ORIGINAL ARTICLE

Short-term effects of ozone air pollution on ischaemic stroke occurrence: a case-crossover analysis from a 10-year population-based study in Dijon, France

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Occup Environ Med 2007;**64**:439–445. doi: 10.1136/oem.2006.029306

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Accepted 15 December 2006
Published Online First 4 April 2007

Objective: To evaluate the association between air pollutants and the occurrence of acute stroke from 10-year population-based study.

Methods: The daily stroke count was obtained from Dijon Stroke Register between March 1994 and December 2004. The register recorded all first-ever strokes among residents of Dijon (150 000 inhabitants) in France, using standard diagnostic criteria. Pollutant concentrations (SO_2, CO, NO_2, O_3 and PM_{10}) were measured hourly. A bi-directional case-crossover design was used to examine the association between air pollutant and stroke onset. The conditional logistic regression model included the meteorological parameters (temperature, relative humidity), influenza epidemics and holidays.

Results: The authors collected 493 large artery infarcts, 397 small artery infarcts, 530 cardio-embolic infarcts, 67 undeterminate infarcts, 371 transient ischaemic attacks and 220 haemorrhagic strokes. For single-pollutant model and for a 10 mg/m^3 increase of O_3 exposure, a positive association was observed only in men, over 40 years of age, between ischaemic stroke occurrence and O_3 levels with 1-day lag, (OR 1.133, 95% CI 1.052 to 1.220) and 0-day lag (OR 1.058, 95% CI 0.987 to 1.134). No significant associations were found for haemorrhagic stroke. In two-pollutant models, the effects of O_3 remained significant after each of the other pollutants were included in the model, in particular with PM_{10}. A significant association was observed for ischaemic strokes of large arteries ($p = 0.02$) and for transient ischaemic attacks ($p = 0.01$). Moreover, the authors found an exposure-response relations between O_3 exposure and ischaemic stroke (test for trend, $p = 0.01$). An increase in association in men with several cardiovascular risk factors (smoker, dyslipidemia and hypertension) was also observed.

Conclusion: These observational data argue for an association between ischaemic stroke occurrence and O_3 pollution levels; these results still need to be confirmed by other studies.

Numerous epidemiological studies have shown the potential deleterious effects of ambient air pollution on health.[1–10] Consequences have been clearly described in terms of cardiovascular mortality and morbidity.[1–11] Several pathophysiological hypotheses have been suggested to explain the increased risk of cardiovascular diseases including effects on blood coagulation, endothelial dysfunction, systemic inflammatory responses, acute arterial vasoconstrictions or a propensity for arrhythmias.[11] Consequences of these effects may be alterations of atheromatous plaque, enhanced potential for acute thrombosis or acceleration of the progression of atherosclerosis.[11] It is reasonable to hypothesise that similar consequences might exist for strokes. However, little is known about occurrence of stroke in relation to air pollution. A few recent studies have reported an association between stroke mortality and pollution.[12–15] However, the significant relative risks observed were weak and one study failed to confirm the association.[4] Furthermore with mortality indicators, we do not know whether air pollution is a causative factor of stroke occurrence or only a precipitating factor of death after stroke, because data are analysed with the date of death rather than that of stroke onset. The studies looking into an association between hospital admissions for stroke and air pollution also generated conflicting results: some studies point to an association,[6 16–18] and others do not.[2 4 19 20] Lastly, the indicators used in these studies (mortality, hospital admission) may be sources of specific biases.[21] For these reasons, these previous results must be confirmed thoroughly.

For this study, we hypothesise that air pollutants may have significant effects on stroke onset. To examine the nature of this relation, we used morbidity indicators from a population-based register in Dijon, France. The aim of the study was to investigate the relation in the short term between urban air pollution and stroke incidence.

MATERIALS AND METHODS

Register data

The daily stroke count and stroke subtype counts were obtained from Dijon Stroke Register between 1 March 1994 and 31 December 2004. The register recorded all first-ever strokes among residents of Dijon, France. A detailed description concerning data collection has been published.[22] In brief, stroke was defined according to World Health Organization recommendations and uniform criteria (TOAST classification) for diagnosis were used for the duration of this study.[23 24] Several information sources declared the occurrence of strokes: departments of the university hospital, private hospitals, private radiological centres, neurologist, general practitioners, death certificates. The ischaemic or haemorrhagic mechanism was identified by CT scan or by magnetic resonance imaging (MRI) in more than 98% of the cases. We also collected vascular risk factors:[22] sex, age, hypertension (previous history of known hypertension with or without antihypertensive treatment, based on >160/095 mmHg before stroke), ischaemic heart disease (history of angina pectoris or myocardial infarction or ischaemic features on ECG), cardiac arrhythmia (history of

