Short term associations of ambient nitrogen dioxide with daily total, cardiovascular, and respiratory mortality: multilocation analysis in 398 cities

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ABSTRACT

OBJECTIVE

To evaluate the short term associations between nitrogen dioxide (NO_2) and total, cardiovascular, and respiratory mortality across multiple countries/regions worldwide, using a uniform analytical protocol.

DESIGN

Two stage, time series approach, with overdispersed generalised linear models and multilevel meta-analysis.

SETTING

398 cities in 22 low to high income countries/regions.

MAIN OUTCOME MEASURES

Daily deaths from total (62.8 million), cardiovascular (19.7 million), and respiratory (5.5 million) causes between 1973 and 2018.

RESULTS

On average, a 10 μg/m^3 increase in NO_2 concentration on lag 1 day (previous day) was associated with 0.46% (95% confidence interval 0.36% to 0.57%), 0.37% (0.22% to 0.51%), and 0.47% (0.21% to 0.72%) increases in total, cardiovascular, and respiratory mortality, respectively. These associations remained robust after adjusting for co-pollutants (particulate matter with aerodynamic diameter ≤10 μm or ≤2.5 μm (PM_{10} and $PM_{2.5}$, respectively), ozone, sulfur dioxide, and carbon monoxide). The pooled concentration-response curves for all three causes were almost linear without discernible thresholds. The proportion of deaths attributable to NO_2 concentration above the counterfactual zero level was 1.23% (95% confidence interval 0.96% to 1.51%) across the 398 cities.

CONCLUSIONS

This multilocation study provides key evidence on the independent and linear associations between short term exposure to NO_2 and increased risk of total, cardiovascular, and respiratory mortality, suggesting that health benefits would be achieved by tightening the guidelines and regulatory limits of NO_2.

WHAT IS ALREADY KNOWN ON THIS TOPIC

Evidence for the short term association between ambient nitrogen dioxide (NO_2) and mortality is limited, especially for cause specific associations

Previous investigations have been mostly conducted in a small number of locations that cover limited geographical areas, and use different study designs and modelling approaches that lead to heterogeneous results

WHAT THIS STUDY ADDS

This large multilocation study in 398 cities found that each 10 μg/m^3 increase in NO_2 concentrations on lag 1 day (previous day) was significantly associated with increased risk of total (0.46%), cardiovascular (0.37%), and respiratory (0.47%) mortality

The concentration-response curves were almost linear without discernible thresholds even below the current WHO air quality guidelines, suggesting a need to revise the currently recommended values

The uniform analytical approach allows a direct comparison of estimates across global regions

Introduction

Nitrogen dioxide (NO_2) is a common air pollutant that has adverse effects on population health, is a precursor to ground level ozone with its own harmful effects on health, and is involved in the secondary formation of fine particulate matter. Most ambient NO_2 has an anthropogenic origin, such as fuel combustion and traffic.[1] Many studies have reported the effects of short term exposure to NO_2 on human mortality or morbidity,[2 3] but these are disputed. The Integrated Science Assessment conducted by the United States Environmental Protection Agency reviewed the existing evidence linking NO_2 exposure with health outcomes in 2008[4] and 2016,[5] and concluded that there is a causal relation between short term exposure to NO_2 and respiratory effects. The review highlighted important gaps in knowledge, resulting in different limits for NO_2 in air quality guidelines being adopted by various governmental and intergovernmental institutions.[1 6-8]

Several epidemiological studies have investigated the association between NO_2 and total mortality,[9] but with important limitations. Firstly, most focused on total non-accidental deaths, with only a few assessing cause specific mortality, such as respiratory and cardiovascular outcomes. Moreover, potential features of associations between NO_2 and mortality, such as non-linearity, thresholds, and lag structures, remain to be clarified. More importantly, questions remain about whether NO_2 has an independent effect on mortality, or if the observed associations can be explained by confounding effects of co-pollutants. Finally, current publications mainly include studies performed in single cities or countries, with the problem of publication bias and difficulties in integrating results from different analytical approaches. Furthermore, emissions in different contexts, population characteristics, and the reliability of routine health data and vital statistics limited the generalisability of such evidence.

This study aimed to examine these limitations by investigating the short term association between NO_2 and mortality across numerous countries/regions worldwide, using data from the Multi-City Multi-Country Collaborative Research Network (https://mccstudy.lshtm.ac.uk/). The assessment takes advantage of a uniform analytical framework based on state-of-the-art epidemiological methodologies to estimate flexible exposure-response associations, to integrate results across cities and countries, and to quantify the excess mortality attributable to short term NO_2 exposure.

