies is toward increased airway responsiveness following NO_2 exposure with some studies reaching statistical significance. Fewer studies showed no effect or a tendency for decreased airway responsiveness following NO_2. One study reported a statistically significant decrease in airway responsiveness following NO_2, but the authors attributed the protective effect of NO_2 to chance (Riedl et al., 2012).

Tables 3, 4 and 5 present the fraction of individuals experiencing a NO_2-induced increase in airway responsiveness to non-specific agents, specific allergens and all challenge types, respectively. Footnotes for these tables indicate the groups from Tables 1 and 2 that were included in the analyses. For example, in Table 3, Footnote c, the results for resting exposures (Table 1) to 100 ppb NO_2 are for the 33 individuals having an increase in non-specific responsiveness and the 17 individuals having a decrease in non-specific responsiveness in the studies by Ahmed et al. (1983a), Hazucha et al. (1983) and Orehek et al. (1976).

Table 3 shows statistically significant increases in non-specific airway responsiveness (following resting NO_2 exposures) across all NO_2 concentrations in individuals with asthma. Increases in airway responsiveness were not observed following the exercising exposures to NO_2. In general, Table 4 does not show significant effects of NO_2 exposure on airway responsiveness to allergen challenge, except at NO_2 concentrations over 300 ppb. This may be, in part, due to the small number of individuals in the analysis. Considering both specific and non-specific challenges, Table 5 shows significant effects of NO_2 on airway responsiveness for resting but not exercising exposures as was also shown for non-specific challenges in Table 3. However, given differing mechanisms of effect (see Bronchial Challenge Agent in ''Discussion'' section), preference should be given to the analysis of non-specific responsiveness (Table 3) over the combined analysis of specific and non-specific agents (Table 5).

Magnitude and distribution of NO_2-induced increase in airway responsiveness

The dPD for each individual was calculated as the PD following NO_2 divided by the PD following air exposure. Hence, a dPD greater than one suggests reduced responsiveness, whereas a dPD less than one suggests increased responsiveness following NO_2 exposure. The dPD from the five studies providing individual PD data following resting exposures to NO_2 and filtered air are illustrated in Figure 1. All of the median responses illustrated in Figure 1 show an increased responsiveness following NO_2 exposure, i.e. an NO_2-induced reduction in the PD. It should be noted in Figure 1 that the dPD are on a log scale. The untransformed dPD data from Bylin et al. (1988) and Mohsenin (1987b) were positively skewed with a few individuals having large values of dPD. This results in a large difference between the median dPD and arithmetic mean dPD. For example, at the 140 ppb concentration in the Bylin et al. (1988) study, the median dPD of 0.73 suggests NO_2 increased responsiveness which is consistent with 14 individuals having an increase in responsiveness versus 6 having a decrease, whereas the arithmetic mean dPD of 1.15 suggests a reduction in responsiveness. The untransformed dPD data from Bylin et al. (1985), Jörres & Magnussen (1990), and Orehek et al. (1976) were more symmetric than Bylin et al. (1988) and Mohsenin (1987b). For the full dataset in Figure 1, untransformed dPD had a skew of 3.0 (using Excel® SKEW function), whereas the log-transformed data had a skew of only 0.2.

A clinically relevant change in dPD is indicated by a doubling dose change, i.e. dPD >2 or <0.5. A clinically relevant, doubling dose, NO_2-induced increase in responsiveness (dPD < 0.5) was observed in 24% of the data, while 8% had a double dose decrease in responsiveness (dPD > 2). Of the 28 responses where dPD was <0.5, 17 were from the Bylin et al. (1988) study. Of the nine responses where dPD was >2, eight were again from the Bylin et al. (1988) study. Subject 1 in the Bylin et al. (1988) study had the three highest dPD in Figure 1 which generally reflects the reproducibility

Table 3. Fraction of individuals with asthma having NO_2-induced increase in airway responsiveness to a non-specific challenge.

NO_2 Concentration, ppb	All exposures[a,b]	Exposure with exercise[a,b]	Exposure at rest[a,b]
$[NO_2] = 100$	0.66 (50; $p = 0.033$)	–	0.66 (50; $p = 0.033$)[c]
$100 \leq [NO_2] < 200$	0.66 (87; $p = 0.005$)	0.59 (17; n.s.)[d]	0.67 (70; $p = 0.006$)[e]
$200 \leq [NO_2] \leq 300$	0.59 (199; $p = 0.011$)	0.55 (163; n.s.)[f]	0.78 (36; $p = 0.001$)[g]
$[NO_2] > 300$	0.57 (94; n.s.)	0.49 (61; n.s.)[h]	0.73 (33; $p = 0.014$)[i]
All $[NO_2]$	0.60 (380; $p < 0.001$)	0.54 (241; n.s.)	0.71 (139; $p < 0.001$)

n.s., less than marginal statistical significance ($p > 0.10$).
[a]Data are the fraction of subjects with asthma having an increase in airway responsiveness following NO_2 versus air exposure. Values in parentheses are number of individuals with asthma having a change (+/−) in responsiveness and the p value for a two-tailed sign test.
[b]Analysis is for the 380 subjects with asthma in Tables 1 and 2 having a change (+/−) in non-specific airway responsiveness.
[c]33 increases, 17 decreases; 100 ppb data from Ahmed et al. (1983a), Hazucha et al. (1983) and Orehek et al. (1976).
[d]10 increases, 7 decreases; 150 ppb data from Roger et al. (1990).
[e]47 increases, 23 decreases; 100 ppb data from Ahmed et al. (1983a), Hazucha et al. (1983), and Orehek et al. (1976); 140 ppb data from Bylin et al. (1988).
[f]90 increases, 73 decreases; 200 ppb data from Kleinman et al. (1983); 250 ppb data from Jörres & Magnussen (1991); 260 ppb data from Strand et al. (1996); 300 ppb data from Avol et al. (1988), Avol et al. (1989), Bauer et al. (1986), Morrow & Utell (1989a), Roger et al. (1990), and Rubinstein et al. (1990).
[g]28 increases, 8 decreases; 200 ppb data from Orehek et al. (1976); 250 ppb data from Jörres & Magnussen (1990); 270 ppb data from Bylin et al. (1988).
[h]30 increases, 31 decreases; 350 ppb data from Riedl et al. (2012); 600 ppb data from Avol et al. (1988) and Roger et al. (1990).
[i]24 increases, 9 decreases; 480 ppb data from Bylin et al. (1985); 500 ppb data from Mohsenin (1987b); 530 ppb data from Bylin et al. (1988).

Table 4. Fraction of individuals with asthma having NO_2-induced increase in specific airway responsiveness to an allergen challenge.

NO_2 Concentration, ppb	All exposures[a,b]	Exposure with exercise[a,b]	Exposure at rest[a,b]
$[NO_2] = 100$	0.50 (26; n.s.)	–	0.50 (26; n.s.)[c]
$100 \leq [NO_2] < 200$	0.50 (26; n.s.)	–	0.50 (26; n.s.)[c]
$200 \leq [NO_2] \leq 300$	0.55 (56; n.s.)	0.55 (11; n.s.)[d]	0.56 (45; n.s.)[e]
$[NO_2] > 300$	0.56 (48; n.s.)	0.48 (40; n.s.)[f]	1.00 (8; $p = 0.008$)[g]
All $[NO_2]$	0.55 (130; n.s.)	0.49 (51; n.s.)	0.58 (79; n.s.)

n.s., less than marginal statistical significance ($p > 0.10$).
[a]See Footnote ''a'' of Table 3.
[b]Analysis is for the 130 subjects with asthma in Tables 1 and 2 having a change (+/−) in specific allergen airway responsiveness.
[c]13 increases, 13 decreases; 100 ppb data from Ahmed et al. (1983b) and (Tunnicliffe et al., 1994).
[d]6 increases, 5 decreases; 200 ppb data from Jenkins et al. (1999).
[e]25 increases, 20 decreases; 260 ppb data from Barck et al. (2002), Strand et al. (1997) and Strand et al. (1998).
[f]19 increases, 21 decreases; 350 ppb data from Riedl et al. (2012); 400 ppb data from Jenkins et al. (1999) and Witten et al. (2005).
[g]8 increases, 0 decreases; 400 ppb data from Tunnicliffe et al. (1994).

