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Do Individuals with Asthma Experience Airway Hyper-responsiveness After Exposure to Nitrogen Dioxide?

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Abstract

The current 100 ppb short-term National Ambient Air Quality Standard for NO₂, and EPA's determination of a causal association for respiratory effects, are based in part on controlled human exposure studies evaluating airway hyper-responsiveness (AHR). A meta-analysis by Goodman *et al.* (2009) found increased AHR at 100 ppb NO₂ but no clear concentration-response relationship up to 600 ppb, and an overall lack of an AHR effect for studies involving exercise or exposure to allergens. Several factors have been suggested to explain why effects on AHR are observed while people are at rest, but not during exercise or after exposure to allergens. These include an exercise-induced refractory period; partial reversal of bronchospasm from use of forced expiration maneuvers; and greater airway responsiveness of participants exposed to NO₂ at rest. We reviewed the scientific evidence to determine whether there is biological support for these factors and found that none sufficiently explained the lack of an effect during exercise or after exposure to allergens. In the absence of either a consistent concentration-response or a plausible explanation for the paradoxical AHR findings, the biological significance of these findings is uncertain and provides equivocal support for NO₂ as a causal factor of AHR at these exposure levels.

Keywords: nitrogen dioxide; asthma; airway hyper-responsiveness; national ambient air quality standards; controlled exposure studies; criteria air pollutants

1 Introduction

2
3 The current 1-hour primary National Ambient Air Quality Standard (NAAQS) for nitrogen dioxide (NO₂)
4 – 100 parts per billion (ppb) – is partly based on the results of controlled human exposure studies that
5 evaluated airway hyper-responsiveness (AHR). The United States Environmental Protection Agency's
6 (EPA's) recent Integrated Science Assessment (ISA) for NO₂ also partly relied on the results of these
7 studies for its determination that there is a causal association between short-term NO₂ exposure and
8 respiratory effects (US EPA 2016). These controlled exposure studies evaluated the ability of NO₂ to
9 enhance bronchoconstriction in response to either a non-specific airway challenge (*e.g.*, a
10 pharmacological agent such as carbachol, methacholine, or histamine; sulfur dioxide [SO₂]; or cold air) or
11 to a specific airway challenge (*i.e.*, an allergen).

12
13 A meta-analysis by Goodman *et al.* (2009) found that although AHR was increased at 100 ppb NO₂, there
14 was no clear concentration-response up to 600 ppb, as would be expected if NO₂ were causally associated
15 with AHR. Further, increased AHR following NO₂ exposure was observed in studies in which
16 participants were exposed to NO₂ while at rest but not, paradoxically, in studies in which participants
17 were exposed to NO₂ while exercising. Moreover, increased AHR following NO₂ exposure was generally
18 not observed in studies involving specific airway challenges and studies in which study participants were
19 exposed to NO₂ in a chamber.

20
21 It has been hypothesized that various methodological factors related to study design and AHR assessment
22 limited the ability to detect an effect of NO₂ on AHR (Brown 2015). Below, we explore these factors,
23 specifically in terms of whether they provide plausible explanations regarding findings from studies
24 involving exposure at rest vs. exposure while exercising, and from studies involving exposure to specific
25 airway challenges. For this analysis, we relied on the studies included in the Goodman *et al.* (2009) meta-
26 analysis, as well as an additional study by Reidl *et al.* (2012) that was reviewed by US EPA in its most

recent ISA (US EPA, 2016) and by Brown (2015). Table 1 summarizes 35 exposure scenarios from 26 studies evaluated in this current analysis.

Refractory Period Following Exercise

In contrast to controlled human exposure studies of ozone and SO₂, for which exercise augments effects on pulmonary function (US EPA 2013, 2015b), Goodman *et al.* (2009) reported that NO₂ did not have a significant effect overall on AHR for studies in which participants were exposed to NO₂ while exercising. The paradoxical lack of a significant effect on AHR for studies in which participants were exposed to NO₂ while exercising (vs. at rest) has been attributed to a potential refractory period following exercise (*e.g.*, Brown 2015; Bylin 1993; Folinsbee 1992).

A refractory period following exercise, during which bronchoconstriction to a subsequent bout of exercise is diminished, is an established phenomenon that is likely due to release of inhibitory prostaglandins (O'Byrne *et al.* 2009). It is not clear, however, whether the refractory effect extends to challenges other than exercise. As discussed by McFadden and Gilbert (1994), individuals may be refractory to exercise-induced bronchoconstriction but still maintain responsiveness to other airway challenges. This has been demonstrated in several other studies, in which individuals maintained post-exercise sensitivity to non-specific airway challenges (such as histamine or methacholine) despite being refractory to exercise-induced bronchoconstriction following repeated bouts of exercise (*e.g.*, Boulet *et al.* 1987; Hahn *et al.* 1984; Hamielec *et al.* 1988; Magnussen *et al.* 1986). Although Magnussen *et al.* (1986) found that a diminished airway response to exercise correlated with a diminished response to a methacholine challenge, Magnussen *et al.* (1986) also reported that the methacholine provocative doses (PDs) that caused a 50% decrease in specific airway conductance before and after exercise were not significantly different.

A study by Jorres and Magnussen (1990), which found statistically significant increases in AHR to SO₂ following a 30-minute exposure to 250 ppb NO₂ at rest, and a study by Rubinstein *et al.* (1990), which found no change in responsiveness to SO₂ following 30-minute exposures to 300 ppb NO₂ with 20 minutes of exercise, have been noted as providing support for the hypothesis that an exercise-induced refractory period diminishes the response to an airway challenge (Brown 2015). However, this paradoxical pattern of an AHR effect when study participants were exposed at rest but not while exercising may be a function of the study design. For example, Jorres and Magnussen (1990) exposed study participants *via* a mouthpiece, while Rubinstein *et al.* (1990) used an exposure chamber. As discussed by Paek and McCool (1992), relative to normal oronasal breathing, breathing *via* a mouthpiece increases the minute ventilation, the tidal volume, and the inspiration time, thus increasing both the amount of NO₂ delivered to the lower airways and the time that NO₂ is in contact with the lower airways. Although the overall effect of increased delivery and contact time is not clear, it is likely that airway effects would be enhanced by breathing *via* a mouthpiece relative to normal oronasal breathing. Hence, the significant effect observed by Jorres and Magnussen (1990) could be related, at least in part, to the exposure method used in this study rather than the hypothesized lack of an exercise-induced refractory period.

