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The effect of short-term exposure to O_3, NO_2, and their combined oxidative potential on mortality in Rome

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Abstract

There is large epidemiological evidence on the short-term health effects of O_3 and NO_2. These gaseous pollutants induce oxidative stress through their oxidative potential. Therefore, the evaluation of their combined oxidative capacity (O_x) has been proposed rather than studying the effect of either gas individually. To study the short-term effects of daily concentrations of O_3, NO_2, and O_x on mortality in Rome, in 2002–2015, daily deaths from the city mortality registry were analyzed along with O_3 and NO_2 levels observed in Rome and with estimated O_x and O_{wt} (O_x, weighted by the redox potential of O_3 and NO_2). A Poisson regression model was used considering trends, and meteorological and population changes. The effects on mortality were estimated at lag 0–1 and 0–5 for 10 μg/m^3. O_3 and NO_2 were associated with mortality, with the highest effects at lag 0–5, 0.81% (0.45–1.17) and 2.72% (2.07–3.37), respectively. O_x had an intermediate effect between the two gases. After adjusting for PM_{10}, O_{wt} had a stronger effect (1.72%; 1.14–2.30) than either gas, 0.86% (0.50–1.22) for O_3 and 1.61% (1.15–2.06) for NO_2. Both O_x and O_{wt} were associated with cerebrovascular, respiratory and, to a lesser extent, cardiac mortality more than either gas. These results suggest that the use of O_x (or O_{wt}) can provide a better assessment of the combined role of O_3 and NO_2 on mortality and can avoid the uncertainty of the threshold level for ozone. The brain and lungs seem to be the main targets of O_3 and NO_2.

Keywords O_3 · NO_2 · Combined oxidative potential · Daily mortality · Short-term exposure

Introduction

The potential to cause a redox reaction refers to the capacity of environmental factors to oxidize target molecules generating reactive oxygen species (ROS) (Ayres et al. 2008; Künzli et al. 2006; Møller et al. 2014). An increase of ROS could be considered as an early effect of air pollution (Thurston et al. 2017), but also as a first defense against oxidative potential, like inflammation. Recent research shows that a small increase of ROS activates biological processes to preserve physiological functions (Schieber and Chandel 2014). Therefore, oxidative stress could be considered as beneficial at low doses and harmful at moderate to high doses (Schieber and Chandel 2014).

Gaseous pollutants, such as gaseous ions, organic components, and secondary radicals, induce oxidative stress through their oxidative potential, but few studies (Delfino et al. 2013; Pavlovic et al. 2015; Steenhof et al. 2014; Weichenthal et al. 2016; Williams et al. 2014) have dealt with this topic due to objective difficulties in estimating oxidative potential from atmospheric gases. On the other hand, agreement has been reached on the ability of particulate matter (PM) to elicit oxidative potential by means of both chemical and biochemical tests (Ayres et al. 2008). The difficulty regarding gases consists essentially in the measurement given that redox active semi-volatile compounds such

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as ozone (O_3) and nitrogen dioxides (NO_2) cannot be fully captured by filters used to sample PM (Pavlovic et al. 2015).

As a result, different approaches—such as modeling oxidative potential (Yang et al. 2015) or estimating the intensity of oxidative stress in subjects exposed to air pollutants (Kelly 2003; Rodríguez-Cotto et al. 2015; Strak et al. 2012; Yang et al. 2016)—have been adopted to assess exposure to the oxidative potential of gases. It has been observed also that estimating the oxidative potential of gaseous pollutants separately, such as O_3 and NO_2, which interchange rapidly in the atmosphere in daylight, might weaken the estimate of their effects because the real exposure is the result of their combined oxidant capacity (Williams et al. 2014). This last point implicitly suggests moving beyond the limits of separate estimates, by assessing the effects of combined oxidant capacity of O_3 and NO_2, that is hypothesized as the true exposure to both gases, combining their mechanisms of action. Another approach estimates the joint effect from a multi-pollutant model by adding their coefficients, multiplied by their respective level increases (Winquist et al. 2014).

There is large epidemiological evidence on the health effects of O_3 and NO_2. Long-term exposure to high levels of O_3 has been related to cardiovascular (CV) and respiratory mortality (Schwartz 2016). In addition, two recent meta-analyses highlighted that both long-term (Faustini et al. 2014) and short-term exposures (Mills et al. 2016) to NO_2 are associated with mortality independent of PM. Some uncertainty persists about the ozone concentration–response relationship (Bae et al. 2015), supporting the hypothesis that combined oxidant capacity of O_3 and NO_2 could be a better measure of exposure and could be used in the exposure assessment of these gases.

We studied the short-term effects of daily concentrations of O_3, NO_2, and their combined oxidant capacity (O_x) on mortality in Rome, in 2002–2015. We also assessed potential confounding induced by co-exposures to PM_{10} and $PM_{2.5}$.

Methods

Deaths that occurred in the city of Rome (Italy) from 2002 to 2015 from natural (International Classification of Diseases, 9th revision—ICD-9: 1–799), cardiac (ICD-9 390–429), cerebrovascular (ICD-9 430–438), and respiratory causes (ICD-9 460–519) were collected from the mortality registry of Rome, which captures nearly 100% of the deaths that occur in the city among residents (personal communication of the registry operators). We selected deaths among subjects aged 35+ years only.