Table 5. Fraction of individuals with asthma having NO_2-induced increase in airway responsiveness regardless of challenge types.

NO_2 Concentration, ppb	All exposures[a,b]	Exposure during exercise[a,b]	Exposure at rest[a,b]
$[NO_2] = 100$	0.61 (76; $p = 0.08$)	–	0.61 (76; $p = 0.08$)[c]
$100 \leq [NO_2] < 200$	0.62 (113; $p = 0.014$)	0.59 (17; n.s.)[d]	0.63 (96; $p = 0.018$)[e]
$200 \leq [NO_2] \leq 300$	0.58 (255; $p = 0.008$)	0.55 (174; n.s.)[f]	0.65 (81; $p = 0.007$)[g]
$[NO_2] > 300$	0.57 (142; n.s.)	0.49 (101; n.s.)[h]	0.78 (41; $p < 0.001$)[i]
All $[NO_2]$	0.59 (510; $p < 0.001$)	0.53 (292; n.s.)	0.67 (218; $p < 0.001$)

n.s., less than marginal statistical significance ($p > 0.10$).
[a]See Footnote ''a'' of Table 3.
[b]Analysis is for the 510 subjects with asthma in Tables 1 and 2 having a change (+/−) in airway responsiveness.
[c]46 increases, 30 decreases; 100 ppb data from Ahmed et al. (1983a), Ahmed et al. (1983b), Hazucha et al. (1983), Orehek et al. (1976), and Tunnicliffe et al. (1994).
[d]10 increases, 7 decreases; 150 ppb data from Roger et al. (1990).
[e]60 increases, 36 decreases; 100 ppb data from Ahmed et al. (1983a), Hazucha et al. (1983), Orehek et al. (1976); 140 ppb data from Bylin et al. (1988).
[f]96 increases, 78 decreases; 200 ppb data from Kleinman et al. (1983) and Jenkins et al. (1999); 250 ppb data from Jörres & Magnussen (1991); 260 ppb data from Strand et al. (1996); 300 ppb data from Avol et al. (1988), Avol et al. (1989), Bauer et al. (1986), Morrow & Utell (1989a), Roger et al. (1990), and Rubinstein et al. (1990).
[g]53 increases, 28 decreases; 200 ppb data from Orehek et al. (1976); 250 ppb data from Jörres & Magnussen (1990); 260 ppb data from Barck et al. (2002), Strand et al. (1997), and Strand et al. (1998); 270 ppb data from Bylin et al. (1988).
[h]49 increases, 52 decreases; 350 ppb data from Riedl et al. (2012); 400 ppb data from Jenkins et al. (1999) and Witten et al. (2005); 600 ppb data from Avol et al. (1988) and Roger et al. (1990).
[i]32 increases, 9 decreases; 400 ppb data from Tunnicliffe et al. (1994); 480 ppb data from Bylin et al. (1985); 500 ppb data from Mohsenin (1987b); 530 ppb data from Bylin et al. (1988).

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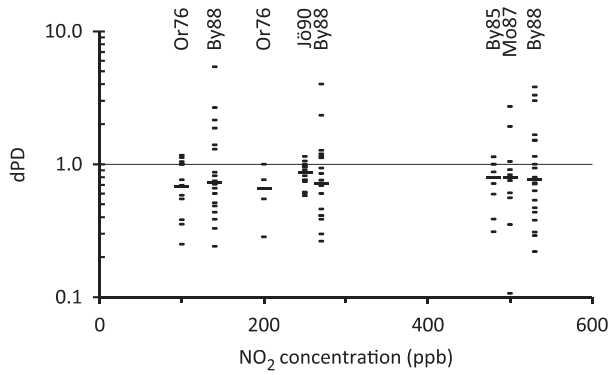

Figure 1. Change in provocative dose (dPD) due to exposure to NO_2 in resting individuals with asthma. Points illustrate the responses of 72 individual subjects and 116 NO_2 exposures. Bars are median responses. Doubling dose changes are illustrated by horizontal dotted lines. Data are from Or76 (Orehek et al., 1976), By88 (Bylin et al., 1988), Jö90 (Jörres & Magnussen, 1990), By85 (Bylin et al., 1985) and Mo87 (Mohsenin, 1987b).

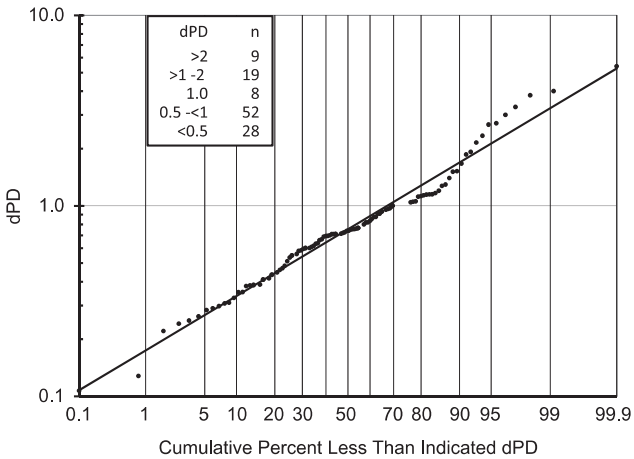

Figure 2. Log-normal distribution of change in provocative dose (dPD) due to exposure to NO_2 in resting individuals with asthma. Data are for 72 individuals and 116 NO_2 exposures illustrated in Figure 1. Line is log-normal fit (0.75, median dPD; 1.88, geometric standard deviation). Table within figure is the number of observations within intervals of dPD. Doubling dose changes are illustrated by horizontal dotted lines. The discontinuity between the 70th and 77th percentiles is due to eight responses equal to one.

of responses. For all subjects in the Bylin et al. (1988) study, the Spearman's rank correlation between the 140 and 530 ppb exposures was 0.56 ($p = 0.01$) and was 0.48 ($p = 0.03$) between the 270 ppb exposure and both the 140 and 530 ppb exposures. Clearly this study has the potential to affect both the assessment of a doubling dose change in dPD as well as the distribution of responses.

Figure 2 illustrates a log-probability plot of the dPD data. The data are log-normally distributed with an estimated (from fitted line on plot) median of 0.75 and a GSD of 1.88. The lowest and highest dPD were assigned the cumulative probabilities of 0.1% and 99.9%. Removing these two values did not affect the median and only slightly reduced the geometric standard deviation from 1.88 to 1.87. Most of the data (namely 69%) suggests a NO_2-induced increase in responsiveness (dPD < 1) due to NO_2 exposure, while 24% of the data suggests decreased responsiveness (dPD > 1). Consistent with the results in Table 3, a two-tailed sign test shows a significant ($p < 0.001$) reduction in the dPD in 74% of the 108 dPD responses not equal to one. Of the 37 dPD having more than a doubling dose change, 76% show a clinically relevant NO_2-induced reduction in dPD ($p = 0.003$; two-tailed sign test).