Methods

Data collection

We obtained health and environmental data from the Multi-City Multi-Country database, which has been described in previous publications.[10 11] Our analysis was limited to locations where ground level NO_2 measurements were available—a total of 398 cities in 22 countries/regions, with different study periods based on data availability. The geographical distribution of cities with NO_2, and the corresponding annual mean concentrations during the study periods are shown in eFigure 1. Mortality data were obtained from local authorities within each country/region. Causes of death were classified according to the international classification of diseases, 9th and 10th revision (ICD-9 and ICD-10) codes, where available. In each location, mortality is represented by daily counts of either non-external causes (ICD-9: 0-799; ICD-10: A0-R99) or, where not available, all cause mortality. We also collected mortality data for two main causes in 16 countries: cardiovascular disease (ICD-10, codes I00-I99) and respiratory disease (ICD-10, codes J00-J99).[12]

Daily concentrations of particulate matter and other gaseous pollutants, including particulate matter with aerodynamic diameter ≤10 µm or ≤2.5 µm (PM_{10} and $PM_{2.5}$, respectively), ozone (O_3), sulfur dioxide (SO_2), and carbon monoxide (CO), were obtained from the same fixed site monitoring stations as for NO_2, where available, to adjust for potential confounding by co-pollutants. We also collected daily data on weather variables, including mean temperature and mean relative humidity from local meteorological bureaux or other statistical authorities. The overall missing rate for all cause mortality, NO_2, and temperature time series was 0.13%, 4.4%, and 1.2%, respectively. Detailed information on missing data is summarised in the appendix (eMethod 1.1 and eTable 7).

Statistical analysis

The associations of NO_2 with daily total, cardiovascular, and respiratory mortality were assessed using the same analytical protocol in all locations, based on extension of a two stage design widely used in previous multilocation time series studies.[13 14]

In the first stage, we estimated the city specific associations using a time series quasi-Poisson generalised linear regression model featuring a natural cubic spline function of time with seven degrees of freedom (df) per year to control for unmeasured temporal trends and indicators for day of the week. Temperature was adjusted using a natural spline function with 6 df. Relative humidity was adjusted with the same spline function with 3 df in cities where such data were available. For the potential lag effect of NO_2 and temperature, we a priori selected the moving average of lag 0-3 days to control temperature according to modelling choices of many previous studies.[14 15] We modelled the association between NO_2 and mortality using a distributed lag model with a linear lag response function, inspecting the lag structure on a single lag day of 0 to 3, and moving average of the present and previous day (lag 0-1) to identify the optimal lag choices.

In the second stage, we used a new multilevel meta-analytical approach to summarise the city specific associations.[16] Briefly, this model defines more complex random effects structures that account for the hierarchical structure of the data—namely, cities nested within countries, and provides the best linear unbiased predictors for the associations between NO_2 and mortality at both levels.[16] We computed the global, country, and city specific estimates and 95% confidence intervals as percentage change in daily mortality per 10 µg/m^3 increase of NO_2 concentrations. Potential heterogeneity across cities was assessed with Cochran Q tests and I^2 statistics. Finally, we computed the proportion of deaths attributable to NO_2 exposure above the counterfactual zero level (since there was no obvious threshold level) and presented the population attributable fraction (%) at the country level, which can be calculated as follows:

$$RR=\exp(\hat{a}\times\Delta X)$$
$$PAF\%=(RR-1/RR)\times 100\%$$

Where RR is the relative risk of mortality associated with NO_2 for each country, ΔX is the difference between the current annual concentration of NO_2 and the counterfactual zero level, $\hat{a}$ is the country specific mortality estimate.

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We extended the main models to assess specific features of the association. Firstly, we fitted models with two pollutants using the other co-pollutants, assessing the robustness of the estimates through likelihood ratio tests. Countries/regions with unavailable data for co-pollutants were excluded accordingly; therefore, the number of countries/regions in eTable 3 varied by co-pollutants. Secondly, we conducted regional analyses (eTable 2 and eTable 4) and explored potential effect modifiers (such as long term air pollution concentrations, temperature, and gross domestic product) on the associations between NO_2 and total mortality based on the main models. Finally, we explored potential non-linearity in the associations and pooled the concentration-response relation curve using a meta-smoothing approach applied in previous studies,[14 17] modelling NO_2 as a non-linear term through a natural spline function with knots at 20 and 40 $\mu g/m^3$.