Hourly NO_2 and O_3 concentrations were available from three fixed monitoring stations of the Regional Environmental Protection Agency network, chosen to represent background levels of these pollutants throughout the whole city, for the entire period (1-1-2002 to 31-12-2015). Daily mean concentrations of PM_{10} (2002–2015) and $PM_{2.5}$ (only available for 2006–2015) were also collected. We selected the same stations for O_3 and NO_2 for the whole period. Daily monitored data needed to be 75% complete for inclusion. Missing values from a monitor were imputed using the average measurements from the other monitors on the same day, weighted by the ratio of the yearly average at that monitor to the yearly average of the others (Stafoggia et al. 2010).

Combined oxidative potential (O_x) was obtained by adding hourly NO_2 and O_3 levels from each station. Weighted oxidative capacity (O_{wt}) was obtained by adding gaseous concentrations, weighted by the oxidative potential of each gas according to the following formula:

$$O_{wt} = ((1.07\ V*NO_2) + (2.075\ V*O_3))/3.145\ V)$$

Daily means of each pollutant were calculated by averaging hourly monitor-specific measurements of NO_2, O_3, O_x, and O_{wt}. Finally, daily means for the whole city were estimated by averaging monitor-specific daily mean concentrations. Daily maximum 1-h levels of O_3, NO_2, and O_x were also calculated.

Daily temperature, humidity, and barometric pressure readings were provided by the Italian Air Force Meteorological Service. Apparent temperature was calculated from air temperature and dew point temperature from relative humidity, as indicated by Steadman (1979).

We used a Poisson regression model allowing for over-dispersion (McCullagh and Nelder 1989) to estimate the short-term effect of daily 10 $\mu g/m^3$ (20 ppm) rise of O_3, NO_2 levels and their combined oxidant capacity on daily natural and cause-specific mortality among 35-year-old residents in Rome at a cumulative lag interval of 0–1 day (consistent with an immediate effect), 2–5 days (delayed effect), and 0–5 days (prolonged effect). We modeled time trends with penalized regression splines (Wood 2000) with a number of six effective degrees of freedom per year to control for both long-term trends and seasonality.

Both apparent temperature and barometric pressure were adjusted for by including non-linear terms. High temperatures were defined as days with lag 0–1 temperature above the median and were modeled with a penalized spline with degrees of freedom chosen by a

generalized cross-validation method (Wood 2000). Low temperatures were defined as lag 1–6 days with temperature below the median and were modeled with another penalized spline. Barometric pressure was adjusted for with a penalized spline (lag 0) of the original variable (Stafoggia et al. 2010).

Influenza epidemics and population decreases during vacation periods were adjusted for using indicator variables: dichotomous variables were used for influenza days (as identified by the weekly national influenza surveillance) and for single-day holidays, while a three-level variable was used for summer vacation periods assuming value "2" in the 2-week period around the 15th of August, the value "1" from mid-July to the end of August, and the value "zero" elsewhere.

All results are expressed as percentage changes in mortality (and 95% CIs) per 10 μg/m^3 increment of the pollutants.

In addition, a two-pollutant model of O_3 and NO_2 was fitted to estimate the independent effects of O_3 and NO_2. Then, the joint effect of O_3 and NO_2 was computed from the two-pollutant model as the sum of their coefficients multiplied by 10 (Winquist et al. 2014). Results were compared with those from the combined O_x approach.

We performed a few sensitivity analyses: (1) we expressed the main estimates of O_3, NO_2, and combined O_x effects by IQR increases as an alternative metric in order to allow better comparison of effects across pollutants. Interquartile ranges (IQRs) were calculated as the difference between the third and first quartile of daily concentrations or combined O_x; (2) we adjusted the effects of O_3, NO_2, and combined O_x for daily PM_{10} or $PM_{2.5}$ (daily increases of 10 μg/m^3 at lag 0–5); (3) we excluded the year 2003, when summer temperatures in Rome were extremely high and persisted throughout the season.

All analyses were performed using STATA software (V.13.0, 2013, Texas, USA) and R software (V.3.1.3; R Core Team, 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/).

Results

Table 1 shows the distributions of the daily death counts, daily levels of ozone, nitrogen dioxide, their combined oxidant capacity (both 1-h maximum and daily 24-h mean), PM_{10} concentrations, and daily means of meteorological parameters. The bottom of the table displays Pearson correlation coefficients between pairs of all environmental variables.

In total, 300,644 natural deaths were included in the analysis, 30% from cardiac causes, 9% from cerebrovascular, and 6% from respiratory causes. The two gases showed similar daily means and 1-h maximum concentrations. O_3 displayed higher variability than NO_2, while O_x variability was halfway between the two gases. O_3 and NO_2 levels were negatively correlated. O_3 was positively correlated with O_x, while NO_2 was only marginally correlated with it. PM_{10} was positively correlated with NO_2 and negatively with O_3. The daily distribution of O_3, NO_2, and O_x levels (Supplementary Fig. 1) shows O_x levels higher than both gases, with NO_2 making the larger proportion of O_x at lower O_x and O_3 making inversely the larger proportion of O_x at higher O_x, suggesting that O_x levels might be most influenced by O_3 in hot seasons. In the summer (Supplementary Table 1), the levels of O_3, NO_2, and O_x (1-h) increase, while the variability of all pollutants decreases and O_3 (1-h) becomes positively related to PM_{10}. Figure 1 shows the relationship between daily mean O_3 and NO_2 concentrations, year-round and by season. $PM_{2.5}$ shows the same relationship with O_3, NO_2, and O_x as PM_{10} (Supplementary Table 2) though assessed over a shorter period (2006–2015).