Table 6 provides sensitivity analyses for the distribution of responses and NO_2-induced increases in responsiveness. The first row of the table provides the results based on all dPD for all 72 individuals and 116 NO_2 exposures in five studies (Bylin et al., 1985, 1988; Jörres & Magnussen, 1990; Mohsenin, 1987b; Orehek et al., 1976). Subsequent rows show results with specific studies excluded. Both Bylin et al. (1988) and Orehek et al. (1976) included multiple exposure concentrations. For rows examining results with exclusion of these two studies, the first row excludes the entire study (all exposure concentrations) with subsequent rows excluding data for specific exposure concentrations from these studies. The last row of study exclusion section in Table 6 provides results excluding all but the lowest exposure concentration from both Bylin et al. (1988) and Orehek et al. (1976). The sensitivity analysis shows that the NO_2-induced increase in airway responsiveness overall and the clinically relevant, doubling dose increase in responsiveness were robust to exclusion of entire studies and subparts of studies with multiple exposures. Also evaluated in this sensitivity analysis, the concentration range of the dataset was split into roughly halves and thirds to determine if effects were more marked for a specific range of concentrations. Dividing the dataset in half, effects were slightly stronger when concentrations >250 ppb were excluded than when concentrations ≤250 ppb were excluded. Dividing the dataset in thirds, effects were least evident when excluding concentrations <480 ppb and doubling dose changes were found only for the lowest concentration range (i.e. >140 ppb excluded), although those doubling dose changes were only marginally significant ($p = 0.057$). These findings suggest more of an NO_2 effect on airway responsiveness following lower concentration exposures.

Using the full dPD dataset of 116 exposures, linear regression did not show an association between log-transformed dPD and either NO_2 concentration ($p = 0.44$) or concentration × exposure duration ($p = 0.89$).

Discussion

The analyses conducted here show the airway responsiveness of individuals with asthma is increased by brief (0.5 to 1 h) exposures to NO_2. There was a statistically significant fraction of individuals with asthma exposed to NO_2 at rest which experienced an increase in responsiveness. About 70% had an increase in non-specific airway responsiveness following 30-min exposures to NO_2 in the range of 200 to 300 ppb and following 60-min exposures to 100 ppb. The median response of these individuals is a NO_2-induced reduction in dPD to 0.75 (1.88, geometric standard deviation). About a quarter of the exposed individuals experienced a clinically relevant, doubling dose reduction in their dPD due to NO_2 exposure. The fraction experiencing a doubling dose increase in responsiveness was also statistically significant and robust to exclusion of individual studies. Results showed

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Table 6. Sensitivity analyses for distribution of responses and NO_2-induced increase in responsiveness to a non-specific challenge.

Population	Distribution[b]	All dPD responses[c]	Doubling dose dPD only[d]
All five studies[a]	0.75 (1.88)	0.74 (108; $p<0.001$)	0.76 (37; $p=0.003$)
Studies excluded from analysis:			
Bylin et al. (1985)[e]	0.76 (1.89)	0.74 (102; $p<0.001$)	0.74 (35; $p=0.006$)
Bylin et al. (1988)			
NO_2 of 140, 270, and 270 ppb[e]	0.70 (1.64)	0.82 (49; $p<0.001$)	0.92 (12; $p=0.006$)
NO_2 of 140 and 270 ppb[f]	0.73 (1.81)	0.76 (68; $p<0.001$)	0.81 (21; $p=0.007$)
NO_2 of 140 and 530 ppb[f]	0.71 (1.72)	0.78 (69; $p<0.001$)	0.85 (20; $p=0.003$)
NO_2 of 270 and 530 ppb[f]	0.73 (1.78)	0.78 (69; $p<0.001$)	0.80 (20; $p=0.012$)
Jörres & Magnussen (1990)[e]	0.74 (1.94)	0.73 (95; $p<0.001$)	0.76 (37; $p=0.003$)
Mohsenin (1987b)[e]	0.76 (1.83)	0.74 (98; $p<0.001$)	0.76 (34; $p=0.003$)
Orehek et al. (1976)			
NO_2 of 100 and 200 ppb[e]	0.80 (1.89)	0.72 (88; $p<0.001$)	0.70 (30; $p=0.043$)
NO_2 of 100 ppb[f]	0.79 (1.89)	0.73 (91; $p<0.001$)	0.71 (31; $p=0.029$)
NO_2 of 200 ppb[f]	0.76 (1.89)	0.73 (105; $p<0.001$)	0.75 (36; $p=0.004$)
Bylin et al. (1988)			
NO_2 of 270 and 530 ppb[f] and Orehek et al. (1976)	0.74 (1.78)	0.77 (66; $p<0.001$)	0.79 (19; $p=0.019$)
NO_2 of 200 ppb[f]			
Concentrations excluded from analysis:			
>140 ppb	0.71 (1.81)	0.77 (37; $p=0.003$)	0.79 (14; $p=0.057$)
≤140 and >270 ppb	0.77 (1.56)	0.78 (36; $p=0.001$)	0.78 (9; n.s.)
<480 ppb	0.78 (1.93)	0.69 (35; $p=0.041$)	0.71 (14; n.s.)
>250 ppb	0.73 (1.71)	0.79 (53; $p<0.001$)	0.80 (15; $p=0.035$)
≤250 ppb	0.77 (1.93)	0.69 (55; $p=0.006$)	0.73 (22, $p=0.052$)

n.s., less than marginal statistical significance ($p>0.10$).

[a]Data are for 72 individuals and 116 NO_2 exposures illustrated in Figures 1 and 2 and from Orehek et al. (1976), Bylin et al. (1988), Jörres & Magnussen (1990), Bylin et al. (1985), and Mohsenin (1987b).

[b]Median (geometric standard deviation) of dPD data.

[c]Data are the fraction of subjects with asthma having an increase in airway responsiveness following NO_2 versus air exposure. Values in parentheses are number of individuals with asthma having a change (+/−) in non-specific airway responsiveness and the p value for a two-tailed sign test.

[d]Data are the fraction of subjects with asthma having a doubling dose reduction in dPD due to NO_2 exposure. Values in parentheses are number of individuals with asthma having at least a doubling dose change (+/−) in non-specific airway responsiveness and the p value for a two-tailed sign test.

[e]Entire study deleted.

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minimal change in airway responsiveness for individuals exposed to NO_2 during exercise. The remainder of this discussion considers a variety of factors that may affect the assessment of airway responsiveness and how those factors may have directionally biased the results of individual studies and the analyses conducted as part of this assessment.

Exercise

In considering why increases in airway responsiveness occurred only after resting exposure to NO_2, Folinsbee (1992) and Bylin (1993) suggested that exercise itself may affect the mechanisms responsible for increased responsiveness. Based on the literature at that time, both of these authors noted that exercise may cause a refractory period during which airway responsiveness to challenge is diminished. Specifically, airway responsiveness to methacholine had been observed to be reduced following exercise (Inman et al., 1990). A more rapid reversal of methacholine-induced bronchoconstriction had also been observed following periods of exercise as compared to rest (Freedman et al., 1988). Additionally, the refractory period from exercise had been found to correlate with the responsiveness to methacholine; i.e. individuals who experienced a smaller bronchoconstrictive response following repeated bouts of exercise subsequently also had a smaller response to methacholine challenge (Magnussen et al., 1986). Recent literature continues to support the possibility that exercise may lead to a period of reduced airway responsiveness. The review by O'Byrne et al. (2009) noted with repeated bouts of exercise, the bronchoconstrictive response to exercise can be abolished in many individuals with asthma. The most probable mechanism explaining this exercise refractory period is the release of inhibitory prostaglandins that partially protect the airways. Refractory periods following exercise of 40 min to 3 h has been reported (Dryden et al., 2010).