In further exploring the concept of an exercise-induced refractory period, Inman *et al.* (1990) and Freedman *et al.* (1988) have been cited to support the hypothesis that exercise may diminish the responsiveness to a bronchial challenge agent (Brown 2015). These studies, however, raise an interesting question concerning the impact and importance of when the challenge agent is administered relative to exercise (*i.e.*, before, during, or after). Inman *et al.* (1990) found that the protection afforded by exercise (in terms of reduced bronchoconstriction) increased with the level of exercise, as indicated by a concomitant increase in methacholine concentration required to reduce the forced expiratory volume in 1 second (FEV₁) by 20% (*i.e.*, the provocation concentration causing a 20% decrease in FEV₁, or PC₂₀). Freedman *et al.* (1988) found that methacholine-induced bronchoconstriction was reversed more rapidly

1 following periods of exercise or hyperventilation than following periods of rest. However, published
2 NO₂/AHR studies in which the challenge agent was administered after a period of exercise are not
3 analogous to the studies by Inman *et al.* and Freeman *et al.* in which methacholine was administered
4 during or before exercise, respectively. Thus, results from Inman *et al.* and Freeman *et al.* may not apply
5 to the NO₂/AHR studies. This is further supported by studies in which post-exercise sensitivity to non-
6 specific airway challenges was maintained when the bronchial challenge agent was administered after a
7 period of exercise (*e.g.*, Boulet *et al.* 1987; Hahn *et al.* 1984; Hamielec *et al.* 1988; Magnussen *et al.*
8 1986), which is analogous to the study design used for the NO₂/AHR studies.

9
10 In addition to the aforementioned studies, results from several of the NO₂ AHR studies do not provide
11 evidence of a refractory period for AHR following exercise. Rather, the PD that caused a 100% increase
12 in specific airway resistance (PD₁₀₀) when measured without prior exercise was either comparable to or
13 greater than that measured following exposure to filtered air, with exercise. In the study by Jorres and
14 Magnussen (1991), the PD₁₀₀ (geometric mean $\pm$ standard deviation, as mg/mL methacholine) measured
15 without prior exercise was 0.46 ± 1.47 , vs. 0.41 ± 1.62 measured following exposure to filtered air, with
16 exercise. In the study by Strand *et al.* (1996), the PD₁₀₀ (average $\pm$ standard deviation, as μ g histamine) at
17 study inclusion (without prior exercise) was 251 ± 192 vs. 296 ± 331 vs. following exposure to filtered
18 air, with exercise. In the study by Roger *et al.* (1990), the PD₁₀₀ (average $\pm$ standard error of the mean, as
19 cumulative inhalation units) was significantly greater ($p < 0.05$) when measured at study inclusion.
20 without prior exercise (5.1 ± 1.1) than when measured following exposure to filtered air, with exercise
21 (3.3 ± 0.7).

22
23 Even assuming that some individuals may experience an exercise-induced refractory period to a
24 subsequent airway challenge, and that exercise intensity in the NO₂ AHR studies was sufficient to elicit
25 exercise-induced bronchoconstriction, the refractory effect would not necessarily abolish the AHR effect
26 altogether, but rather would increase the threshold for the effect (*e.g.*, Inman *et al.* 1990). This means that

any effect of exercise would occur on both NO₂ and filtered air exposure days. If AHR in response to an airway challenge is observed following inhalation of filtered air, then one should observe a more pronounced effect following NO₂ exposure if NO₂ were associated with AHR. In fact, AHR (as evidenced by either decreased FEV₁ or airway conductance in response to an airway challenge, or a decrease in the airway challenge doubling dose) was observed in six NO₂ studies that provided pulmonary function data both before and after administration of the airway challenge (*e.g.*, Avol *et al.* 1988, 1989; Bauer *et al.* 1986; Morrow and Utell 1989; Roger *et al.* 1990; Witten *et al.* 2005). Only one of these six studies (Bauer *et al.* 1986) observed a significant difference in AHR following exposure to filtered air vs. NO₂.

Overall, the available evidence does not indicate that the lack of an NO₂ effect on AHR for studies in which individuals were exposed to NO₂ while exercising can be explained, at least in part, by an exercise-induced refractory period.

Use of Deep Inspiration to Deliver Non-Specific Challenge Agents and Assess AHR

Use of deep inspiration to total lung capacity (TLC) has also been hypothesized as a factor that may have contributed to the lack of a significant NO₂ effect on AHR for studies in which participants were exposed to NO₂ while exercising, or for studies evaluating specific airway challenges (Brown 2015). Specifically, airway challenge methods in which delivery of a non-specific airway challenge involves deep inspiration to TLC can result in bronchoprotection for some individuals with asthma, and may thus yield higher provocative doses than methods involving submaximal inhalations or tidal breathing (*e.g.*, Cockcroft and Davis 2005; Cockcroft *et al.* 2006). In addition, studies have shown that a deep inspiration, as is undertaken immediately prior to measuring forced vital capacity (FVC) and FEV₁ (*i.e.*, an FVC maneuver), might cause a partial reversal of bronchospasm in response to an airway challenge (as indicated by a reduction in FEV₁) without a corresponding decrease in airway resistance (*e.g.*, Beaupré

and Orehek 1982; Jackson *et al.* 2004; Orehek *et al.* 1981a). Moreover, deep inspiration was involved (for either delivery of airway challenge or assessing AHR) for a greater percentage of participants exposed to NO₂ while exercising vs. while at rest (72% [154/213] vs. 46% [95/205]), and for a greater percentage of participants exposed to specific vs. non-specific airway challenges (85% [107/126] vs. 49% [142/292]).