Table 2 reports the results of the short-term effects of NO_2, O_3, and O_x on mortality. One-hour maximum ozone was associated with higher mortality, with the greatest effect seen (0.81%) at lag 0–5; NO_2 (both 1-h maximum and 24-h mean) showed a stronger association with mortality than O_3 at any lag, the greatest effect (2.72%) being at lag 0–5. The combined O_x levels (both 1-h and 24-h) showed greater effects than ozone and lower than NO_2, at any lag interval. In the summer, effects on mortality were higher for both gases but remained much higher for NO_2; O_x also presented values halfway between the individual gases, but still closer to ozone effects. No difference was found between the effects of O_x and O_{wt} on natural mortality, except for O_{wt} (1 h) effects in the hot season, which were higher than those of both gases.

Concentration–response curves for 1-h O_3, and 24-h mean NO_2, O_x, and O_{wt} (Fig. 2) show a non-linear shape between O_3 and mortality at low levels (plot a), as well as between NO_2 and mortality (plot b) at high levels. The concentration–response curve for O_x is suggestive of a linear relationship, while that for O_{wt} is more similar to O_3.

Results of the two-pollutant models for O_3 and NO_2 are reported in Fig. 3. Both O_3 and NO_2 were significantly associated with natural mortality, with ozone effects greater than single-pollutant models, whereas NO_2 effects were attenuated. Adding PM_{10} (Supplementary Fig. 2) or $PM_{2.5}$ (Supplementary Table 3) marginally

Table 1 Daily counts of natural and cause-specific mortality and distribution of daily environmental variables, Rome 2002–2015

	Days	Mean	SD	Minimum	Percentiles					Maximum	IQR	% missing
					5th	25th	50th	75th	95th			
Natural mortality	5113	58.8	10.5	25.0	43.0	52.0	58.0	65.0	77.0	106.0	13.0	0.0
Cardiac mortality	5113	17.5	5.1	3.0	10.0	14.0	17.0	21.0	27.0	43.0	7.0	0.0
Cerebrovascular mortality	5113	5.0	2.3	0.0	2.0	3.0	5.0	6.0	9.0	17.0	3.0	0.0
Respiratory mortality	5113	3.6	2.2	0.0	1.0	2.0	3.0	5.0	8.0	14.0	3.0	0.0
O_3 (daily mean)	5111	86.6	46.0	0.3	15.0	47.1	88.7	122.0	160.0	235.3	74.8	0.0
O_3 (daily 1-h maximum)	5111	172.7	76.9	2.0	49.0	115.0	174.0	224.0	302.0	477.0	109.0	0.0
NO_2 (daily mean)	5110	82.6	30.7	14.2	37.3	59.5	80.2	103.1	136.3	194.6	43.5	0.1
NO_2 (daily 1-h maximum)	5110	159.0	46.6	31.5	87.9	126.1	155.7	186.6	241.6	345.4	60.6	0.1
O_x (daily mean)	5107	170.7	34.4	79.7	118.4	145.8	169.0	192.7	230.5	333.1	46.9	0.1
O_x (daily 1-h maximum)	5107	234.8	59.3	93.5	153.6	191.8	225.8	268.2	345.0	591.2	76.3	0.1
PM_{10} (daily mean)	5111	32.9	14.7	4.0	14.9	22.9	30.0	40.0	60.7	174.1	17.0	0.0
Air temperature (°C)	5064	15.9	7.0	− 1.0	4.9	10.2	15.6	21.9	27.3	32.5	11.7	1.0
Barometric pressure (hPa)	5090	1014.3	7.7	982.3	1002.3	1010.3	1014.3	1018.3	1026.0	1253.4	8.0	0.4
	O_3 mean	O_3 maximum		NO_2 mean	NO_2 maximum		O_x mean	O_x maximum		PM_{10}	Temperature	Pressure
O_3 (daily mean)	1.00											
O_3 (daily 1-h maximum)	0.89	1.00										
NO_2 (daily mean)	− 0.68	− 0.46		1.00								
NO_2 (daily 1-h maximum)	− 0.44	− 0.22		0.86	1.00							
O_x (daily mean)	0.75	0.81		− 0.03	0.18		1.00					
O_x (daily 1-h maximum)	0.55	0.74		0.11	0.31		0.86	1.00				
PM_{10} (daily mean)	− 0.37	− 0.23		0.60	0.55		0.04	0.21		1.00		
Air temperature (°C)	0.69	0.74		− 0.51	− 0.35		0.48	0.50		− 0.15	1.00	
Barometric pressure (hPa)	− 0.19	− 0.06		0.34	0.35		0.06	0.22		0.40	− 0.06	1.00