We conducted several sensitivity analyses to test the robustness of our estimates, including alternative choices for controlling for temperature, alternative placements of knots for testing non-linear exposure-response between NO_2 and mortality, a comparison of models with and without adjustment for humidity, a test for seasonal differences modelled through an interaction with an indicator of warm/cold season (May-September *v* October-April for northern hemisphere, and vice versa for southern hemisphere), and comparing the associations within different time periods with a cutoff point at year 2000 (around the median year of each country's time period).

We conducted all statistical analyses in R software (version 3.3.1), using the stats and dlnm packages for fitting first stage models and the mixmeta package for performing multilevel meta-analyses. We presented the percentage change of mortality for a 10 $\mu g/m^3$ increase in NO_2 concentrations. P values less than 0.05 were considered statistically significant in all analyses.

Patient and public involvement

This analysis used health data at the aggregated level, and thus there is no patient or public involvement. No patients were involved in setting the research question or the outcome measures, nor were they involved in developing plans for recruitment, design, or implementation of the study. No patients were asked to advise on interpretation or writing up of results. There are no plans to disseminate the results of the research to study participants or relevant patients.

Results

Descriptive statistics

This analysis included 62.8 million deaths from total or non-external causes, including 19.7 and 5.5 million deaths from cardiovascular diseases and respiratory diseases, respectively (table 1), covering the study period from 1973 to 2018. Cardiovascular deaths accounted for 31.4% of total deaths among all countries, ranging from 19.9% in Thailand to 50.4% in the Czech Republic; while respiratory deaths accounted for 8.8%, ranging from 4.8% in the Czech Republic to 15.7% in Japan. On average, the median annual mean NO_2 concentration across 398 cities was 26.9 $\mu g/m^3$ (25th to 75th centiles 19.5 to 36.2 $\mu g/m^3$), which was lower than the air quality guidelines for NO_2 (annual mean 40 $\mu g/m^3$) of the World Health Organization.[6] The median annual mean temperature was 14.4°C (25th to 75th centiles 7.8°C to 20.6°C). A detailed summary of exposure data is provided in eTable 1. The Spearman correlation coefficients of NO_2 were 0.34 with PM_{10}, 0.38 with $PM_{2.5}$, −0.24 with O_3, 0.42 with SO_2, and 0.56 with CO. On average, NO_2 was negatively correlated with mean temperature (Pearson r=−0.29) and relative humidity (Pearson r=−0.15).

Regression results

Figure 1 illustrates the estimated pooled associations between NO_2 and total, cardiovascular, and respiratory mortality on different lag days. Results suggested that the magnitude of associations increased from the current (lag 0) to the previous day (lag 1) and then decreased on lag 2 day for all three endpoints. In addition, considering its causal respiratory effect,[5] we used NO_2 concentration on lag 1 day as the main lag value for NO_2 in subsequent analysis.

Table 2 displays the country specific and pooled estimates for the associations of NO_2 (lag 1) with total, cardiovascular, and respiratory mortality. Across 398 cities, a 10 $\mu g/m^3$ increase in NO_2 concentrations was associated with an increase of 0.46% (95% confidence interval 0.36% to 0.57%) in total mortality; while across 362 cities with available data, the corresponding increases were 0.37% (95% confidence interval 0.22% to 0.51%) and 0.47% (95% confidence interval 0.21% to 0.72%), respectively, for cardiovascular and respiratory mortality. The country specific estimates showed considerable variations. Consistently positive associations of NO_2 with total mortality were seen, ranging from 0.17% to 0.70% for total mortality. Estimates were less precise for cause specific associations, although generally indicating an increased risk.

Figure 2 shows the results of the model with non-linear terms for estimating the effect of the association between NO_2 and mortality, indicating positive and almost linear concentration-response curves for total, cardiovascular, and respiratory mortality, with no discernible thresholds. Thus a counterfactual scenario at 0 $\mu g/m^3$ was defined to assess the mortality impacts in each country based on the current study sample (table 3). Although we recognise that this level is not practical from a policy standpoint, our analysis provides insight into the overall health burden from NO_2. The population attributable fraction (%) ranged from 0.28% (95% confidence interval 0.05% to 0.51%) in Finland and 3.05% (95% confidence interval 1.79% to 4.29%) in Greece, with a pooled population attributable fraction (%) of 1.23% (95% confidence interval: 0.96% to 1.51%) across the 398 cities.