If use of deep inspiration to deliver non-specific airway challenges resulted in bronchoprotection for some of the study participants, then studies that delivered airway challenges using deep inspiration should have been less likely to observe increased AHR. To evaluate whether this is the case, we meta-analyzed studies (using STATA 14) to compare the pooled standardized mean differences (SMDs) in AHR across different approaches used to deliver non-specific airway challenges (*i.e.*, deep inspiration vs. tidal breathing or submaximal inhalation) using random-effects models. As shown in Table 2, the AHR response to NO₂ did not differ significantly between studies that used deep inspiration vs. tidal breathing or submaximal inhalation (pooled SMD = 0.239 vs. 0.253; *p* for between-group heterogeneity = 0.943). Thus, it does not appear that studies that delivered non-specific airway challenges were less likely to observe increased AHR.

Using a similar approach, we also conducted meta-analyses to evaluate whether use of FVC maneuvers (*i.e.*, those that assessed AHR using FEV₁) reduced the likelihood of observing increased AHR for studies in which participants were exposed to NO₂ while exercising (listed in Table 3). If it is true that the lack of a significant NO₂ effect on AHR is due to the use of FVC maneuvers to evaluate AHR, we would expect that the use of FVC maneuvers would reduce the likelihood of observing increased AHR. Considering all studies for which participants were exposed to NO₂ while exercising, the AHR response did not differ significantly between studies that assessed AHR using FVC maneuvers vs. those using airway resistance or conductance (pooled SMD = 0.017 vs. 0.033; *p* for between-group heterogeneity = 0.967). When we excluded the exposure scenario from the study by Reidl *et al.* (2012) involving

1 exposure to cat allergen, for which AHR was significantly decreased, the difference in response between
2 studies that assessed AHR using FVC maneuvers vs. airway resistance or conductance remained not
3 significant (p for between-group heterogeneity = 0.262), but AHR was significantly increased for studies
4 that assessed AHR using FVC maneuvers (pooled SMD = 0.23, 95% CI: 0.034-0.426). Thus, although it
5 may be biologically plausible that the use of FVC maneuvers could mask an AHR effect, results from the
6 NO₂/AHR studies for which study participants were exposed to NO₂ while exercising do not indicate that
7 use of FVC maneuvers biased studies towards not finding an AHR effect.

8
9 Only two studies that evaluated specific airway challenges assessed AHR using airway resistance or
10 conductance (Ahmed *et al.* 1983; Strand *et al.* 1997), thus it is not possible to assess whether studies that
11 assessed AHR using airway resistance or conductance were more likely to observe increased AHR than
12 studies that assessed AHR using FVC maneuvers. However, based on findings for studies involving
13 exercise, it does not appear that use of FVC maneuvers biased studies towards not observing increased
14 AHR.

Subject Inclusion/Exclusion Criteria

Another hypothesized explanation for the lack of an NO₂ effect on AHR in studies involving exercise is that participants with more responsive airways may have been excluded, thus biasing results from these studies towards the null (Brown 2015).

To assess whether participants differed with respect to airway responsiveness, we compared baseline airway function based on individuals' percentage of the predicted FEV₁ (% predicted FEV₁), as well as the FEV₁/FVC ratio, both of which are used to classify asthma severity. For example, according to the National Asthma Education and Prevention Program, asthma is classified as mild, moderate, or severe if the % predicted FEV₁ is >80%, 60-80%, or <60%, respectively; or if the FEV₁/FVC ratio is >80%, 75-80%, or <75%, respectively (NIH 2012). If the hypothesis that individuals with more responsive airways may have been excluded from studies involving exercise is correct, we would expect both % predicted FEV₁ at baseline and the FEV₁/FVC ratio to be lower for studies in which participants were exposed to NO₂ while at rest. Percent predicted FEV₁ and FEV₁/FVC ratios are provided in Tables 4a and 4b, for subjects exposed to NO₂ while at rest or while exercising, respectively. For % predicted FEV₁, we based our analysis on % predicted FEV₁, if provided in a study, or calculated it if not, using predicted values from Hankinson (1999) and the study participants' sex, age, and height. Eight studies in which participants were exposed at rest and five studies in which participants were exposed while exercising did not provide either % predicted FEV₁ or age and height information. Based on average % predicted FEV₁ for each study (when available), we found that asthma status was mild and comparable for studies in which participants were exposed to NO₂ at rest (% predicted FEV₁ weighted average of 101 ± 4) and while exercising (% predicted FEV₁ weighted average of 87 ± 3). Only two studies in which participants were exposed to NO₂ at rest provided FEV₁/FVC ratios, of 71% (Jorres and Magnussen 1990) and 72% (Mohsenin *et al.* 1987). These ratios are comparable to the weighted average ratio of 74 ± 5%, for studies in which participants were exposed to NO₂ while exercising.

We also considered asthma severity, which is associated with non-specific airway responsiveness (*e.g.*, Grootendorst and Rabe 2004). As shown in Table 4a, the asthma severity of participants (as classified by the study authors) was mild in all but three studies involving exposures at rest, for which information regarding participants' asthma severity was not provided. Among studies in which participants were exposed while exercising (Table 4b), participants' asthma status was mild in six studies, asymptomatic in two studies, mild to moderate in one study, and moderate to severe in one study. Information regarding asthma severity was not provided in the remaining two studies in which participants were exposed while exercising. Thus, based on asthma status as classified by the study authors, asthma severity, and by extension airway responsiveness, was comparable for studies in which participants were exposed to NO₂ while at rest vs. while exercising. Although asthma severity was comparable for studies in which participants were exposed to NO₂ while at rest vs. while exercising, AHR could not be assessed for half of the participants in the study by Morrow and Utell (1989), which the authors presumed is due to preexisting exercise-induced bronchoconstriction. Thus, individuals with more severe asthma may not have been assessed in the study by Morrow and Utell.

With the exception of the study by Morrow and Utell (1989), there is no indication that participants in studies involving exposure to NO₂ while at rest were more responsive to airway challenges. Hence, the lack of a significant NO₂ effect on AHR for studies in which participants were exposed to NO₂ while exercising does not seem to be explained by preferential inclusion of participants with less responsive airways in these studies.