A comparison of two studies that utilized the same challenge agent following the same duration of NO_2 exposure and nearly the same exposure concentration supports the conclusion that exercise may diminish the subsequent responsiveness to bronchial challenge. Jörres & Magnussen (1990) found a statistically significant increase in airway responsiveness to a SO_2 challenge in subjects with asthma following exposure to 250 ppb NO_2 for 30 min at rest; whereas, Rubinstein et al. (1990) found no change in responsiveness to a SO_2 challenge following exposure of subjects with asthma to 300 ppb NO_2 for 30 min with 20 min of exercise.

Overall, the literature on airway responsiveness supports the development of a refractory period following bouts of exercise. An effect of exercise refractoriness is consistent with greater increases in airway responsiveness

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following resting than exercising exposures to NO_2 as shown in Table 3.

Bronchial challenge delivery and assessment

Variations in methods for administering the bronchoconstricting agents may substantially affect the results (Cockcroft & Davis, 2006c; Cockcroft et al., 2005). A repeated measures study of 55 subjects with asthma evaluating two ATS, recommended methods of methacholine delivery found a highly significant ($p < 0.00001$), two-fold difference in PC_{20} (concentration producing a 20% reduction in FEV_1), which was attributable to the delivery method (Cockcroft & Davis, 2006c). Even in the same subjects exposed by the same investigators in the same facility to the same bronchial challenge agent, there can be a doubling dose difference due to the delivery method. The difference observed by Cockcroft & Davis (2006c) may, in part, be due to the use of full vital capacity inspirations with breath-hold as part of the delivery technique that yielded the higher PC_{20}. The maximal lung inflations are recognized to induce bronchodilation.

The full vital capacity inspiration required for FEV_1 measurements when assessing airway response to challenge may cause a partial reversal of bronchospasm versus the use of other measures, such as specific airway resistance (sRaw) or conductance (Beaupré & Orehek, 1982; Jackson et al., 2004; Orehek et al., 1981). It is likely that the use of forced vital capacity (FVC) maneuvers contributed to the lack of significant effects in NO_2 studies employing exercising exposures and specific allergen challenges. For non-specific challenges (Table 3), responsiveness was assessed using FVC maneuvers in only 6% of 139 individuals exposed at rest versus 62% of 241 individuals exposed during exercise. For specific allergen challenges (Table 4), responsiveness was assessed using FVC maneuvers in 54% of 79 individuals exposed at rest and 100% of 51 individuals exposed during exercise. Thus, the preferential use of FVC maneuvers in studies exposing individuals to NO_2 during exercise as well as in studies evaluating responsiveness to specific allergens could have contributed to not finding statistically significant effects of NO_2 exposure on airway responsiveness. Where statistically significant effects were observed, generally the studies using resting exposures and non-specific challenge agents, FVC maneuvers were seldom used to assess responsiveness. Consistent with the results in Tables 3 and 4, the use of FVC maneuvers may have biased NO_2 studies using exercise and specific allergen challenges toward the null.

Bronchial challenge agent

Bronchial challenge agents differ in the mechanisms by which they cause bronchoconstriction, acting either ''directly'' or ''indirectly'' on bronchial smooth muscle receptors. Even similarly delivered non-specific, direct acting agents may affect the lung differently. In a comparison of responses to methacholine and histamine in healthy volunteers not having airway hyper-responsiveness, Verbanck et al. (2001) reported that histamine caused an overall narrowing of the airways (i.e. similar between parallel lung regions), whereas methacholine caused a differential narrowing of parallel airways which altered ventilation distribution. The differential effects of these two direct acting agents may, in part, be due to their differing target receptors and the distribution of these receptors in the airways (O'Byrne et al., 2009). Comparison of the airway responsiveness among bronchial challenge agents is complicated by the differing mechanisms by which they initiate bronchoconstriction.

The lack of statistical significance in Table 4 does not necessarily diminish the potential importance of allergen exposures. First, as described above, use of FVC maneuvers in NO_2 studies may have biased results toward not finding an effect on airway responsiveness. Second, 80% of children with asthma are thought to be sensitized to common household allergens (O'Byrne et al., 2009). Third, individuals with asthma may experience an early phase response to allergen challenge with declines in lung function within 30 min which primarily reflects release of histamine and other mediators by airway mast cells; and, approximately half of those having an early phase response also have a late phase response with a decline in lung function 3–8 h after the challenge which reflects enhanced airway inflammation and mucous production (Cockcroft & Davis, 2006b; O'Byrne et al., 2009). The early response may be reversed with bronchodilators; whereas, the late response requires steroidal treatment. Studies have reported NO_2-induced effects on allergen responsiveness for both the early phase (Jenkins et al., 1999; Strand et al., 1998; Tunnicliffe et al., 1994) and late phase (Strand et al., 1998; Tunnicliffe et al., 1994). These effects were observed following 30-min resting exposures to concentrations as low as 260 ppb NO_2. Finally, the response to an allergen is not only a function of the concentration of inhaled allergen, but also the degree of sensitization as measured by the level of allergen-specific IgE and responsiveness to non-specific agents (Cockcroft & Davis, 2006a). These factors make it difficult to predict the level of responsiveness to an allergen, and although rare, severe bronchoconstriction can occur with inhalation of very low allergen concentrations (O'Byrne et al., 2009). Given the ubiquity of allergens and potential severity of responses to allergen inhalation, that NO_2 exposure might augment these responses is of concern.

Subject inclusion/exclusion

Exercise is a major trigger of asthma symptoms in between 60 and 90% of people with asthma (Dryden et al., 2010). In their study of NO_2 effects on airway responsiveness, Roger et al. (1990) reported that all their volunteers with asthma experienced either cold air or exercise-induced bronchoconstriction. Morrow & Utell (1989a) reported that, ''Many of the asthmatic subjects were unable to undertake the carbachol challenge after either NO_2 or air exposures, presumably because of pre-existing exercise-induced bronchoconstriction''. Consequently, in their study, data on changes in airway responsiveness were only available for nine of 20 subjects. Thus, the existence of exercise-induced bronchospasm and symptoms may have caused an underlying difference in the health status of subjects for which airway responsiveness was evaluated between studies utilizing resting versus exercising exposures. Assessing those individuals with less responsive airways could bias results toward not finding an effect of NO_2

on airway responsiveness in studies utilizing exercising exposures.

Medication usage

There was a wide range in restrictions on asthma medication usage among NO_2 studies. It is recommended that short-acting bronchodilators be stopped 8 h and long-acting bronchodilators 36 h before the bronchial challenge (Reddel et al., 2009). Even after withholding salmeterol (a long-acting bronchodilator) for 24 h, there is still a greater than two-fold reduction in airway responsiveness relative to an unmedicated baseline (Reddel et al., 2009). In their NO_2 study, Hazucha et al. (1983) required that subjects should not receive steroid therapy or daily bronchodilator therapy for a month prior to bronchial challenge testing. Other NO_2 study investigators recorded asthma medication usage and asked subjects to refrain from usage for defined periods of time depending on the medication, such as 8 h for short-acting bronchodilators (e.g. Avol et al., 1988; Witten et al., 2005). Restrictions were far less in some studies, for example, Kleinman et al. (1983) asked subjects to withhold bronchodilators for at least 4 h prior to exposure, but subjects were not excluded for non-compliance since medication usage was generally balanced between filtered air and NO_2 exposure days. Still other studies provided no indication of asthma medications or prohibitions for study inclusion (e.g. Bylin et al., 1988). Pre-treatment (500 mg, 4 times per day for 3 days) with ascorbic acid was shown to prevent NO_2-induced increases in airway responsiveness of healthy individuals (Mohsenin, 1987a). Thus, the use of asthma medications or dietary supplements may have reduced the ability of studies to identify an effect of NO_2 on airway responsiveness and may have affected observed provocative doses.