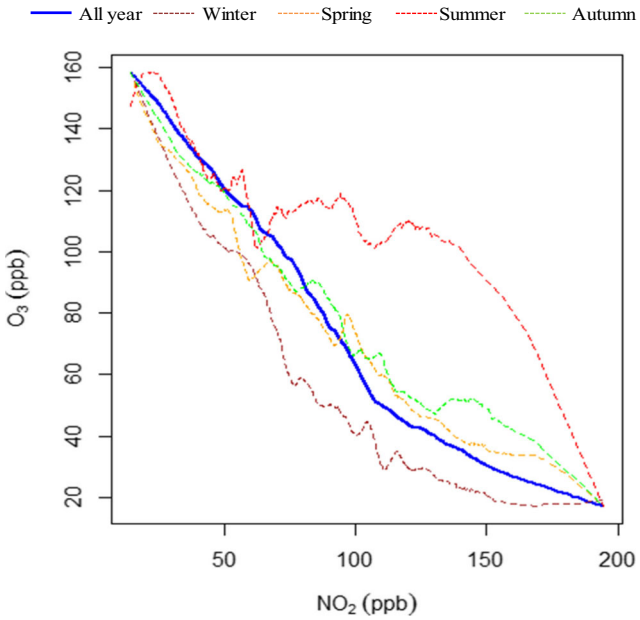

Fig. 1 Scatter plot of daily mean O_3 and NO_2 concentrations, Rome 2002–2005, all year

decreased the effects of both gases and their O_x, suggesting gases and particulate effects are independent of each other. We did not find evidence of effect modification of PM_{10} on the O_x-natural mortality association (Fig. 4).

Mortality from cardiac, cerebrovascular, and respiratory diseases (Table 3) shows characteristics very similar to those of natural mortality: it increases with rising O_3 (1 h only), NO_2, and O_x (1-h and 24-h); the highest estimates are at lag 0–5 for all causes, and O_x shows estimates halfway between O_3 and NO_2. However, mortality from respiratory and cerebrovascular causes is higher than mortality from cardiac mortality and mortality from all natural causes. These effects are more evident when O_{wt} is used. In addition, after adjusting for PM_{10} (Supplementary Fig. 2, Fig. 4), cerebrovascular mortality seems almost entirely due to O_x while respiratory mortality recognizes an independent contribution from O_x and PM_{10}.

Table 2 Associations between NO_2, O_3, and oxidative potential (Ox) with natural mortality: % increase of risk (% IR) and 95% CI, per 10 μg/m^3 increases in pollutant levels

		All year			April–September		
	Lag	% IR	95% CI		% IR	95% CI	
O_3 daily mean	0–1	0.04	−0.37	0.46	0.65	0.06	1.25
	2–5	−0.33	−0.83	0.17	0.09	−0.63	0.82
	0–5	−0.27	−0.86	0.32	0.54	−0.31	1.40
O_3 daily 1-h maximum	0–1	0.66	0.41	0.91	1.13	0.79	1.47
	2–5	0.40	0.09	0.71	0.64	0.25	1.04
	0–5	0.81	0.45	1.17	1.34	0.86	1.82
NO_2 daily mean	0–1	1.67	1.18	2.17	2.74	1.96	3.52
	2–5	1.82	1.29	2.36	2.86	1.96	3.76
	0–5	2.72	2.07	3.37	4.32	3.26	5.39
NO_2 daily 1-h maximum	0–1	0.89	0.61	1.16	1.44	1.02	1.85
	2–5	1.02	0.71	1.32	1.18	0.69	1.68
	0–5	1.57	1.20	1.95	2.06	1.47	2.65
O_x daily mean	0–1	1.35	0.92	1.78	1.98	1.43	2.53
	2–5	1.33	0.84	1.82	1.55	0.92	2.18
	0–5	1.98	1.42	2.56	2.56	1.83	3.29
O_x daily 1-h maximum	0–1	0.83	0.59	1.06	1.22	0.91	1.52
	2–5	0.82	0.55	1.08	0.91	0.56	1.26
	0–5	1.28	0.96	1.60	1.60	1.19	2.02
O_{wt} daily mean	0–1	1.36	0.65	2.08	2.49	1.55	3.43
	2–5	1.16	0.31	2.02	1.75	0.66	2.85
	0–5	1.93	0.94	2.93	3.17	1.89	4.46
O_{wt} daily 1-h maximum	0–1	1.26	0.85	1.67	1.78	1.28	2.28
	2–5	1.20	0.72	1.68	1.25	0.68	1.82
	0–5	1.92	1.36	2.49	2.30	1.62	2.99