Medication Usage

As discussed by Brown (2015), insufficient withholding times for asthma medication may have reduced airway responsiveness, and thus reduced the ability of studies to detect an effect of NO₂ exposure on

AHR. We thus assessed whether the lack of an NO₂ effect on AHR in studies involving exercise might be related to shorter medication withholding time prior to the participants' exposure, which may have masked or blunted the effect of NO₂.

To assess whether differences in medication withholding time prevented the observation of NO₂-induced AHR in studies involving exercise, we assigned studies as having a sufficient, borderline, or insufficient withholding time, according to the type of medication used by the study participants. Adequate information was provided so that we could assign studies according to withholding time for 14 studies in which participants were exposed to NO₂ while at rest and 14 studies in which participants were exposed to NO₂ while exercising. Based on duration of action, we considered withholding times of 6 and 24 hours to be sufficient for short- and long-acting β 2-agonists, respectively (Cazzola *et al.* 2012; Currie *et al.* 2013; Palmqvist *et al.* 1997; Tamm *et al.* 2012), and a withholding time of 48 hours to be sufficient for corticosteroids (Tamm *et al.* 2012). We considered withholding times to be borderline for one study that withheld β 2-agonists for 6 hours without specifying whether the β 2-agonists were short- or long-acting, and for seven studies that withheld β 2-agonists for 8 hours without specifying whether the β 2-agonists were short- or long-acting. Withholding times were also considered to be borderline for two studies that withheld corticosteroids with relatively short half-lives (approximately 3 hours) on the day of exposure.

If the lack of an NO₂ effect on AHR in studies involving exercise is related to shorter medication withholding times, then we would expect that withholding times are more likely insufficient or borderline for studies in which participants were exposed to NO₂ while exercising. Although the numbers in each category are small, and there are a few for which information is missing, as shown in Table 5, the distribution of studies according to medication withholding time was similar (p value for homogeneity = 1.0) for studies in which participants were exposed to NO₂ at rest vs. while exercising. This cursory analysis suggests that shorter or insufficient medication withholding times did not contribute to the

1 difference in NO₂-induced AHR for studies in which participants were exposed to NO₂ while exercising
2 vs. at while at rest.

3
4 Brown (2015) also indicated that use of dietary supplements such as vitamin C, which may prevent NO₂-
5 induced AHR, may have been more prevalent in studies involving exercise. However, information on
6 vitamin C intake is not provided for the NO₂/AHR studies, and thus it is not possible to assess the extent
7 to which vitamin C intake differed between studies in which participants were exposed to NO₂ while
8 exercising vs. at rest.

9 10 **Conclusions**

11
12 It has been hypothesized that several factors may have biased studies towards not observing an NO₂ effect
13 on AHR when participants were exposed to NO₂ while exercising and when participants were exposed to
14 specific airway challenges (Brown 2015). These hypotheses include the existence of a refractory period
15 following exercise, the use of FVC maneuvers, and the exclusion of participants with greater airway
16 responsiveness from studies involving exercise. Use of asthma medication or dietary supplements has
17 also been hypothesized as a factor that could attenuate NO₂'s AHR effect (Brown 2015).

18
19 Our evaluation indicates that the available evidence does not indicate that any of these factors explain the
20 overall lack of an NO₂ effect on AHR for studies involving exercise or exposure to specific airway
21 challenges. In the absence of a biologically plausible explanation for the paradoxical AHR findings, the
22 biological significance of these findings is difficult to interpret.

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25

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Table 2 AHR as a Function of Airway Challenge Delivery Method (Deep Inspiration vs. Tidal Breathing/Submaximal Inhalation) for Non-specific Airway Challenges

NO ₂ Concentration (ppb)	Delivery of Airway Challenge			
	Deep Inspiration		Tidal Breathing/Submaximal Inhalation	
	Study	SMD (95% CI) ¹	Study	SMD (95% CI) ¹
100			Orehek <i>et al.</i> (1976)	0.666 (0.029, 1.304)
			Hazucha <i>et al.</i> (1983)	-0.036 (-0.805, 0.732)
140			Bylin <i>et al.</i> (1988)	0.406 (-0.220, 1.033)
150			Roger <i>et al.</i> (1990)	0.066 (-0.571, 0.702)
200	Kleinman <i>et al.</i> (1983)	0.457 (-0.157, 1.070)	Orehek <i>et al.</i> (1976)	0.972 (-0.519, 2.463)
250	Jorres and Magnussen (1990)	0.539 (-0.216, 1.294)		
	Jorres and Magnussen (1991)	0.029 (-0.807, 0.865)		
260			Strand <i>et al.</i> (1996)	0.230 (-0.408, 0.868)
270			Bylin <i>et al.</i> (1988)	0.593 (-0.041, 1.227)
300	Morrow and Utell (1989)	0.051 (-0.826, 0.928)	Roger <i>et al.</i> (1990)	0.000 (-0.636, 0.636)
	Rubinstein <i>et al.</i> (1990)	-0.096 (-1.020, 0.829)		
350	Riedl <i>et al.</i> (2012)	0.154 (-0.563, 0.870)		
480			Bylin <i>et al.</i> (1985)	0.283 (-0.702, 1.269)
500			Mohsenin (1987)	0.381 (-0.505, 1.266)
530			Bylin <i>et al.</i> (1988)	0.147 (-0.474, 0.767)
600			Roger <i>et al.</i> (1990)	-0.100 (-0.736, 0.537)
	Pooled²	0.239 (-0.073, 0.551)	Pooled²	0.253 (0.050, 0.455)
	p-value for between-group heterogeneity³: 0.943			

Notes:

AHR = Airway Hyper-responsiveness; CI: Confidence Interval; NO₂ = Nitrogen Dioxide; ppb = Parts Per Billion; SMD = Standardized Mean Difference.

(1) The SMDs and their 95% CIs were calculated using the study-specific data presented in Table 1, with the function "metan" in STATA 14.

(2) The pooled SMDs were calculated using random-effect meta-analyses, with the function "metan" in STATA 14.

(3) The p-value for between-group heterogeneity was calculated using meta-regression, with the function "metareg" in STATA 14.