Airway caliber

Bylin (1993) suggested that NO_2 may have a direct effect on airway smooth muscle, possibly relaxing and inducing mild bronchodilation at higher NO_2 doses. Consistent with this supposition, Bylin et al. (1985) reported statistically significant decreases in sRaw following exposure to 480 ppb NO_2 in healthy individuals and a similar trend for sRaw decreases in individuals with asthma. Bronchoconstriction shifts the deposition site of challenge agents proximally, whereas bronchodilation shifts the deposition site more distally. Decreasing the surface dose in the bronchi may in turn decrease the responsiveness to the challenge.

The importance of particle dosimetry (which is affected by factors, such as inhaled particle size, airway dimensions and breathing rates) on airway responsiveness has been investigated in numerous studies. Some of the more conclusive findings are described here. Moss & Oldham (2006) found that the dose of methacholine producing a 200% increase in airway resistance in Balb/c mice and B6C3F1 mice was equivalent in terms of the amount deposited within the first six generations of airways. Wanner et al. (1985) found a strong correlation between the decrease in FEV_1 following histamine challenge and the estimated histamine dose to the airways of 10 smokers ($r = -0.82$, $p < 0.005$) and 10 non-smokers ($r = -0.83$, $p < 0.005$). In a study of 19 individuals with asthma, Casset et al. (2007) found that the PD_{20} (dose causing a 20% reduction in FEV_1) of house dust mite (HDM) allergen decreased with increasing inhaled particle size from 1 to 10 μm (mass median aerodynamic diameter). As inhaled particle size was increased, the pattern of particle deposition would be expected to move toward the larger more central airways. These studies demonstrate lower airway responsiveness for distal versus proximal deposition of challenge agents; and thus, are supportive of the supposition proposed by Bylin (1993).

Simply considering airway caliber may not adequately capture the complexity and anatomical heterogeneity of lung disease from asthma. In a comparison of individuals with asthma and healthy controls, Laube et al. (1992) reported that increasing heterogeneity in particle deposition was significantly associated with decreasing PD_{20} to methacholine. Heterogeneity in deposition is, in part, due to heterogeneity in ventilation distribution. In another study of individuals with asthma, Downie et al. (2007) found heterogeneity in ventilation distribution to be a predictor of airway responsiveness independent of airway inflammation and airway caliber.

The literature supports an effect of the surface dose of challenge agents to the conducting airways on airway responsiveness. The dose of bronchial challenge agents to the conducting airways may have been affected by numerous factors within and among studies evaluating the effect of NO_2 on airway responsiveness. Although it is clear that such factors could contribute to variability within and among studies, the available information is insufficient to support an effect, such as decreased airway responsiveness at higher NO_2 concentrations due to bronchodilation.

Effect of challenge time following NO_2 exposure

With respect to the data in Tables 1 and 2, bronchial challenges were delivered an average of 60 min post-exposure. For non-specific agents, on average, challenges were delivered 16 min following resting exposures and 67 min following exercise exposures ($p < 0.01$). Although challenges may take upwards of 40 min to complete (Mohsenin, 1987b), the difference in the time when challenge agents were delivered could plausibly affect differences in airway responsiveness among studies.

Strand et al. (1996) exposed exercising adults with asthma to 260 ppb NO_2 for 30 min. Responsiveness to histamine was assessed at 30 min, 5 h, 27 h and 7 days post-exposure. The provocative dose causing a 100% increase in specific airway resistance (PD_{100}) tended ($p = 0.08$) to decrease after 30 min, became significantly decreased by 5 h ($p = 0.03$), and returned to baseline by 27 h post-NO_2 exposure compared to filtered air. Although the PD_{100} following NO_2 exposure was fairly constant between 30 min and 5 h, the PD_{100} following filtered air was increased at the 5-h time point, which may have contributed to the significant difference between NO_2 and filtered air after 5 h. This 5-h time point is just beyond reported refractory periods following exercise of 40 min to 3 h (Dryden et al., 2010). A comparison across other NO_2 studies of human subjects for an effect of challenge delivery timing is not possible due to differences in NO_2 concentration and exposure duration. Silbaugh et al. (1981) found a rapid return

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to baseline responsiveness in guinea pigs by 2 h post-exposure.

Although there is strong evidence for a refractory period following exercise and the preferential use of full vital capacity maneuvers which may relax constricted airways in studies using exercise, the existing data on airway responsiveness following NO_2 exposure are insufficient to assess the influence of challenge delivery timing on airway responsiveness in those studies.

Effect of repeated NO_2 exposures

To mimic a daily commute, Strand et al. (1998) exposed adults with asthma on four sequential days to either filtered air or 260 ppb NO_2 for 30 min during rest. The early phase response to allergen challenge was significantly increased by NO_2 exposure; the 4-day mean change in FEV_1 was -2.5 after NO_2 versus -0.4% after air ($p = 0.018$). The late phase response to allergen challenge was also significantly greater after NO_2 with a 4-day average change in FEV_1 of -4.4 after NO_2 versus -1.9% after air ($p = 0.009$). This study suggests that the effect of NO_2 exposure on airway responsiveness to allergen challenge is relatively constant over several contiguous days of repeated NO_2 exposure. Recently, Ezratty et al. (2014) demonstrated increases in eosinophils and eosinophil cationic protein after repeated NO_2 exposures which could increase airway responsiveness. Repeated ambient NO_2 exposures could potentially augment responses observed in the controlled exposure studies.

Extraneous factors

Although some early studies progressively increased NO_2 exposure concentrations for safety purposes, the majority of controlled human exposure studies investigating the effects of NO_2 are of a randomized, controlled, cross-over design in which subjects were exposed, without knowledge of the exposure condition and in random order to clean filtered air (the control) and, depending on the study, to one or more NO_2 concentrations. The filtered air control exposure provides an unbiased estimate of the effects of the experimental procedures on the outcome(s) of interest. Comparison of responses following this filtered air exposure to those following NO_2 exposure allows for estimation of the effects of NO_2 itself on an outcome measurement, while controlling for independent effects of the experimental procedures. Furthermore, the studies by Hazucha et al. (1983) and Strand et al. (1997) provided airway responsiveness data at the time of enrollment in their study and airway responsiveness data following resting exposures to filtered air. Little to no discernible change was observed between airway responsiveness at inclusion and following the resting exposure which suggests that experimental procedures (other than exposure to NO_2) did not affect airway responsiveness.

Dose-response

Folinsbee (1992) noted that greater NO_2 doses occur with exercise due to both the increased ventilation rates and a tendency for increased exposure duration. However, in his meta-analyses, the effects of NO_2 exposure on airway responsiveness were found following resting, but not exercising exposures to NO_2.

The dose-response of NO_2 on airway responsiveness may be modulated by a number of factors that have already been described above. The finding of greater airway responsiveness following exposures at rest than exercise, despite a lower intake dose of NO_2 during the resting exposures, is consistent with an effect of exercise refractoriness. Greater airway responsiveness following exposures at rest than exercise is also consistent with the preferential usage of full vital capacity maneuvers in studies having exercise to assess airways responsiveness. Issues related to subject selection and medication may also have reduced observed effects of NO_2 on airway responsiveness and contributed to variability within and among studies. Both the choice of bronchial challenge agent and method of delivery would have likely contributed to variability among studies. Limited evidence also suggests airway dilation at higher intake doses could reduce airway responsiveness. Overall, the effects of exercise refractoriness, use of vital capacity maneuvers, and potential for some individuals with asthma with exercise-induced bronchoconstriction to be excluded from the evaluation of airway responsiveness appear to be the most likely contributors to not readily finding effects of NO_2 on airway responsiveness at higher intake doses occurring with exercise. Other methodological differences, if randomly occurring, among studies such as the choice of challenge agents, challenge delivery method, severity of disease and asthma medication usage would likely add variability to assessment of airway responsiveness and, thereby, bias data toward the null of no discernible dose-response.