In the models with two pollutants (eTable 3), the associations of NO_2 with total mortality were robust

Table 1 | Mortality and environmental data in 398 cities of 22 countries/regions

Country /region	No of cities	Period	Number of deaths (in thousands)*			Median (interquartile range)	
			Total	Cardiovascular	Respiratory	NO_2 (μg/m^3)	Temperature (°C)
Australia	3	1988-2009	1178.0	NA	NA	21.4 (14.1-27.9)	18.1 (14.7-21.2)
Brazil	1	1997-2011	916.2	NA	NA	84.9 (62.6-115.3)	20.6 (18-22.9)
Canada	25	1986-2015	3617.6	1220.7	307.8	23.7 (15.6-33.8)	7.4 (−0.9-15.7)
Chile	3	2005-13	316.8	NA	NA	21.6 (13.9-32.4)	13.7 (10.7-17.2)
China	15	1996-2015	1201.7	468.0	164.7	46.5 (36.2-60.4)	16.3 (6.5-23.5)
Colombia	1	1998-2013	426.3	123.8	46.3	30.5 (23.3-37.9)	13.9 (13.2-14.5)
Czech Republic	1	1994-2015	287.5	145.0	13.9	30.8 (24.2-38.7)	9.2 (2.7-15.3)
Estonia	4	1997-2015	133.8	NA	NA	11.4 (7.6-16.7)	6.0 (−0.1-13.6)
Finland	1	1994-2014	153.3	57.4	9.7	6.8 (4.3-11.7)	5.9 (0.0-13.8)
Germany	12	1993-2015	3105.9	NA	NA	29.6 (21.8-38.4)	10.5 (4.8-15.9)
Greece	1	2001-10	288	136.2	28.8	50.2 (39.6-61.6)	17.9 (12.9-24.9)
Japan	47	2011-15	1885	496.7	296.0	16.7 (12.1-23.4)	16.1 (7.6-22.7)
Portugal	5	1990-2018	1750.7	659.8	165.9	14.9 (10.0-21.7)	15.4 (11.5-19.9)
Romania	8	1994-2016	951.1	NA	NA	25.6 (17.7-36.2)	11.4 (3.4-18.9)
South Korea	7	1992-2015	2245.3	547.5	133.3	43.7 (33.0-57.5)	14.9 (5.7-21.9)
Spain	48	1990-2014	2929.6	1011.8	330.8	26.4 (20.2-33.9)	14.9 (10.2-20.7)
Sweden	1	1990-2010	201.2	91.3	15.9	26.8 (20.0-34.8)	6.8 (1.2-13.9)
Switzerland	8	1995-2013	243.6	90.7	16.0	32.3 (24.0-42.0)	10.7 (4.4-16.5)
Taiwan	3	1994-2014	1209.6	269.4	116.5	42.2 (31.6-54.7)	24.9 (20.4-28.0)
Thailand	18	1999-2008	843.4	167.8	110.2	22.0 (16.3-30.4)	28.0 (26.4-29)
United Kingdom	39	1990-2016	5413.4	1978.9	799.2	25.6 (17.9-35.6)	10.4 (6.6-14.7)
United States	147	1973-2006	33 502.8	12 303.5	2904.9	28.7 (20.8-38.6)	14.7 (7.2-21.8)
Pooled	398	1973-2018	62 800.8	19 768.5	5459.9	26.9 (19.5-36.2)	14.4 (7.8-20.6)

NO_2=nitrogen dioxide; NA=not available.
*Mortality data from cardiovascular and respiratory diseases were not available in Australia, Brazil, Chile, Estonia, Germany, and Romania.

to the adjustment of co-pollutants. The estimates of NO_2 mortality associations increased slightly with adjustment of SO_2, O_3, and CO. Although the effect estimates decreased by 19% (P=0.08) and 21% (P=0.08) after adjusting for PM_{10} and $PM_{2.5}$, respectively, the associations were still positive and statistically significant.

In the sensitivity analyses, compared with main models, the estimates for the associations of NO_2 with mortality were generally similar when adjusting for temperature with different lag structures, except for a smaller association when using the lag of the present and mean of the previous three days (eFigure 2). The use of alternative knots did not substantially change the shape of the concentration-response curves (eFigure 3). The associations of NO_2 with total and cause specific mortality did not change with or without adjustment of relative humidity (eTable 5). We found no evidence of seasonal difference in the association of NO_2 with mortality (P=0.68), with the estimate for the cold season (0.51%, 95% confidence interval 0.41% to 0.60%) similar to that of the warm season (0.43%, 95% confidence interval 0.31% to 0.50%). Finally, the estimates for all three endpoints were nearly the same for different time periods (eTable 6).