CI confidence interval

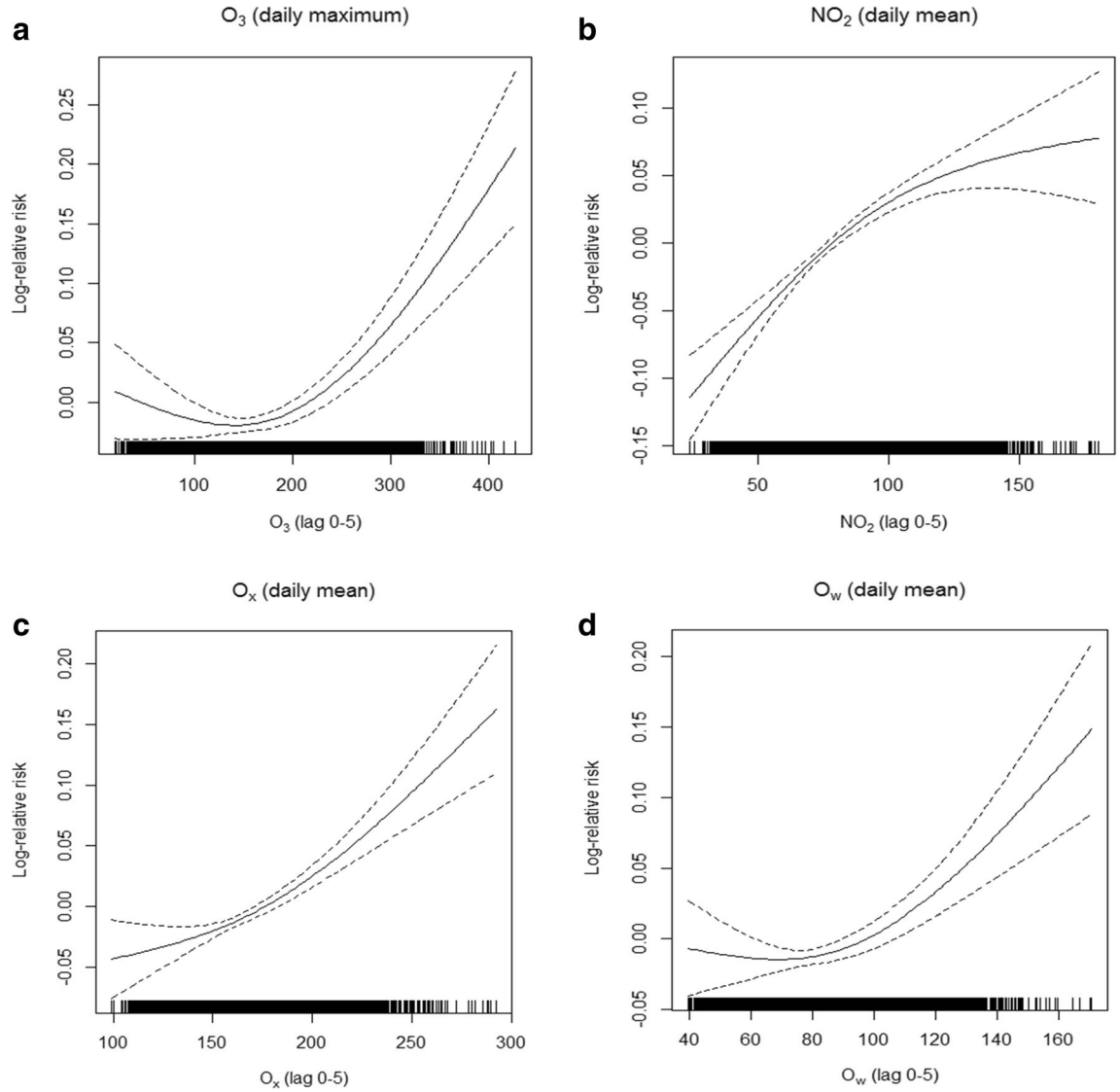

Fig. 2 Association between daily O_3, NO_2, oxidative potential (O_x), and oxidative potential after redox weighting (O_{wt}) with natural mortality: dose–response relationships. Pollutants at lag 0–5. **a** Daily 1-h maximum O_3, lag 0–5; (**b**) daily mean NO_2, lag 0–5; (**c**) daily mean O_x, lag 0–5; (**d**) daily mean O_{wt}, lag 0–5

Figure 3 shows that O_3 and NO_2 have an independent effect on mortality when added together in a model, and the effect is very similar for both gases, but differs greatly from the single pollutant model, due to the complex relationship between these gases during the day. It is interesting in this respect to highlight that the lag 0–5 and the daily mean levels of the pollutants prove to give a smoothed but realistic measure of gases' single effect in this study. The effect of combined oxidative potential is almost twice what was expected, when calculated both as O_x or as O_{wt}. Finally, the joint effect obtained using the combined O_x and that of summing the estimates from the two-pollutant model show very similar results.

Sensitivity analyses show that using IQR as the metric of exposure (Supplementary Fig. 3) gives higher estimates but does not change the characteristics of O_3, NO_2, or O_x impact, while excluding the year 2003 gives a bit lower estimate but does not significantly modify the result.

Discussion

Our results show that ozone (1-h) and nitrogen dioxides (both 1-h and 24-h) are associated with mortality, reaching the highest effects at lag 0–5 and in the hot season. Combined O_x has an effect comprised between that of the two gases, while weighted oxidative capacity has a stronger effect on mortality than either of the two gases when 1-h levels are considered, which remains significant after adjusting for PM_{10} or $PM_{2.5}$. Our