A few studies have investigated the effects of NO_2 exposure on airway responsiveness at more than one concentration. Intra-study evaluation of a potential dose-response reduces the inherent variability and uncertainty occurring with inter-study comparisons. Tunnicliffe et al. (1994) found a significant and larger increase in airway responsiveness at 400 ppb as compared to tendency for increased responsiveness at 100 ppb. Orehek et al. (1976) provided responsiveness data for four individuals following exposure to both 100 and 200 ppb NO_2. Of these four individuals, three had similar PD_{100} between the two exposures, one individual had a doubling difference in the PD_{100} (0.42 mg at 200 ppb versus 0.94 mg at 100 ppb). Bylin et al. (1988) found statistically significant effects of NO_2 on airway responsiveness at 270 ppb, but not at 140 ppb or 530 ppb. These three studies (Bylin et al., 1988; Orehek et al., 1976; Tunnicliffe et al., 1994), for resting exposure to NO_2 provide limited support for increasing airway responsiveness with increasing NO_2 concentration in individuals with asthma. Additionally, conducted as part of this assessment, the regression of individual log-transformed dPD data against dose in terms of both concentration and concentration $\times$ exposure duration did not show a dose-response relationship. The dose-response evidence from studies that used exercising protocols is less compelling. Roger et al. (1990) did not find a change in airway responsiveness at either 150 or 300 ppb NO_2. Jenkins et al. (1999) found significant increases in airway responsiveness to allergens following a 3-h exposure to 400 ppb NO_2, but not following a 6-h exposure to 200 ppb NO_2 despite equivalence

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in terms of the total intake dose (concentration × exposure duration).

Several inter-study differences likely contribute to variability and uncertainty in cross-study comparisons of provocative dose and lung function response to bronchial challenge agents. Evaluation of the proportional change in these outcomes following NO_2 and filtered air exposure as performed by Goodman et al. (2009) and herein should allow for a valid comparison across studies since the air control would, theoretically, adjust for many methodological differences among studies. However, even after this adjustment, clear differences between resting and exercising exposures exist. Exercise itself, the preferential use of full vital capacity maneuvers to assess responsiveness, and exclusion of individuals with exercise-induced bronchospasm would all act to reduce the measured NO_2 effect on airway responsiveness in the studies with exercise. Not using log-transformed data may also affect the validity of statistical analysis requiring homoscedasticity and normally distributed data. It may not be possible to adequately remove the influence of some methodological factors that so substantially affect the airways or the determination of airway responsiveness in individuals with asthma. Thus, it is not clear to what extent inter-study assessments of the dose-response relationship between NO_2 exposure and airway responsiveness are affected by methodological biases of studies. The few studies having evaluated effects at multiple NO_2 concentrations, using resting exposure, are somewhat supportive of a dose-response relationship showing increasing airway responsiveness with increasing NO_2 exposure concentration.

Summary and conclusions

There is a wide range in airway responsiveness due to many factors, including exercise, medications, cigarette smoke, air pollutants, respiratory infections, disease status and respiratory irritants. In the general population, airway responsiveness is log-normally distributed with individuals having asthma generally being more responsive than healthy age-matched controls. Non-specific bronchial challenge agents causing bronchoconstriction may act directly (i.e. histamine, carbachol and methacholine) on airway smooth muscle receptors or act indirectly (i.e. exercise, cold air) through intermediate pathways, especially via inflammatory mediators. Specific challenge agents (i.e. allergens) also act indirectly on smooth muscle to initiate bronchoconstriction.

Likely affecting the observed changes in airway responsiveness due to NO_2 exposure, there are methodological differences among NO_2 studies including subject activity level (rest versus exercise) during NO_2 exposure, asthma medication usage, choice of airway challenge agent (e.g. direct and indirect nonspecific stimuli), method of administering the bronchoconstricting agents, and physiological endpoint used to assess airway responsiveness. Most of these intra-study differences likely contributed to variability and uncertainty in comparison among studies of provocative doses and lung function responses to bronchial challenge agents. A few factors, such as exercise, the use of full vital capacity maneuvers and exclusion of subjects with exercise-induced bronchospasm may have preferentially biased studies toward observing minimal NO_2 effect on airway responsiveness.

The analyses provided in this paper show that in individuals with asthma exposed to NO_2 at rest, statistically significant increases in non-specific airway responsiveness occur in the range of 200 and 300 ppb NO_2 for 30-min exposures and at 100 ppb NO_2 for 60-min exposures. Following exposure to NO_2, relative to filtered air exposure, there was a median decrease of 25% (1.88 geometric standard deviation) in the provocative dose. A clinically relevant, doubling dose increase (halving of the provocative dose) due to NO_2 occurred in a quarter of these individuals with asthma exposed to NO_2 during rest. A sensitivity analysis showed these findings to be robust and not driven by individual studies. Consistent with the majority of studies which did not find statistically significant changes in airways responsiveness when exposing individuals to NO_2 during exercise, the meta-analyses also showed no effect for exposures during exercise. Effects of exercise refractoriness and methodological aspects of these studies likely contributed to not finding effects of NO_2 on airway responsiveness in these studies. Analyses of the available data show clinically relevant and statistically significant effects of NO_2 on the airway responsiveness of individuals with asthma exposed to NO_2 during rest but not exercise.

Declaration of interest

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use. The views expressed in this article are those of the author and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency.

References

Ahmed T, Dougherty R, Sackner MA. (1983a). Effect of 0.1 ppm NO_2 on pulmonary functions and non-specific bronchial reactivity of normals and asthmatics [final report]. (CR-83/11/BI). Warren, MI: General Motors Research Laboratories.

Ahmed T, Dougherty R, Sackner MA. (1983b). Effect of NO_2 exposure on specific bronchial reactivity in subjects with allergic bronchial asthma [final report]. (CR-83/07/BI). Warren, MI: General Motors Research Laboratories.

Avol EL, Linn WS, Peng RC, et al. (1988). Laboratory study of asthmatic volunteers exposed to nitrogen dioxide and to ambient air pollution. Am Ind Hyg Assoc J 49:143–9.

Avol EL, Linn WS, Peng RC, et al. (1989). Experimental exposures of young asthmatic volunteers to 0.3 ppm nitrogen dioxide and to ambient air pollution. Toxicol Ind Health 5:1025–34.

Barck C, Sandstrom T, Lundahl J, et al. (2002). Ambient level of NO_2 augments the inflammatory response to inhaled allergen in asthmatics. Respir Med 96:907–17.

Bauer MA, Utell MJ, Morrow PE, et al. (1986). Inhalation of 0.30 ppm nitrogen dioxide potentiates exercise-induced bronchospasm in asthmatics. Am Rev Respir Dis 134:1203–8.

Beaupré A, Orehek J. (1982). Factors influencing the bronchodilator effect of a deep inspiration in asthmatic patients with provoked bronchoconstriction. Thorax 37:124–8.

Bylin G. (1993). Health risk evaluation of nitrogen oxide: controlled studies on humans. Scand J Work Environ Health 19:37–43.

Bylin G, Hedenstierna G, Lindvall T, Sundin B. (1988). Ambient nitrogen dioxide concentrations increase bronchial responsiveness in subjects with mild asthma. Eur Respir J 1:606–12.

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Bylin G, Lindvall T, Rehn T, Sundin B. (1985). Effects of short-term exposure to ambient nitrogen dioxide concentrations on human bronchial reactivity and lung function. Eur Respir J 66:205–17.