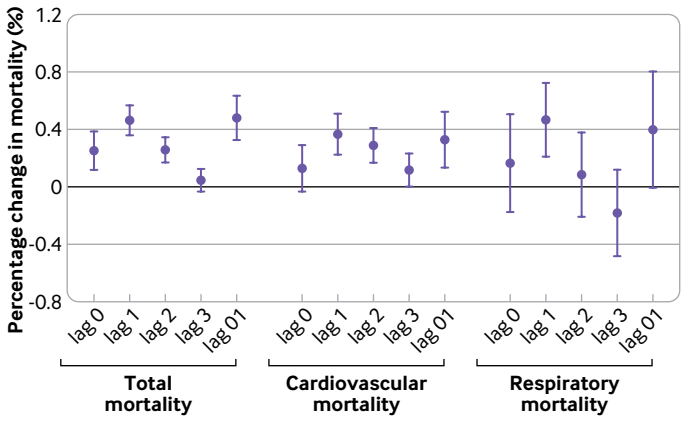

Fig 1 | Relative risks of total, cardiovascular, and respiratory mortality associated with a 10 μg/m^3 increase in nitrogen dioxide (NO_2) on different lag days for NO_2. Lags indicate the time difference between the NO_2 exposure and the outcome. Lag 0=the present day; lag 1=the previous day; lag 2=the day before lag 1; lag 3=the day before lag 2; lag 0-1=the two day moving average of the present day and the previous day

Discussion

Principal findings

A key advantage of this epidemiological study on the short term association of NO_2 with daily mortality is the analysis of a large dataset using the same analytical protocol, which allows a valid comparison among countries and regions of the world. We found robust associations of NO_2 with daily mortality from total, cardiovascular, and respiratory causes that were independent of concomitant exposures to other air pollutants. More importantly, we pooled a concentration-response curve for NO_2 at the global level, suggesting an almost linear association, with no discernible thresholds. This result suggests that NO_2 is associated with considerable health risks even at levels below health based standards and guidelines, including the current WHO air quality guidelines.

Among the 398 cities, the risk of total mortality increased by 0.46% for every 10 μg/m^3 increase in NO_2 concentrations. The magnitude of the association is comparable with the result from one previous systematic review, which included studies in 26 cities world-

Table 2 | Percentage changes in total, cardiovascular, and respiratory mortality associated with increase of 10 μg/m³ in nitrogen dioxide on lag 1 day. Results are shown as percentage (95% confidence interval)

	Total mortality		Cardiovascular mortality		Respiratory mortality	
Country/region	No*	Mean change (%; 95% CI)	No*	Mean change (%; 95% CI)	No*	Mean change (%; 95% CI)
Australia	3	0.64 (0.36 to 0.93)	0	NA	0	NA
Brazil	1	0.34 (0.22 to 0.47)	0	NA	0	NA
Canada	25	0.62 (0.47 to 0.76)	24	0.45 (0.23 to 0.67)	24	0.52 (0.10 to 0.94)
Chile	3	0.53 (0.39 to 0.67)	0	NA	0	NA
China	15	0.57 (0.31 to 0.83)	13	0.54 (0.34 to 0.73)	13	0.76 (0.41 to 1.12)
Colombia	1	0.31 (0.01 to 0.61)	1	0.28 (−0.09 to 0.66)	1	−0.05 (−0.75 to 0.67)
Czech Republic	1	0.46 (0.16 to 0.76)	1	0.37 (0.01 to 0.73)	1	0.42 (−0.34 to 1.18)
Estonia	4	0.40 (0.06 to 0.74)	0	NA	0	NA
Finland	1	0.41 (0.08 to 0.75)	1	0.39 (−0.01 to 0.79)	1	0.50 (−0.27 to 1.28)
Germany	12	0.62 (0.47 to 0.78)	0	NA	0	NA
Greece	1	0.62 (0.36 to 0.88)	1	0.47 (0.15 to 0.80)	1	0.84 (0.20 to 1.49)
Japan	47	0.36 (0.19 to 0.54)	47	0.18 (−0.10 to 0.45)	47	0.70 (0.27 to 1.12)
Portugal	5	0.52 (0.37 to 0.66)	5	0.22 (−0.07 to 0.51)	5	0.13 (−0.40 to 0.66)
Romania	8	0.27 (0.07 to 0.48)	0	NA	0	NA
South Korea	7	0.41 (0.12 to 0.70)	7	0.48 (0.25 to 0.71)	7	0.48 (0.05 to 0.91)
Spain	48	0.70 (0.51 to 0.88)	47	0.53 (0.25 to 0.81)	47	0.88 (0.40 to 1.35)
Sweden	1	0.55 (0.27 to 0.83)	1	0.21 (−0.16 to 0.59)	1	0.41 (−0.34 to 1.15)
Switzerland	8	0.24 (−0.07 to 0.55)	8	0.52 (0.16 to 0.88)	8	0.24 (−0.47 to 0.96)
Taiwan	3	0.52 (0.28 to 0.76)	3	0.37 (0.07 to 0.67)	3	0.22 (−0.29 to 0.74)
Thailand	18	0.32 (0.13 to 0.51)	18	0.38 (0.03 to 0.74)	18	0.69 (0.10 to 1.28)
United Kingdom	39	0.17 (0.04 to 0.30)	39	0.10 (−0.09 to 0.28)	39	0.11 (−0.21 to 0.43)
United States	147	0.57 (0.46 to 0.68)	146	0.45 (0.30 to 0.61)	146	0.62 (0.34 to 0.90)
Pooled	398	0.46 (0.36 to 0.57)	362	0.37 (0.22 to 0.51)	362	0.47 (0.21 to 0.72)