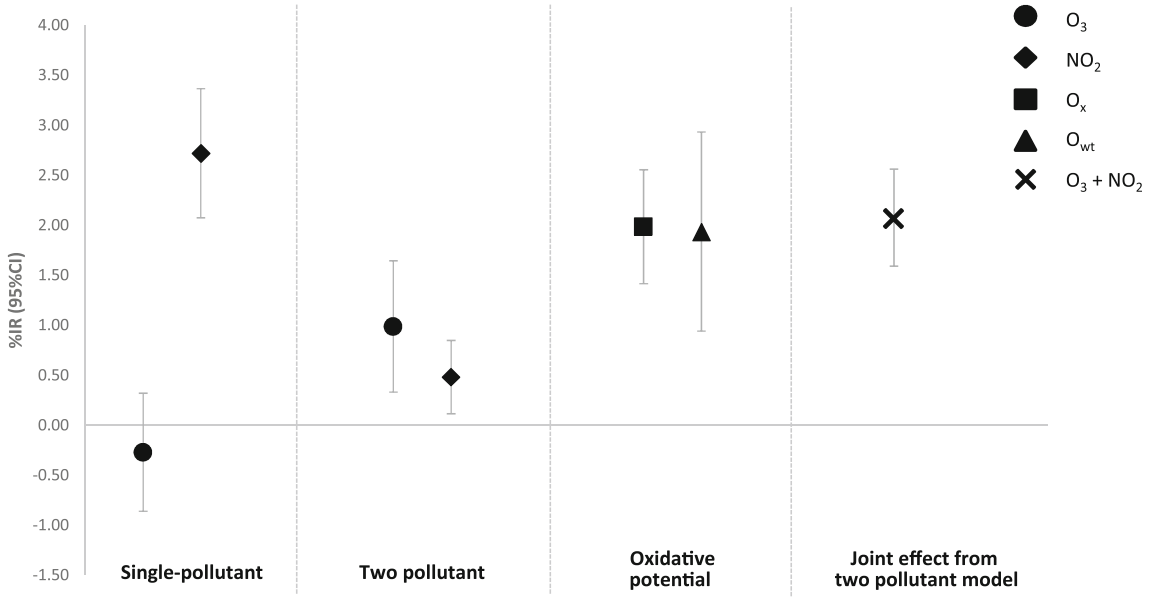

Fig. 3 Associations between O_3, NO_2, O_x, and O_{wt} (daily mean) with natural mortality (lag 0–5): % increase of risk (% IR), and 95% CI, per 10 mg/m^3 increases in the pollutants. "Single pollutant" refers to models where O_3 and NO_2 are added one at a time; "two-pollutant" refers to a model where O_3 and NO_2 are added together; "oxidative potential" is a single-pollutant model where the sum of O_3 and NO_2 (un-weighted, O_x; or redox-weighted, O_{wt}) is added as exposure term; "joint effect from two-pollutant model" uses the joint effect of O_3 and NO_2 computed as the sum of their coefficients multiplied by 10, from two-pollutant model

results suggest that both O_x and O_{wt} are associated with high cerebrovascular and respiratory mortality and, to a lesser extent, cardiac mortality, more than either gas, at both 1-h and 24-h exposures.

The effects of both O_3 and NO_2 on mortality today are widely recognized (Schwartz 2016). Yet their oxidative potential has been studied less, although it deserves more attention given their ability to trigger oxidative stress, which is an early effect of air pollution (Thurston et al. 2017) and, in turn, a mediator of epigenetic damage (Møller et al. 2014). Moreover, using cumulative oxidative potential of more than one pollutant streamlines the process since it is one exposure factor, yet estimates the toxicity of all the pollutants (Ito et al. 2007).

Our results show that the correlation between O_3 and NO_2 is basically negative, while that of O_3 with PM_{10} is negative and between NO_2 and PM_{10} is positive. These relationships should be interpreted by referring to the three fundamental reactions between O_3 and NO_x (Williams et al. 2014). These reactions interchange O_3 and NO_2, but lead to no net production of O_3 in the absence of "smog" episodes in hot weather when the correlations between the two gases and with PM change, as we also observed in this study. Zeka and Schwartz (2004) underlined the consequence that these relationships could have on the interpretation of two-pollutant effects: when pollutants are correlated and affected by measurement errors (as in the case of air pollutants), the measurement error of the second pollutant affects that of the first and the direction of the bias depends on the sign of the pollutants' correlations. Finally, Ito et al. (2007) offer the more general comment that if multi-pollutant effects could be predicted from the model specifications of their interactions, it is likely that multi-pollutant effects could reflect the toxicity of the mixture for which the pollutants are a surrogate, rather than the relative effects of the pollutant.

In our results, the effect on mortality of weighted oxidative potential is greater than it was from either pollutant by itself, as has been reported in previous papers (Weichenthal et al. 2016; Williams et al. 2014); however, the exposure metric differs among studies: O_x effects were larger than those of single pollutants as observed for 24-h levels in previous studies, while we found larger effects of O_x for 1-h levels. Williams et al. (2014) found higher effects for O_3 than for NO_2 (for both 24-h and 1-h), while we found larger effects for NO_2 than for O_3, in spite of the higher redox potential of O_3. Possible explanations are that NO_2 acts via other mechanisms on health effects (Williams et al. 2014) or even that the 1-h maximum levels of the two gases do not occur at the same time of day. On the other hand, the results we obtained for O_{wt} are consistent with those observed in a London study of O_x (Williams et al. 2014).

We chose to study 1-h levels when exposures get the highest values and 24-h levels when the complex reactions between O_3 and NO_2 occur, allowing a greater assessment of population exposure. Other European studies such as APHEA-1 and 2 (Gryparis et al. 2004; Samoli et al. 2006) mostly used 8-h mobile means for both O_3 and NO_2; however, 8-h mobile