Casset A, Purohit A, Birba E, et al. (2007). Bronchial challenge test in asthmatics sensitized to mites: role of particle size in bronchial response. J Aerosol Med 20:509–18.

Cockcroft DW. (2010). Direct challenge tests: airway hyperresponsiveness in asthma: its measurement and clinical significance [Review]. Chest 138:18S–24S.

Cockcroft DW, Berscheid BA, Murdock KY. (1983). Unimodal distribution of bronchial responsiveness to inhaled histamine in a random human population. Chest 83:751–4.

Cockcroft DW, Davis BE. (2006a). Airway hyperresponsiveness as a determinant of the early asthmatic response to inhaled allergen. J Asthma 43:175–8.

Cockcroft DW, Davis BE. (2006b). Mechanisms of airway hyperresponsiveness [Review]. J Allergy Clin Immunol 118:551–9.

Cockcroft DW, Davis BE. (2006c). The bronchoprotective effect of inhaling methacholine by using total lung capacity inspirations has a marked influence on the interpretation of the test result [Review]. J Allergy Clin Immunol 117:1244–8.

Cockcroft DW, Davis BE, Todd DC, Smycniuk AJ. (2005). Methacholine challenge: comparison of two methods. Chest 127:839–44.

Currell G, Dowman AA. (2014). Sign test (Web). Available from: http://calcscience.uwe.ac.uk/w2/am/Ch12/12_6_SignTest.pdf [Last accessed 30 June 2014].

Dawson SV, Schenker MB. (1979). Health effects of inhalation of ambient concentrations of nitrogen dioxide [Editorial]. Am Rev Respir Dis 120:281–92.

Delfino RJ, Gone H, Linn WS, et al. (2003). Asthma symptoms in Hispanic children and daily ambient exposures to toxic and criteria air pollutants. Environ Health Perspect 111:647–56.

Delfino RJ, Zeiger RS, Seltzer JM, et al. (2002). Association of asthma symptoms with peak particulate air pollution and effect modification by anti-inflammatory medication use. Environ Health Perspect 110: A607–17.

Downie SR, Salome CM, Verbanck S, et al. (2007). Ventilation heterogeneity is a major determinant of airway hyperresponsiveness in asthma, independent of airway inflammation. Thorax 62:684–9.

Dryden DM, Spooner CH, Stickland MK, et al. (2010). Exercise-induced bronchoconstriction and asthma. (AHRQ Publication No. 10-E001). Rockville, MD: Agency for Healthcare Research and Quality.

Ezratty V, Guillossou G, Neukirch C, et al. (2014). Repeated nitrogen dioxide exposures and eosinophilic airway inflammation in asthmatics: a randomized crossover study. Environ Health Perspect 122: 850–5.

Folinsbee LJ. (1992). Does nitrogen dioxide exposure increase airways responsiveness? Toxicol Ind Health 8:273–83.

Freedman S, Lane R, Gillett MK, Guz A. (1988). Abolition of methacholine induced bronchoconstriction by the hyperventilation of exercise or volition. Thorax 43:631–6.

Goodman JE, Chandalia JK, Thakali S, Seeley M. (2009). Meta-analysis of nitrogen dioxide exposure and airway hyper-responsiveness in asthmatics. Crit Rev Toxicol 39:719–42.

Hazucha MJ, Ginsberg JF, McDonnell WF, et al. (1983). Effects of 0.1 ppm nitrogen dioxide on airways of normal and asthmatic subjects. J Appl Physiol 54:730–9.

Inman MD, Watson RM, Killian KJ, O'Byrne PM. (1990). Methacholine airway responsiveness decreases during exercise in asthmatic subjects. Am Rev Respir Dis 141:1414–17.

Iskandar A, Andersen ZJ, Bønnelykke K, et al. (2012). Coarse and fine particles but not ultrafine particles in urban air trigger hospital admission for asthma in children. Thorax 67:252–7.

Jackson AC, Murphy MM, Rassulo J, et al. (2004). Deep breath reversal and exponential return of methacholine-induced obstruction in asthmatic and nonasthmatic subjects. J Appl Physiol 96: 137–42.

Jalaludin B, Khalaj B, Sheppeard V, Morgan G. (2008). Air pollution and ED visits for asthma in Australian children: a case-crossover analysis. Int Arch Occup Environ Health 81:967–74.

Jenkins HS, Devalia JL, Mister RL, et al. (1999). The effect of exposure to ozone and nitrogen dioxide on the airway response of atopic asthmatics to inhaled allergen: dose- and time-dependent effects. Am J Respir Crit Care Med 160:33–9.

Jörres R, Magnussen H. (1990). Airways response of asthmatics after a 30 min exposure, at resting ventilation, to 0.25 ppm NO_2 or 0.5 ppm SO_2. Eur Respir J 3:132–7.

Jörres R, Magnussen H. (1991). Effect of 0.25 ppm nitrogen dioxide on the airway response to methacholine in asymptomatic asthmatic patients. Lung 169:77–85.

Kjaergaard SK, Rasmussen TR. (1996). Clinical studies of effects of nitrogen oxides in healthy and asthmatic subjects. Cent Eur J Public Health 4:23–6.

Kleinman MT, Bailey RM, Linn WS, et al. (1983). Effects of 0.2 ppm nitrogen dioxide on pulmonary function and response to bronchoprovocation in asthmatics. J Toxicol Environ Health 12:815–26.

Kudo M, Melton AC, Chen C, et al. (2012). IL-17A produced by αβ T cells drives airway hyper-responsiveness in mice and enhances mouse and human airway smooth muscle contraction. Nat Med 18: 547–54.

Laube BL, Norman PS, Adams GK. (1992). The effect of aerosol distribution on airway responsiveness to inhaled methacholine in patients with asthma. J Allergy Clin Immunol 89:510–18.

Magnussen H, Reuss G, Jörres R. (1986). Airway response to methacholine during exercise induced refractoriness in asthma. Thorax 41:667–70.

Mann JK, Balmes JR, Bruckner TA, et al. (2010). Short-term effects of air pollution on wheeze in asthmatic children in Fresno, California. Environ Health Perspect 118:1497–502.

Mohsenin V. (1987a). Effect of vitamin C on NO_2-induced airway hyperresponsiveness in normal subjects: a randomized double-blind experiment. Am Rev Respir Dis 136:1408–11.

Mohsenin V. (1987b). Airway responses to nitrogen dioxide in asthmatic subjects. J Toxicol Environ Health 22:371–80.

Morrow PE, Utell MJ. (1989a). Responses of susceptible subpopulations to nitrogen dioxide [HEI]. (ISSN 1041–5505 Research Report 23). Cambridge, MA: Health Effects Institute.

Morrow PE, Utell MJ. (1989b). Responses of susceptible subpopulations to nitrogen dioxide: appendix H. Selected raw data [HEI]. (Research Report 23). Cambridge, MA: Health Effects Institute.

Moss OR, Oldham MJ. (2006). Dosimetry counts: molecular hypersensitivity may not drive pulmonary hyperresponsiveness. J Aerosol Med 19:555–64.

O'Byrne PM, Gauvreau GM, Brannan JD. (2009). Provoked models of asthma: what have we learnt? [Review]. Clin Exp Allergy 39:181–92.

Orehek J, Massari JP, Gayrard P, et al. (1976). Effect of short-term, low-level nitrogen dioxide exposure on bronchial sensitivity of asthmatic patients. J Clin Invest 57:301–7.

Orehek J, Nicoli MM, Delpierre S, Beaupre A. (1981). Influence of the previous deep inspiration on the spirometric measurement of provoked bronchoconstriction in asthma. Am Rev Respir Dis 123: 269–72.

Postma DS, Boezen HM. (2004). Rationale for the Dutch hypothesis. Allergy and airway hyperresponsiveness as genetic factors and their interaction with environment in the development of asthma and COPD [Review]. Chest 126:96S–104S; discussion 159S–161S.