Table 3 AHR as a Function of Assessment Method (FVC Maneuver vs. Airway Resistance/Conductance) in Participants Exposed to NO₂ While Exercising

NO ₂ Concentration (ppb)	Assessment of AHR			
	FVC Maneuver ¹		Resistance or Conductance ²	
	Study	SMD (95% CI) ³	Study	SMD (95% CI) ³
150			Roger <i>et al.</i> (1990)	0.066 (-0.571, 0.702)
200	Kleinman <i>et al.</i> (1983) Jenkins <i>et al.</i> (1999)	0.457 (-0.157, 1.070) 0.023 (-0.813, 0.859)		
250			Jorres and Magnussen (1991)	0.029 (-0.807, 0.865)
260			Strand <i>et al.</i> (1996)	0.230 (-0.408, 0.868)
300	Bauer <i>et al.</i> (1986) ⁴ Avol <i>et al.</i> (1988) Avol <i>et al.</i> (1989) Morrow and Utell (1989)	0.750 (-0.080, 1.580) 0.198 (-0.259, 0.655) 0.000 (-0.475, 0.475) 0.051 (-0.826, 0.928)	Roger <i>et al.</i> (1990) Rubinstein <i>et al.</i> (1990)	0.000 (-0.636, 0.636) -0.096 (-1.020, 0.829)
350	Riedl <i>et al.</i> (2012) ⁵ Riedl <i>et al.</i> (2012) ⁶	0.154 (-0.563, 0.870) -3.765 (-4.983, -2.546)		
400	Jenkins <i>et al.</i> (1999) Witten <i>et al.</i> (2005)	0.389 (-0.497, 1.274) 0.562 (-0.168, 1.293)		
600	Avol <i>et al.</i> (1988)	0.161 (-0.295, 0.618)	Roger <i>et al.</i> (1990)	-0.100 (-0.736, 0.537)
	Pooled⁷	0.017 (-0.412, 0.446)	Pooled⁷	0.033 (-0.250, 0.316)
	p-value for between-group heterogeneity⁸: 0.967			
	Pooled⁷ – Excluding Riedel <i>et al.</i> (2012) cat allergen exposure	0.230 (0.034, 0.426)	Pooled⁷ – Excluding Riedel <i>et al.</i> (2012) cat allergen exposure	0.033 (-0.250, 0.316)
	p-value for between-group heterogeneity⁸ – Excluding Riedel <i>et al.</i> (2012) cat allergen exposure: 0.262			

Notes:

AHR = Airway Hyper-responsiveness; CI = Confidence Interval; FEV₁ = Forced Expiratory Volume in 1 Second; FVC = Forced Vital Capacity; NO₂ = Nitrogen Dioxide; ppb = Parts Per Billion; SMD = Standardized Mean Difference.

(1) AHR assessed using FEV₁.

(2) AHR assessed using specific airway resistance (sRAW) or specific airway conductance (sGAW).

(3) The SMDs and their 95% CIs were calculated using the study-specific data presented in Table 1, with the function "metan" in STATA 14.

(4) AHR assessed using PD10RHE (respiratory heat exchange causing a 10% decrease in FEV₁).

(5) Study participants challenged with methacholine.

(6) Study participants challenged with cat allergen.

(7) The pooled SMDs were calculated using random-effect meta-analyses, with the function "metan" in STATA 14.

(8) The p-value for between-group heterogeneity was calculated using meta-regression, with the function "metareg" in STATA 14.

Table 4a Baseline Lung Function, Asthma Status, Medication Status, and Medication Withholding Time for Studies in Which Participants Were Exposed to NO₂ at Rest

Study	N	Baseline % Predicted FEV ₁ (Av.)	FEV ₁ /FVC (Av.)	Asthma Status	Medication Status ¹	Medication Withholding Time	Withholding Time Category ²
Ahmed <i>et al.</i> (1982)	20	N/A		N/A	N/A	N/A	N/A
Ahmed <i>et al.</i> (1983, as cited in Folinsbee 1992)	19	N/A		N/A	N/A	N/A	N/A
Barck <i>et al.</i> (2002)	13	97		Mild	β ₂ -agonists as needed	No use of inhaled steroids during study period	Insufficient
Barck <i>et al.</i> (2005)	18	100		Mild	β ₂ -agonists as needed	2 months without steroids; no steroid or anti-inflammatory use during study	Insufficient
Bylin <i>et al.</i> (1985)	8	N/A		N/A	Occasional use of bronchodilating aerosol (3)	6 hours for bronchodilating aerosol	Borderline
Bylin <i>et al.</i> (1988)	20	N/A		Mild	β ₂ -agonist; tablet or aerosol (2)	12 hours for oral β ₂ -agonist; 8 hours for inhaled β ₂ -agonists	Borderline
Devalia <i>et al.</i> (1994)	10	N/A		Mild	Only inhaled salbutamol (short-acting β ₂ -agonist); no use of sodium cromoglycate or inhaled/oral corticosteroids	N/A	N/A
Hazucha <i>et al.</i> (1983) ³	13	77		Mild	No current steroid therapy; no daily bronchodilator therapy for the previous month	48 hours for bronchodilators, antihistamines, and vitamins; 12 hours for coffee, tea, colas, alcohol, and chocolate	Sufficient
Jorres and Magnussen (1990) ⁴	14	82	71	Mild	Inhaled β ₂ -agonists; ipratropium bromide (anti-cholinergic agent); disodium cromoglycate; inhaled/oral corticosteroids	10 hours for sympathomimetic, anticholinergic agents, and disodium cromoglycate; 16 hours for theophylline	Insufficient
Mohsenin (1987)	10	83	72	Mild	β ₂ -agonist; tablet or aerosol (2)	No therapy for at least 24 hours prior to each study day	Sufficient
Orehek <i>et al.</i> (1976) ⁵	20	N/A		Mild	None receiving long-term steroid therapy	No symptomatic medication for at least 24 hours prior to study	Sufficient
Strand <i>et al.</i> (1997)	18	102		Mild	Inhaled β ₂ -agonists as needed; inhaled steroids (4)	N/A	N/A

Study	N	Baseline % Predicted FEV ₁ (Av.)	FEV ₁ /FVC (Av.)	Asthma Status	Medication Status ¹	Medication Withholding Time	Withholding Time Category ²
Strand <i>et al.</i> (1998)	16	93		Mild	Inhaled β 2-agonists as needed; inhaled steroids during pollen season (9)	4 weeks for inhaled corticosteroids	Insufficient
Tunnicliffe <i>et al.</i> (1994)	8	94		Mild	Inhaled medication only (<400 μ g/24 hours [of the steroids] beclomethasone or budesonide)	No inhaled corticosteroids on day of challenge; 1 week for antihistamine medication	Borderline

Notes:

FEV₁ = Forced Expiratory Volume in 1 Second; FVC = Forced Vital Capacity; N/A = Information Not Available; NO₂ = Nitrogen Dioxide.