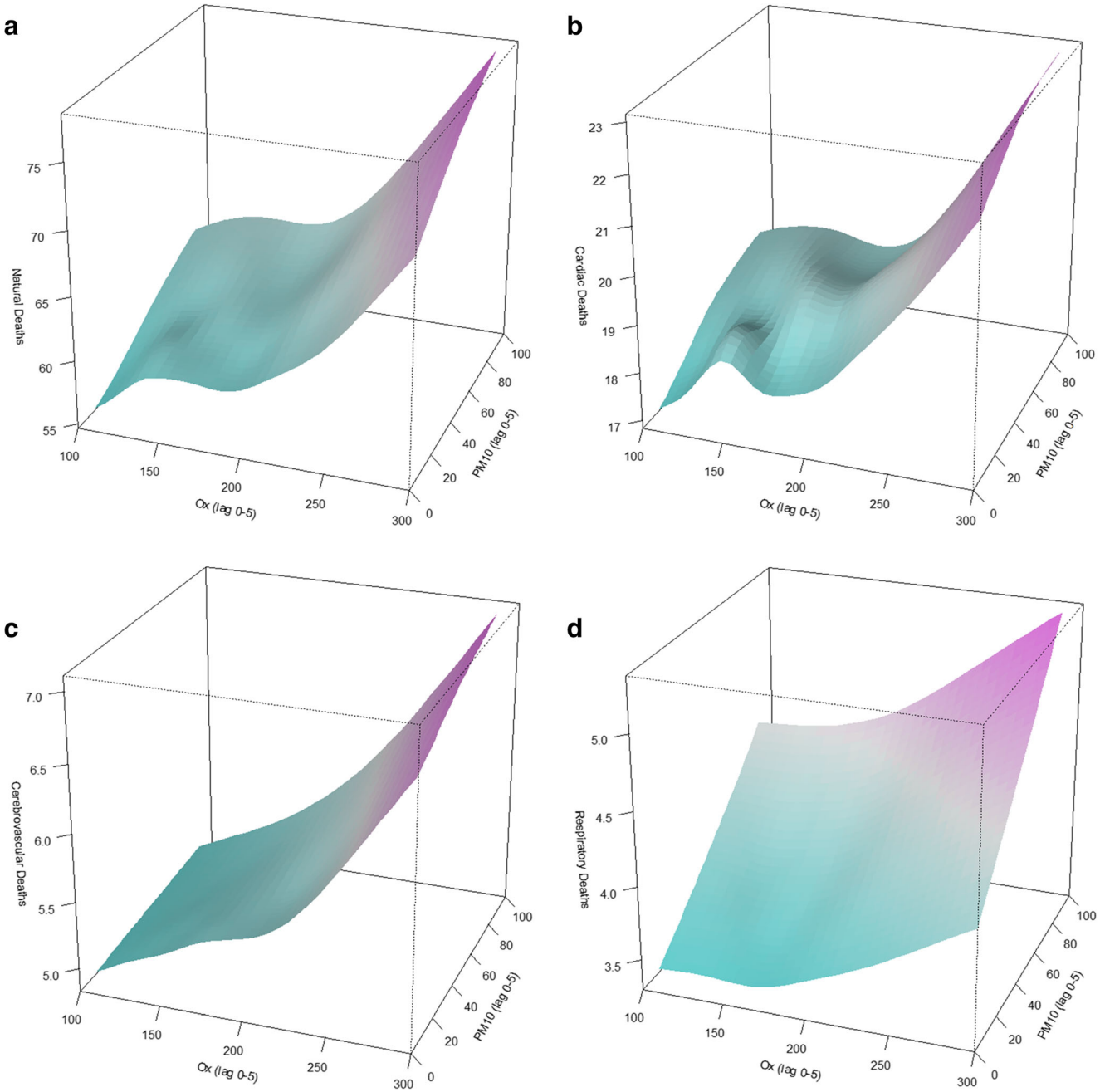

Fig. 4 Joint association between daily mean O_x and PM_{10} (lag 0–5) with cause-specific mortality. **a** Natural mortality; (**b**) cardiac mortality; (**c**) cerebrovascular mortality; (**d**) respiratory mortality. All the figures were performed using R, version 3.4.2 (R Core Team 2011. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. Available: http://www.R-.project.org (accessed 18 January 2017)).

means are only slightly lower than 1 h (Supplementary Fig. 1) in our data, and even so would not have avoided assessing O_3 and NO_2 at different times of the day.

Our two-pollutant analysis shows that both gases individually have an effect on mortality, with O_3 having a larger impact, possibly due to its stronger oxidative potential. Previous studies do not present homogeneous results. Mortality due to NO_2 did not change substantially when analyzed in the same model with O_3 8-h in European studies (Gryparis et al. 2004; Samoli et al. 2006), neither was any change observed in a London study (Williams et al. 2014). These observations suggest that the effects of O_3 and NO_2 were independent, but mortality associated with both gases was higher when 24-h levels were used, suggesting prudence in interpreting the relative effects of O_3 and NO_2. In the New York study (Ito et al. 2007), adding O_3 to a multi-pollutant model did not increase the low multi-collinearity of NO_2 among all pollutants. In addition, using a single factor as

Table 3 Associations between NO_2, O_3, and O_x with cause-specific mortality: % increase of risk (% IR), and 95% CI, per 10 μg/m^3 increases in pollutant levels