Reddel HK, Taylor DR, Bateman ED, et al.; Exacerbations, ATSERSTFoACa. (2009). An official American Thoracic Society/European Respiratory Society statement: asthma control and exacerbations: standardizing endpoints for clinical asthma trials and clinical practice. Am J Respir Crit Care Med 180:59–99.

Riedl MA, Diaz-Sanchez D, Linn WS, et al. (2012). Allergic inflammation in the human lower respiratory tract affected by exposure to diesel exhaust [HEI] (pp. 5–43; discussion 45–64). (ISSN 1041–5505 Research Report 165). Boston, MA: Health Effects Institute.

Roger LJ, Horstman DH, Mcdonnell W, et al. (1990). Pulmonary function, airway responsiveness, and respiratory symptoms in asthmatics following exercise in NO_2. Toxicol Ind Health 6:155–71.

Rubinstein I, Bigby BG, Reiss TF, Boushey Jr, HA. (1990). Short-term exposure to 0.3 ppm nitrogen dioxide does not potentiate airway responsiveness to sulfur dioxide in asthmatic subjects. Am Rev Respir Dis 141:381–5.

Sandström T, Andersson MC, Kolmodin-Hedman B, et al. (1990). Bronchoalveolar mastocytosis and lymphocytosis after nitrogen dioxide exposure in man: a time-kinetic study. Eur Respir J 3:138–43.

Sandström T, Stjernberg N, Eklund A, et al. (1991). Inflammatory cell response in bronchoalveolar lavage fluid after nitrogen dioxide

exposure of healthy subjects: a dose-response study. Eur Respir J 3: 332–9.

Silbaugh SA, Mauderly JL, Macken CA. (1981). Effects of sulfuric acid and nitrogen dioxide on airway responsiveness of the guinea pig. J Toxicol Environ Health 8:31–45.

Son JY, Lee JT, Park YH, Bell ML. (2013). Short-term effects of air pollution on hospital admissions in Korea. Epidemiology 24: 545–54.

Strand V, Rak S, Svartengren M, Bylin G. (1997). Nitrogen dioxide exposure enhances asthmatic reaction to inhaled allergen in subjects with asthma. Am J Respir Crit Care Med 155:881–7.

Strand V, Salomonsson P, Lundahl J, Bylin G. (1996). Immediate and delayed effects of nitrogen dioxide exposure at an ambient level on bronchial responsiveness to histamine in subjects with asthma. Eur Respir J 9:733–40.

Strand V, Svartengren M, Rak S, et al. (1998). Repeated exposure to an ambient level of NO_2 enhances asthmatic response to a nonsymptomatic allergen dose. Eur Respir J 12:6–12.

Strickland MJ, Darrow LA, Klein M, et al. (2010). Short-term associations between ambient air pollutants and pediatric asthma emergency department visits. Am J Respir Crit Care Med 182:307–16.

Tunnicliffe WS, Burge PS, Ayres JG. (1994). Effect of domestic concentrations of nitrogen dioxide on airway responses to inhaled allergen in asthmatic patients. Lancet 344:1733–6.

U.S. EPA (U.S. Environmental Protection Agency). (2008). Integrated science assessment for oxides of nitrogen Health criteria [EPA Report]. (EPA/600/R-08/071). Research Triangle Park, NC.

Verbanck S, Schuermans D, Noppen M, et al. (2001). Methacholine versus histamine: paradoxical response of spirometry and ventilation distribution. J Appl Physiol 91:2587–94.

Villeneuve PJ, Chen L, Rowe BH, Coates F. (2007). Outdoor air pollution and emergency department visits for asthma among children and adults: a case-crossover study in northern Alberta, Canada. Environ Health Global Access Sci Source 6:40.

Wanner A, Brodnan JM, Perez J, et al. (1985). Variability of airway responsiveness to histamine aerosol in normal subjects. Role of deposition. Am Rev Respir Dis 131:3–7.

Witten A, Solomon C, Abbritti E, et al. (2005). Effects of nitrogen dioxide on allergic airway responses in subjects with asthma. J Occup Environ Med 47:1250–9.

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Appendix

Table A1. Identification of airway responsiveness data in papers.

Study	Data location and type
Ahmed et al. (1983a)	Number of individuals having change in airway responsiveness from p. 10, conclusion 2
Ahmed et al. (1983b)	PD causing 35% decrease in sGaw following NO_2 and air from Table VII
Avol et al. (1988)	Number of individuals having a change in airway responsiveness from Table 1 of Folinsbee (1992)
Avol et al. (1989)	Number of individuals having a change in airway responsiveness from Table 1 of Folinsbee (1992)
Barck et al. (2002)	FEV_1 (% change) to allergen challenge following NO_2 and air from Table 3
Bauer et al. (1986)	PD causing 10% decrease in FEV_1 following NO_2 and air from Figure 3
Bylin et al. (1985)	PD_{100} following NO_2 and air from Table 4; PD_{100} of 0.44 substituted for >0.44 and 64 for >64
Bylin et al. (1988)	PD_{100} following NO_2 and air from Table 2
Hazucha et al. (1983)	Number of individuals having a change in airway responsiveness from text, p. 734, first full paragraph
Jenkins et al. (1999)	PD_{20} following air and NO_2 from Table 5
Jörres & Magnussen (1990)	Provocative ventilation rate of SO_2 following NO_2 and air from Table 3
Jörres & Magnussen (1991)	PC_{100} following NO_2 and air from Table 3
Kleinman et al. (1983)	Number of individuals having a change in airway responsiveness from p. 824, first paragraph
Mohsenin (1987b)	PC of methacholine causing 40% reduction in peak expiratory flow from 60% vital capacity following NO_2 and air from Figure 1
Morrow & Utell (1989a)	FEV_1 (% change) for 0.25% carbachol challenge after NO_2 and air from of Appendix H (PDF p. 10) of Morrow & Utell (1989b)
Orehek et al. (1976)	PD_{100} following 200 ppb NO_2 and air from Figure 1. PD_{100} following 400 ppb NO_2 from text p. 303, right column, first paragraph
Riedl et al. (2012)	PC_{20} for methacholine following NO_2 and air from Table 18. FEV_1 (% change) to cat allergen challenge following NO_2 and air from Table 27
Roger et al. (1990)	Number of individuals having a change in airway responsiveness from Table 1 of Folinsbee (1992)
Rubinstein et al. (1990)	PC for SO_2 causing 8 cm H_20 per L/s increase in sRaw above baseline following NO_2 and air from Table 2
Strand et al. (1996)	PD_{100} at 30 min post-exposure for NO_2 and air from Table 2
Strand et al. (1997)	PD_{100} for allergen following NO_2 and air from Table 2. Histamine data also available but not extracted since this challenge followed the allergen challenge
Strand et al. (1998)	Early phase FEV_1 (% change) for allergen challenge following Day 1 exposure to NO_2 and air from Table 3
Tunnicliffe et al. (1994)	FEV_1 (% change) for allergen challenge following NO_2 and air from Table 3
Witten et al. (2005)	PD_{20} following NO_2 and air from Table 2

FEV_1, forced expiratory volume in 1 s; PC, provocative concentration; PC_{20}, provocative concentration causing 20% decrease in FEV_1; PC_{100}, provocative concentration causing 100% increase in specific airway resistance; PD, provocative dose; PD_{20}, provocative dose causing 20% decrease in FEV_1; PD_{100}, provocative dose causing 100% increase in specific airway resistance; NO_2, nitrogen dioxide; SO_2, sulfur dioxide; sGaw, specific airway conductance; sRaw, specific airway resistance.