(1) Numbers in parentheses indicate the number of study participants on the specified medication.

(2) Withholding times assigned as Sufficient (withholding times of 6, 24, and 48 hours for short-acting β 2-agonists, long-acting β 2-agonists, and corticosteroids, respectively), borderline (β 2-agonist, not otherwise specified, withheld for $\leq$ 8 hours; or corticosteroids with short half lives withheld on day of exposure), or insufficient.

(3) Asthma status classified as "mild atopic."

(4) Baseline percentage of predicted FEV₁ estimated using the National Institute for Occupational Safety and Health (NIOSH) Reference Value Calculator (with values from Hankinson *et al.* 1999).

(5) Asthma status considered "slight to mild."

Table 4b Baseline Lung Function, Asthma Status, Medication Status, and Medication Withholding Time for Studies in Which Participants Were Exposed to NO₂ While Exercising

Study	N	Baseline % Predicted FEV ₁ (Av.)	% FEV ₁ /FVC (Av.)	Asthma Status	Medication Status ¹	Medication Withholding Time	Withholding Time Category ²
Avol <i>et al.</i> (1988)	37	N/A	69	Moderate to Severe	Bronchodilator (inhaled/oral)	48 hours for antihistamines; 8 hours for bronchodilators or steroids	Borderline
Avol <i>et al.</i> (1989)	34	N/A		N/A	Bronchodilator (inhaled/oral)	48 hours for antihistamines; 24 hours for oral bronchodilators; 8 hours for inhaled bronchodilators	Borderline
Bauer <i>et al.</i> (1986)	12	N/A	69	Asymptomatic	No use of chronic corticosteroid therapy	12 hours prior to testing for inhaled agents; 24 hours for oral drugs	Sufficient
Jenkins <i>et al.</i> (1999)	11	89	83	Mild	β ₂ agonists only (<14 inhalations albuterol [100 µg per actuation] per week); no regular use of inhaled corticosteroids or other anti-inflammatory therapy (inhaled sodium cromoglycate or nedocromil sodium; oral theophyllines)	N/A	N/A
Jorres and Magnussen (1991)	11	91	78	Asymptomatic	Occasional use of sympathomimetic (bronchodilators) metered-dose inhalers (5)	8 hours for inhalers	Borderline
Kleinman <i>et al.</i> (1983)	31	N/A	80	N/A	Bronchodilators (14); steroids (2); bronchodilators and steroids (6); bronchodilator and cromolyn (1)	4 hours for bronchodilators (six non-compliant subjects included in the analysis because pre-study medication was same for air and NO ₂)	Insufficient
Morrow and Utell (1989)	10	104	86	Mild	Both inhaled and oral (not further specified)	N/A	N/A

Study	N	Baseline % Predicted FEV ₁ (Av.)	% FEV ₁ /FVC (Av.)	Asthma Status	Medication Status ¹	Medication Withholding Time	Withholding Time Category ²
Roger <i>et al.</i> (1990) ³	19	77	68	Mild	No use of cromolyn sodium (anti-inflammatory agent, prevents release of inflammatory mediators) or steroids	24 hours for oral medications; 12 hours for inhaled bronchodilators	Sufficient
Riedl <i>et al.</i> (2012)	15	N/A		Mild	No use of oral corticosteroids within 4 weeks of screening visit	Allowed use within 2 weeks of exposure	Insufficient
Rubinstein <i>et al.</i> (1990)	9	N/A	72	Mild	Regular use of one or more antihistamine; no subjects using oral corticosteroids, cromolyn sodium or NSAIDs at time of study	12 hours for oral bronchodilators; 8 hours for inhaled bronchodilators; 4 hours for caffeine-containing beverages	Borderline
Strand <i>et al.</i> (1996)	19	N/A		Mild	β ₂ -agonists as needed	No use of inhaled steroids during study period	Insufficient
Witten <i>et al.</i> (2005)	15	82		Mild to Moderate	Inhaled steroids (5), short-acting bronchodilators (9); long-acting bronchodilators (4); leukotriene antagonists (4); antihistamines (4); nasal steroid (2)	2 weeks for inhaled steroids; 2 days for long-acting bronchodilators; 8 hours for short-acting bronchodilators; 5 days for NSAIDs (<i>e.g.</i> , leukotriene agonists, antihistamines)	Sufficient

Notes:

FEV₁ = Forced Expiratory Volume in 1 Second; FVC = Forced Vital Capacity; N/A = Information Not Available; NSAID = Non-steroidal Anti-inflammatory Drug.

(1) Numbers in parentheses indicate number of study participants on specified medication.

(2) Withholding times assigned as Sufficient (withholding times of 6, 24, and 48 hours for short-acting β₂-agonists, long-acting β₂-agonists, and corticosteroids, respectively), borderline (β₂-agonist, not otherwise specified, withheld for ≤ 8 hours; or corticosteroids with short half lives withheld on day of exposure), or insufficient.

(3) Baseline percentage of predicted FEV₁ estimated using the National Institute for Occupational Safety and Health (NIOSH) Reference Value Calculator (with values from Hankinson *et al.* 1999).