NA=not available.
*Number of cities with available data within the country or region.

wide and reported an estimate of 0.78% for all cause mortality with the same NO_2 increment as ours.[18] The other multilocation study conducted in Europe (known as the Air Pollution on Health: a European Approach (APHEA)2 project) found a smaller effect estimate with an increase of 0.30% (95% confidence interval 0.22% to 0.38%) in all cause mortality associated with a 10 μg/m³ increase in NO_2.[19] Our estimate is somewhat smaller than some multilocation studies conducted in a single country. For a 10 μg/m³ increase in NO_2, a multilocation study in China observed a 0.91% (95% confidence interval 0.70% to 1.12%) increase in total mortality[20]; another study in Italy reported an estimate of 2.09% (95% confidence interval 0.96% to 3.24%).[21] The differences in estimates do not necessarily reflect a diverse effect of NO_2 on health. Rather, the coverage of regions and time periods, population characteristics, and exposure patterns might contribute to these various findings. Furthermore, as found in our sensitivity analysis, different modelling specification for temperature would introduce fluctuation in the estimated associations.

The 2016 Integrated Science Assessment conducted by the US Environmental Protection Agency determined a causal relation between short term NO_2 exposure and respiratory effects, whereas for total mortality and cardiovascular effects, the conclusion was "suggestive of, but not sufficient to infer, a causal relation." Our findings from this multilocation analysis add to the supporting evidence for causal associations between short term exposure to NO_2 and non-respiratory endpoints. Experimental investigations and controlled human exposure studies are needed to understand the non-respiratory effect of NO_2.

There has been a long debate on whether exposure to NO_2 independently causes health effects, or whether it serves as a marker for a broader mixture of air pollutants, especially those related to traffic.[5] Findings from controlled human exposure and animal toxicological studies are scarce, but are key for indicating independent associations.[22 23] Although statistically adjusting the NO_2 association for another co-pollutant cannot conclusively show an independent effect, gathering evidence might contribute towards understanding this matter. In our analysis, the NO_2-mortality association decreased after adjustment of PM_{10} and $PM_{2.5}$ but remained positive and statistically significant. A systematic review, including studies in 26 cities worldwide, found similarly that the effect estimates of NO_2 on total mortality decreased from 0.78% to 0.60%, but were still statistically significant after adjusting for PM.[18] Meanwhile, as found in our previous study, the association between PM and mortality was also influenced by NO_2.[15] This association might indicate potential mutual confounding in the PM-mortality and NO_2-mortality associations. On the other hand, the null estimates found in previous reports of NO_2 after adjustment of co-pollutants might be due to the smaller study sample, regional difference, and other uncertainties of study heterogeneity. Our multilocation study and meta-analysis provided robust evidence for the independent association between NO_2 and mortality.