paroxysmal atrial fibrillation or flutter confirmed by electrocardiogram or diagnosed on ECG or holter recording), diabetes mellitus (fasting plasma glucose level ⩾7.8 mmol/l or patients who had been treated with insulin or oral hypoglycemic), dyslipidemia (total cholesterol level ⩾6.0 mmol/l or treatment with lipid-lowering drugs) and smoking history (ex-smoker and current smoker). Two-dimensional echocardiography was performed to detect possible cardio-embolic source. Carotid and vertebral ultrasonography were routinely performed. Diagnosis of the subtypes of stroke was always performed on clinical and cerebral imaging:[24] ischaemic stroke from atheroma of large arteries; ischaemic stroke from atheroma of small arteries, so-called lacunar infarct; ischaemic stroke from cardiac embolism; primary cerebral haemorrhagic stroke; subarachnoid haemorrhagic stroke. When it was difficult to differentiate ischaemic stroke from atheroma of large arteries, lacunar infarct and cardiac embolism, consensus meetings were performed to classify the difficult cases in one of the three groups. A transient ischaemic attack was diagnosed when clinical symptoms disappeared within 24 h without any infarct lesion on imaging.

Air pollution and meteorological parameters measurements

Dijon is located in the Burgundy region (France). The study population (1999 census) was 150 000 inhabitants in the whole area covered by register (40 km^2) with a population density of 3710 people/km^2. Its economic activity mainly consists of a large tertiary sector and services. Dijon is not considered as a particularly polluted town. Air pollution is mainly from motor vehicle exhaust emission and the displaced pollution from other regions for ozone pollution, with industrial pollution playing a smaller part. Dijon has a four-season climate and an annual temperature range of −14.5℃ to 39.3℃. The daily meteorological information (temperature, relative humidity) was obtained from the National Meteorological Office.

Air pollution data were provided by Dijon Environmental Monitoring Center. Exposure measurements during the study period were taken at the background monitoring station located in the town centre. The station was remote from the main sources of urban pollution (major roads, industrial areas). The following atmospheric pollutants were measured: particulate matter of aerodynamic diameter ⩽10 μm (PM_{10}) by β-ray absorption, ozone (O_3) by ultraviolet photometry, sulphur dioxide (SO_2) by ultraviolet fluorescence, nitrogen oxides (NOx) by chemoluminescence and carbon monoxide (CO) by non-dispersive infrared photometry. All pollutant data were available during the whole study period except the PM_{10} data which were available from 15 March 1999. We calculated the hourly mean of each pollutant expressed in μg/m^3 then computed their 24-h averages except O_3 for which the means were calculated over 8-h daytime periods.

The missing values for each pollutant were replaced with predictions from multiple linear regression integrating the following predictive variables: minimum temperature, minimum humidity, year, season and holidays. The proportion of missing data for all the pollutants was 3.4%. Thus the effect of the missing observations was probably small. However, testing was done to control the results with missing values and the results with predictions from this regression. No differences were detected. Furthermore, data on influenza epidemics (weekly count) were obtained from the Sentiweb monitoring network of the Burgundy region.

Statistical analysis

A bi-directional case-crossover design was used to examine the association between air pollutants and stroke onset (Maclure,[25] Jaakkola[26]). This design was developed to study the effects of short-term exposures on the risk of acute events.[25] In our study, cases were all subjects over 40 years of age presenting with a first-ever stroke during the studied period and in Dijon. Here, each subject served as his own control. The design focused on the point in time when the event occurred. Thus, the covariate levels at the time of stroke occurrence (case day) were compared with levels obtained in a period chosen before and after the stroke onset (control days). The control days were selected to represent the usual exposure levels in the source population that produce the strokes. Moreover, we used a bi-directional control period before and after the day of stroke occurrence. This allowed for individual adjustment for seasonality, longer-term trends and days of the week.[26] The control days were selected 7, 14, 21 and 28 days before and after the case day.[26]