		Cardiac mortality			Cerebrovascular mortality			Respiratory mortality		
	Lag	% IR	95% CI		% IR	95% CI		% IR	95% CI	
O_3 daily mean	0–1	0.03	− 0.72	0.78	0.63	− 0.72	1.99	− 0.43	− 2.02	1.19
	2–5	− 0.50	− 1.39	0.41	0.68	− 0.93	2.32	− 0.06	− 1.98	1.90
	0–5	− 0.43	− 1.49	0.63	1.01	− 0.89	2.95	− 0.29	− 2.55	2.02
O_3 daily 1-h maximum	0–1	0.64	0.18	1.09	1.20	0.40	2.01	0.76	− 0.23	1.76
	2–5	0.66	0.11	1.22	1.01	0.04	2.00	1.22	0.01	2.45
	0–5	1.04	0.39	1.70	1.71	0.56	2.87	1.64	0.23	3.08
NO_2 daily mean	0–1	1.02	0.14	1.91	1.53	− 0.04	3.13	1.66	− 0.23	3.58
	2–5	2.26	1.31	3.23	1.39	− 0.30	3.11	3.19	1.14	5.28
	0–5	2.81	1.65	3.97	2.27	0.22	4.35	4.01	1.53	6.55
NO_2 daily 1-h maximum	0–1	0.51	0.02	1.01	1.37	0.49	2.26	0.39	− 0.66	1.45
	2–5	1.28	0.73	1.84	0.87	− 0.11	1.86	1.81	0.64	3.01
	0–5	1.62	0.95	2.30	1.77	0.56	2.99	2.07	0.62	3.53
O_x daily mean	0–1	0.92	0.15	1.70	1.78	0.41	3.16	0.83	− 0.84	2.52
	2–5	1.60	0.72	2.50	2.06	0.50	3.64	2.94	0.99	4.91
	0–5	2.03	1.00	3.06	2.84	1.03	4.69	3.10	0.86	5.40
O_x daily 1-h maximum	0–1	0.60	0.17	1.03	0.78	0.03	1.53	0.78	− 0.15	1.72
	2–5	1.15	0.67	1.63	1.06	0.22	1.90	2.30	1.26	3.36
	0–5	1.48	0.91	2.06	1.47	0.46	2.48	2.70	1.44	3.97
O_{wt} daily mean	0–1	0.94	− 0.34	2.23	2.31	0.03	4.65	0.44	− 2.30	3.25
	2–5	1.31	− 0.23	2.87	2.91	0.17	5.74	3.05	− 0.32	6.53
	0–5	1.85	0.07	3.67	4.02	0.82	7.31	3.02	− 0.86	7.05
O_{wt} daily 1-h max	0–1	0.94	0.20	1.68	0.27	2.85	1.53	1.50	− 0.11	3.13
	2–5	1.72	0.86	2.60	0.41	3.44	1.90	3.31	1.39	5.26
	0–5	2.24	1.22	3.28	0.92	4.52	2.48	4.04	1.78	6.36

CI confidence interval

O_{wt} seems to give a more "true" picture of the effects of the NO_2–O_3 combination because it takes into account the toxicity of both gases (Williams et al. 2014).

We would add that using only one factor as O_{wt} could provide a better estimate because it does not require assumptions of independence or linearity as a multi-pollutant analysis does. In summary, if estimating the joint effect of more pollutants in the same environment is important to assess the true impact on human health (Winquist et al. 2014), the combined oxidative potential of more pollutants presents the advantages of being a single exposure factor and at the same time of summarizing exposure to more pollutants. The high similarity with the joint effect from the two-pollutant model suggests further analyses of these approaches would be beneficial.

The concentration–response functions for O_3 and NO_2 suggest in our results a non-linear relationship for single pollutants, whereas O_x does not deviate significantly from linearity, thus supporting the above hypothesis that it could provide a more accurate estimate of the NO_2–O_3 combination. A non-linear relationship of O_3 and even of O_x effects has been observed in the London study (Williams et al. 2014) and other authors (Atkinson et al. 2012; Bae et al. 2015) found clear evidence of a threshold in the relationship between ozone and mortality at very similar levels of 40 ppb and 65 μg/m^3, respectively. The use of the combined oxidative potential of both gases seems to solve the problem of the threshold of ozone effects since low or zero ozone concentrations in urban areas arise because the ozone is converted to NO_2. Even so, the concentration–response function between ozone and mortality still requires further assessment also to take into account the collinearity between gases.

Finally, the results of cause-specific analyses offer interesting observations. Specific-cause mortality is not yet been exhaustively explored with combined gaseous oxidative potential. One possible explanation for the stronger effects on cerebrovascular and respiratory mortality when compared with cardiovascular mortality is that the brain and the lung are specific targets for O_3 and NO_2 oxidative potential or are more sensitive to the oxidative potential of these gases. In addition, the almost exclusive involvement of O_x in increasing cerebrovascular mortality (compared with PM), as well as the independent contribution of both

O_x and PM to increase respiratory mortality, suggests the important and diverse role of oxidative potential of gases on human health. Also, a clear seasonality of the diseases could explain some of the results. While O_x has higher effects in the hot season, all specific causes of mortality are more frequent in the cold season: more people die in the winter from cardiac disease (von Klot et al. 2012). Seasonal differences in stroke incidence are conflicting and might depend on stroke subtype rather than on season itself (Takizawa et al. 2013). Respiratory mortality is clearly influenced by the cold season: a linear relation between influenza incidence and excess mortality has been demonstrated also considering changes in mortality baselines (Goldstein et al. 2012).

There are possible limits of our analysis: first, the effect modifiers could have not been adequately addressed given that adequate evidence supports ozone-related effects for conditions we did not analyze here, such as certain genotypes, pre-existing asthma or certain nutritional deficiencies, and age (Vinikoor-Imler et al. 2014). Second, other gaseous compounds not analyzed here such as polycyclic aromatic hydrocarbons and quinones also contribute to oxidative potential.

We can conclude that combined O_x is able to capture a combined effect of O_3 and NO_2 on mortality that could be more representative of the true situation than that given by either pollutant individually or the two-pollutant analysis. In addition, the use of O_x can avoid the uncertainty of the threshold level for ozone. The important role that oxidative potential shows in increasing cerebrovascular and respiratory mortality suggests that these systems could be a specific target for O_3 and NO_2 oxidative potential.

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Compliance with ethical standards

Conflict of interests The authors declare that they have no competing interests.

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