Table 5 Medication Withholding Category for Studies in Which Participants Were Exposed to NO₂ at Rest vs. While Exercising

Withholding Category	Number of Studies	
	Exposed at Rest (n = 14)	Exposed While Exercising (n = 12)
Sufficient	3 (21%)	3 (25%)
Borderline	3 (21%)	4 (33%)
Insufficient	4 (29%)	3 (25%)
N/A	4 (29%)	2 (17%)

Notes:

N/A = Information Not Available; NO₂ = Nitrogen Dioxide.

Table 1 Summary of Clinical Studies Assessing Airway Hyper-responsiveness Following NO₂ Exposure in Subjects with Asthma

Study	NO ₂ (ppm)	Exp. Time (min)	Challenge Type	N	PFT	AHR Assessment	Delivery of Non-specific Airway Challenge	Exercise	Test Type	Fraction Affected	PD (Average ± SD)		
											Metric	Air	NO ₂
Orehek <i>et al.</i> (1976)	0.10	60	CARB	20	SRaw	PD100	Inhalation of fixed volume (860 mL) followed by breath holding	No	WC	14/20	PD	0.56 ± 0.35	0.36 ± 0.24
Ahmed <i>et al.</i> (1983b) ¹	0.10	60	RAG	19	SGaw	NA		No	NA	10/19	PD	9 ± 24.7	11.7 ± 32.9
Ahmed <i>et al.</i> (1983a) ²	0.10	60	CARB	20	SGaw	PD35	NA	No	NA	13/20	PD	6 ± 2.4	2.7 ± 0.8
Hazucha <i>et al.</i> (1983)	0.10	60	METH	13	SRaw	PD100SRaw	Inhale to volume of 750-1,100 mL, breath holding	No	WC	6/13	PD	1.9 ± 1.44	2 ± 3.61
Tunnicliffe <i>et al.</i> (1994) ³	0.10	60	HDM	8	FEV ₁	ΔFEV ₁		No	MP	5/8	FEV1	-2.85 ± 3.94	-7.75 ± 6.91
Bylin <i>et al.</i> (1988)	0.14	30	HIST	20	SRaw	PC100SRaw	Tidal breathing	No	WC	14/20	PD	0.39 ± 0.32	0.28 ± 0.21
Roger <i>et al.</i> (1990)	0.15	75	METH	19	SRaw	PD100SRaw	Inhale to 25% of VC, breath holding	Yes	WC	10/19	PD	3.3 ± 3.05	3.1 ± 3.05
Orehek <i>et al.</i> (1976)	0.20	60	CARB	4	SRaw	PD100SRaw	Inhalation of fixed volume (860 mL) followed by breath holding	No	WC	1/4	PD	0.54 ± 0.31	0.32 ± 0.08
Jenkins <i>et al.</i> (1999)	0.20	360	HDM	11	FEV ₁	PD20FEV ₁		Yes	WC	6/11	PD	1,413.18 ± 1,473.95	1,377.27 ± 1,675.98
Kleinman <i>et al.</i> (1983) ⁴	0.20	120	METH	21	FEV ₁	PD10FEV ₁	Inhale to VC, breath holding	Yes	MP	20/27	PD	8.6 ± 16.2	3 ± 6.2
Jorres and Magnussen (1990)	0.25	30	SO ₂	14	SRaw	PV100SRaw	Inhale to TLC	No	MP	11/14	PD	46.47 ± 19.09	37.66 ± 13.03
Jorres and Magnussen (1991)	0.25	30	METH	11	SRaw	PC100SRaw	Inhale to TLC	Yes	MP	6/11	PD	1.09 ± 1.43	1.05 ± 1.35
Strand <i>et al.</i> (1996) ⁵	0.26	30	HIST	19	SRaw	PD100SRaw	Tidal breathing	Yes	WC	13/19	PD	295.68 ± 331.56	229 ± 242.11
Strand <i>et al.</i> (1998) ⁶	0.26	30	BIR	16	FEV ₁	ΔFEV ₁		No	WC	11/16	FEV ₁	-0.11 ± 3.32	-2.51 ± 3.73
Barck <i>et al.</i> (2002) ⁷	0.26	30	POL	13	FEV ₁	ΔFEV ₁		No	WC	5/13	FEV ₁	-7.51 ± 9.2	-7.08 ± 7.55
Barck <i>et al.</i> (2005)⁸	0.26	15	POL	18	FEV ₁	ΔFEV ₁		No	WC		FEV ₁	-3 ± 3	-2 ± 3
Strand <i>et al.</i> (1997) ⁹	0.26	30	POL	18	FEV ₁	ΔFEV ₁		No	WC	10/18	PD	856.67 ± 1,922.23	965.33 ± 1,919.27
Bylin <i>et al.</i> (1988)	0.27	30	HIST	20	SRaw	PC100SRaw	Tidal breathing	No	WC	14/20	PD	0.39 ± 0.32	0.24 ± 0.16
Bauer <i>et al.</i> (1986) ¹⁰	0.30	30	COLD	12	RHE	PD10RHE		Yes	MP	9/12	PD	0.83 ± 0.42	0.54 ± 0.35
Avol <i>et al.</i> (1988) ¹¹	0.30	120	COLD	37	FEV ₁	ΔFEV ₁		Yes	WC	11/37	FEV ₁	-8.4 ± 11.2	-10.7 ± 12
Avol <i>et al.</i> (1989)	0.30	30	COLD	34	FEV ₁	ΔFEV ₁		Yes	WC	12/34	FEV ₁	-5 ± 12	-5 ± 13
Morrow and Utell (1989) ¹²	0.30	240	CARB	10	FEV ₁	ΔFEV ₁	Inhale to TLC, breath holding	Yes	WC	4/10	FEV ₁	-14.24 ± 27.52	-15.38 ± 15.54
Roger <i>et al.</i> (1990)	0.30	75	METH	19	SRaw	PD100SRaw	Inhale to 25% of VC, breath holding	Yes	WC	8/19	PD	3.3 ± 3.05	3.3 ± 3.49
Rubinstein <i>et al.</i> (1990)	0.30	30	SO ₂	9	SRaw	PD8USRaw	Inhale at 20 L/min	Yes	WC	4/9	PD	1.24 ± 0.71	1.31 ± 0.75
Riedl <i>et al.</i> (2012) ¹³	0.35	120	METH	15	FEV ₁	ΔFEV ₁	Inhale to VC	Yes	WC	6/7	PD	7.5 ± 2.6	7.0 ± 3.8
Riedl <i>et al.</i> (2012) ¹³	0.35	120	CAT	15	FEV ₁	ΔFEV ₁		Yes	WC	4/11	FEV ₁	-6.9 ± 1.7	-0.5 ± 1.7
Jenkins <i>et al.</i> (1999)	0.40	180	HDM	10	FEV ₁	PD20FEV ₁		Yes	WC	7/10	PD	3,325.7 ± 5,998.27	1,586.4 ± 2,018.88
Witten <i>et al.</i> (2005) ¹³	0.40	180	HDM	15	FEV ₁	PD20FEV ₁		Yes	WC	8/15	PD	550.09 ± 938.75	164.65 ± 242.15
Tunnicliffe <i>et al.</i> (1994) ³	0.40	60	HDM	8	FEV ₁	ΔFEV ₁		No	MP	6/8	FEV1	-2.85 ± 3.94	-8.14 ± 6.65
Devalia <i>et al.</i> (1994)	0.40	360	HDM	8	FEV ₁	PD20FEV ₁		No	WC		PD	3.5 ± 3.03	2 ± 1.46
Bylin <i>et al.</i> (1985) ¹⁴	0.48	20	HIST	8	SRaw	PD100SRaw	Tidal breathing	No	WC	5/8	PD	32.51 ± 29.06	24.42 ± 28.03
Mohsenin (1987)	0.50	60	METH	10	VP40	PD40	Tidal breathing	No	WC	7/10	PD	9.2 ± 15	4.6 ± 8.2
Bylin <i>et al.</i> (1988)	0.53	30	HIST	20	SRaw	PC100SRaw	Tidal breathing	No	WC	12/20	PD	0.39 ± 0.32	0.34 ± 0.36
Roger <i>et al.</i> (1990)	0.60	75	METH	19	SRaw	PD100SRaw	Inhale to 25% of VC, breath holding	Yes	WC	11/19	PD	3.3 ± 3.05	3.7 ± 4.79