Characterising the shape of the concentration-response curve helps to quantify the effect on public health of exposure to NO_2 and establish safe concentration limits. The shape of the concentration-response curve between effect on health and short term

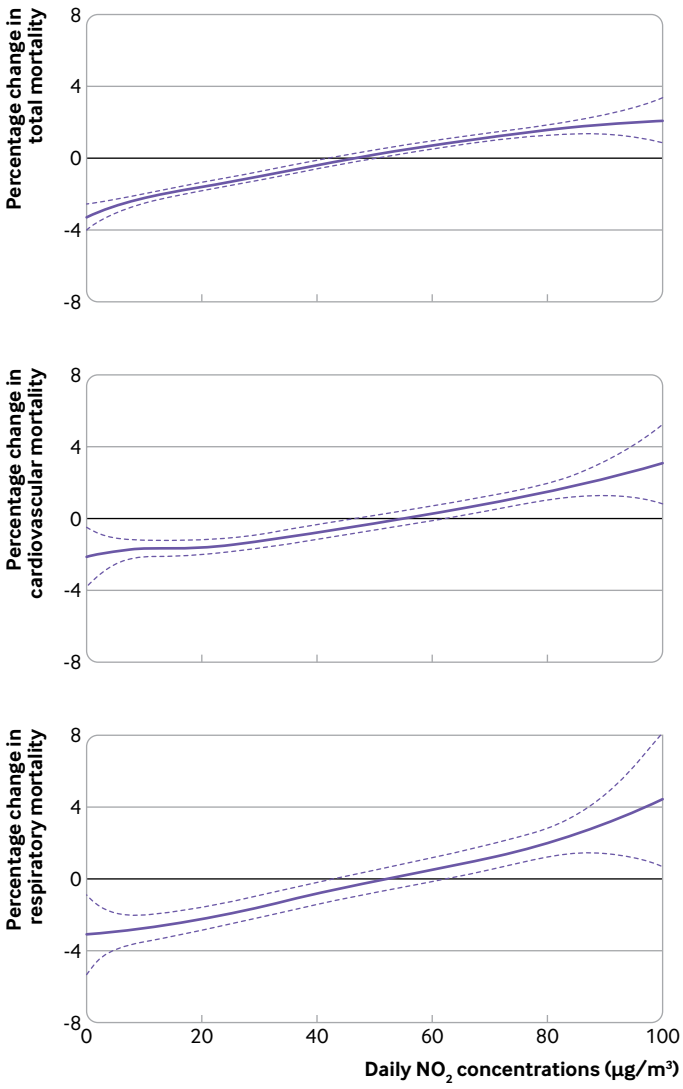

Fig 2 | Concentration-response curve between nitrogen dioxide (NO_2) concentrations (lag 1) and total, cardiovascular, and respiratory mortality. The vertical scale can be interpreted as the relative change of the mean effect of NO_2 on mortality; the fraction of the curve below zero denotes a smaller estimate than the mean effect

exposure to NO_2, however, has only been examined in a small number of epidemiologic studies with a limited spatial scale. Our study provides pooled estimates of the concentration-response relation curves for short term exposure to NO_2 and total, cardiovascular, and respiratory mortality across various cities and countries. Results show that the curves are almost linear with no obvious thresholds, suggesting that the level of NO_2 below the current air quality guidelines is still hazardous to public health. We estimated that reduction in daily NO_2 concentration to a counterfactual zero level would reduce 1.25% of attributable deaths across the 398 cities. Although reduction of NO_2 to zero is infeasible, our analysis provides insight into the public health benefits of substantial reductions in NO_2, suggesting considerable health benefits from stricter control of NO_2 emissions and tightening of the regulatory limits of NO_2 in future revisions of WHO air quality guidelines.

Strengths and limitations of the study

This multilocation study has several advantages. Firstly, the study included 398 cities of 22 countries, providing enormous statistical power and ensuring the stability of the findings. Secondly, we examined the associations of NO_2 with not only total, but also cause specific, mortality. Thirdly, we pooled concentration-response curves covering a wide range of ambient NO_2 concentrations, the evidence from which can be considered generalisable. Fourthly, the uniform analytical approaches can aid in integrating and comparing the results across different regions and populations. Finally, our results contribute to the increasing evidence that supports independent health effects of NO_2 on total, cardiovascular, and respiratory mortality.

We acknowledge some limitations of this study. Firstly, given the vast difference in accessibility, most of the data on health and exposure were obtained from developed areas, such as Europe, North America, and East Asia. Thus global generalisation of the findings should be interpreted with caution, especially for areas with smaller study samples or none (that is, Africa and Latin America).