Study	NO ₂ (ppm)	Exp. Time (min)	Challenge Type	N	PFT	AHR Assessment	Delivery of Non-specific Airway Challenge	Exercise	Test Type	Fraction Affected	PD (Average ± SD)		
											Metric	Air	NO ₂
Avol <i>et al.</i> (1988) ¹¹	0.60	120	COLD	37	FEV ₁	ΔFEV ₁		Yes	WC	13/37	FEV ₁	-8.4 ± 11.2	-10.4 ± 13.5

Notes:
AHR = Airway Hyper-responsiveness; NA = Not Available; NO₂ = Nitrogen Dioxide; PD = Provocative Dose; PFT = Pulmonary Function Test; ppm = Parts Per Million; SD = Standard Deviation; SO₂ = Sulfur Dioxide; TLC = Total Lung Capacity; US EPA = United States Environmental Protection Agency; VC = Vital Capacity.
Studies in ***bold italics*** are not included in the analysis by Brown (2015).
Challenge Type: Specific challenges – BIR = Birch; CAT = Cat Allergen; HDM = House Dust Mite; RAG = Ragweed. Non-specific challenges – CARB = Carbachol; COLD = Cold Dry Air; HIST = Histamine; METH = Methacholine; POL = Pollen; SO₂ = Sulfur Dioxide.
PFT: FEV₁ = Forced Expiratory Volume in 1 Second; RHE = Respiratory Heat Exchange; sGaw = Specific Airway Conductance; sRaw = Specific Airway Resistance; VP40 = Partial Expiratory Flow at 40% Vital Capacity.
AHR Assessment Endpoints: PD100 = Challenge Dose (in mg) Required to Decrease sGaw or Increase sRaw by 100%; PD35 = Challenge Dose (in mg) Required to Decrease sGaw by 35%; ΔFEV₁ = Percentage Change in FEV₁ Before and After Airway Challenge; PC100SRaw = Challenge Concentration (in mg/L) Required to Increase sRaw by 100%; PD100SRaw = Challenge Dose (in mg) Required to Increase sRaw by 100%; PD20FEV₁ = Allergen Dose (in Cumulative Breadth Units) Required to Decrease FEV₁ by 20%; PD10FEV₁ = Challenge Dose (in Cumulative Breadth Units) Required to Decrease FEV₁ by 10%; PD10RHE = Respiratory Heat Exchange (Loss) (in Kcal/min) for a 10% Decrease in FEV₁; PD8USRaw = SO₂ Dose (ppm) Required to Increase SRaw by 8 Times the Baseline Values; PD40 = Methacholine Dose (in mg/mL) Required to Decrease VP40 by 40%; PV100SRaw = Provocative Ventilation of 0.75 ppm SO₂ Required to Increase SRaw by 100%.
Test Type: MP = Mouth Piece; WC = Whole Chamber.
Fraction Affected = Number of subjects with increased AHR/total number of subjects tested.
(1) Information regarding exercise and PD values are as reported by US EPA (2016),
(2) Information regarding exercise and PD values are as reported by US EPA (2016).
(3) Response reported for late-phase response.
(4) 31 subjects were enrolled, but data to evaluate AHR were available for only 21 subjects.
(5) Response reported for 30 min. post-exposure.
(6) Response reported for early-phase response for day 1 exposure.
(7) Response reported for late-phase response.
(8) Response reported for early-phase response for day 1 exposure.
(9) Response reported for late-phase response.
(10) ΔFEV₁ for isocapnic cold air challenge at 60 L/hour.
(11) Response reported for 1 hour post-exposure.
(12) Results for challenge at highest dose of carbachol following air exposure vs. highest dose of carbachol following NO₂ exposure; 20 subjects participated, but data to evaluate AHR were available for only 10 subjects.
(13) Fraction Affected, PD, and FEV₁ are as reported by US EPA (2016).
(14) Response reported for early phase response. When the lower limit of the PD was reported as >X, X was used to calculate the average and the SD.
(15) When the lower limit of PD was reported as >X, X was used to calculate the average and the SD.