Secondly, this analysis was inherently a time series design that used ecological data and environmental measurements from fixed site monitors, and we could not completely rule out ecological fallacy and exposure misclassification. In addition, the assumption of this time series study is that the reconstructed daily levels of air pollution are a good representation of the average exposure across the whole population at the city level.[24 25] Under this condition, the estimates of aggregated analyses are affected by Berkson's bias and not measurement error bias, leading therefore to the correct point estimates and only an inflation of the standard errors.[26 27] Therefore, although the proximity of people to air quality monitors is unclear, the overall results would not be substantially biased.

Thirdly, there might have been slight changes in air pollution measurements in this multilocation study covering multiple decades, and the health data collection might be subject to diagnostic or coding errors. The effects of these problems on our results are difficult to evaluate, adding more uncertainty to cause specific mortality than to all cause mortality. The internal validity of each location specific dataset is presumed to be high, however, in that the changes in operating protocols, diagnostic practices, and coding have been accounted for by the statistical authorities in the various data gathering and preparation stages.

Fourthly, health outcomes were obtained only from their primary causes, and thus our analysis might underestimate the potential effect of NO_2 on total mortality. Finally, missing data were inevitable in such a multilocation study over a long period of time, but the amount of missing data was generally small for both health and exposure data, and its influence on our estimates is unlikely to be substantial (Appendix, eTable 7 and eResults 2.3).

Table 3 | Proportion of deaths attributable to reductions in daily NO_2 concentrations in each country or region

Country/region	Cities	Annual deaths (in thousands)*	Annual NO_2 concentrations ($\mu g/m^3$)†	PAF (%; 95% CI)
Australia	3	53.5	21.4	1.36 (0.76 to 1.96)
Brazil	1	61.1	84.9	2.87 (1.81 to 3.91)
Canada	25	115.9	23.7	1.45 (1.10 to 1.79)
Chile	3	35.2	21.6	1.13 (0.83 to 1.43)
China	15	200.3	46.5	2.61 (1.42 to 3.79)
Colombia	1	23.1	30.5	0.94 (0.04 to 1.84)
Czech Republic	1	12.3	30.8	1.39 (0.48 to 2.30)
Estonia	4	7.0	11.4	0.45 (0.07 to 0.83)
Finland	1	6.6	6.8	0.28 (0.05 to 0.51)
Germany	12	135.0	29.6	1.82 (1.37 to 2.27)
Greece	1	28.8	50.2	3.05 (1.79 to 4.29)
Japan	47	377.0	16.7	0.60 (0.31 to 0.89)
Portugal	5	44.9	14.9	0.76 (0.55 to 0.97)
Romania	8	41.4	25.6	0.70 (0.18 to 1.21)
South Korea	7	21.0	43.7	1.76 (0.51 to 3.00)
Spain	48	117.2	26.4	1.81 (1.34 to 2.29)
Sweden	1	9.6	26.8	1.46 (0.72 to 2.19)
Switzerland	8	12.4	32.3	0.78 (−0.21 to 1.75)
Taiwan	3	32.8	42.2	2.17 (1.17 to 3.15)
Thailand	18	84.3	22	0.70 (0.30 to 1.10)
United Kingdom	39	194.0	25.6	0.45 (0.11 to 0.78)
United States	147	985.4	28.7	1.63 (1.32 to 1.94)
Pooled	398	2598.7	26.9	1.23 (0.96 to 1.51)

NO_2=nitrogen dioxide; PAF%=population attributable fraction.
*Annual death numbers summed from the country specific time series data.
†Reductions in NO_2 concentrations were calculated as the difference between country specific annual NO_2 concentrations and the theoretically zero concentration.

Conclusions and policy implications

In summary, this multilocation time series analysis provides robust evidence for the independent associations of short term exposure to NO_2 with increased risk of total, cardiovascular, and respiratory mortality, although the total mortality burden might be underestimated as the study locations did not fully reach global coverage. The associations remained positive and statistically significant after adjusting for co-pollutants. The concentration-response curves were linear without discernible thresholds, suggesting a need to revise and tighten the current air quality guidelines of NO_2 for greater public health benefit, and to consider a regulation limit for daily mean NO_2 concentration. These findings contribute to a better understanding of how to optimise public health actions and strategies to mitigate air pollution.

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The lead authors (HaK and AG) affirm that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

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Web appendix: Online only supplements