Conditional logistic regression analysis was performed to estimate the association between stroke occurrence and air pollutants.[26 27] Thus, adjusted odds ratios (ORa) and their 95% confidence intervals (95% CI) were calculated. Each case day was matched to eight control days (coded as case day = 1 or control day = 0). As each subject serves as his own control, the individual factors (for example, age, sex) cannot be integrated into a logistic regression model. But stratified analyses by subgroup according to individual factors (sex, age, cardiovascular risk factors) were carried out to identify individuals susceptible to the effects of air pollution. Furthermore, several studies have observed an increase of stroke occurrence during influenza epidemics,[28] fewer strokes during holidays,[29] and variations in stroke incidences according to ambient temperature levels and seasons.[30] This is why the ORs were systematically adjusted for relative humidity (minimum or maximum in percentage), temperature (minimum or maximum values in degrees Celsius), influenza epidemics (number of incident cases per week) and holidays. The quantitative covariates (temperature, humidity, influenza) were entered into the models as continuous variables. Holiday variables were controlled through the use of binary variable (coded as holiday = 1 or no holiday = 0). After inclusion of lagged effects up to three days or averaged on 1–3 days for temperature (minimum or maximum) and relative humidity (maximum or minimum), the choice of logistic models was based on Akaike's information criterion.[31] Finally, minimum temperature and maximum relativity humidity were included with 1-day lag. Holiday and influenza epidemics were included in models without lagged day. Interaction between temperature and humidity was also investigated. For each pollutant, the OR of stroke occurrence was given for a continuous variation with an increment of 10 units (μg/m^3) of pollutant concentration. The pollutant exposure was tested in models for the day of stroke onset (D0), for 1-day lag (D-1), for 2-day lag (D-2) or 3-day-lag (D-3). Also, the assumption of linearity in the logit was specifically checked to O_3 variable.[27] We created a new categorical variable with five levels using four cutpoints based on the quintiles. We then explored (test for trend) the exposure-effect relation across these five exposure levels. The first quintile was used as the reference group. Using the method of fractional polynomials, we used the appropriate scale for O_3 variable in its linear form.[27]

The goodness-of-fit of the model was tested with the likelihood ratio test at the 5% level of significance.[27] The measures $\Delta\chi^2$ of Hosmer–Lemeshow and ?β of Pregibon were used to check the potential outliers.[27] No particular outliers were observed in our data. The absence of excessive co-linearity between the continuous variables (temperature, relative humidity, pollutant, influenza) was also verified using the variance influence factor (VIF<10).[31] No variables involved in a

Table 1 Clinical characteristics of study population, Dijon stroke register (France) from 1994 to 2004

	Ischaemic stroke		Haemorrhagic stroke	
	Men	Women	Men	Women
	n = 689	n = 798	n = 115	n = 105
Age				
Median (years)	73.3	79.6	74	76.9
Medical history	n (%)	n (%)	n (%)	n (%)
Coronary artery disease	152 (22.4)	156 (19.8)	23 (20.0)	13 (12.6)
Cardiac arrhythmia	126 (18.7)	239 (30.4)	22 (19.6)	21 (20.8)
Hypertension	436 (64.3)	506 (64.0)	66 (57.4)	53 (51.5)
Diabetes	121 (17.9)	123 (15.6)	22 (19.1)	12 (11.5)
Dyslipidemia	192 (28.4)	175 (22.3)	25 (21.7)	22 (21.1)
Smoker*	375 (67.4)	55 (9.3)	39 (53.4)	6 (10.7)

*Ex-smokers and current smokers.

significant interaction were detected. For this study, the analysis was addressed to people ⩾40 years of age. The data were analysed using the Stata software package (version 8.2, Stata Corporation 2003).[31]

RESULTS

During the study period, we collected 493 large artery strokes, 397 lacunar strokes, 530 cardioembolic strokes, 371 transient ischaemic attacks and 67 strokes of indeterminate aetiology. There were 220 haemorrhagic strokes with 174 cerebral haemorrhages and 46 subarachnoid haemorrhages. In 23 cases (1.3%) CT scan or MRI was not performed. Table I presents clinical characteristics of the study population. We observed that women were older than men, particularly for ischaemic strokes (median of 79.6 vs 73.3 years). Women were also characterised by a lower proportion of smokers (9.3% vs 67.4%) and by a higher proportion of cardiac arrhythmia (30.4% vs 18.7%).

The distribution of air pollutants and meteorological parameters is shown in table 2. Spearman correlation coefficients (r_{sp}) were ranged from 0.58 to 0.70 between PM_{10}, SO_2, NO_x measures, from −0.58 to −0.62 with O_3 and these pollutants and $r_{sp} = -0.29$ between O_3 and PM_{10}. Also, correlation coefficient between temperature minimal and maximal relative humidity was $r_{sp} = -0.23$, and between minimal temperature and O_3 the correlation coefficient was $r_{sp} = 0.57$.

The associations between air pollutants and the occurrence of stroke are presented in table 3. We observed a significant association between ischaemic stroke occurrence and O_3 levels with 1-day lag (ORa 1.075, 95% CI 1.022 to 1.131). This result was confirmed for ischaemic strokes in men (ORa 1.133, 95% CI 1.052 to 1.220) (table 3). In women, the strength of association was weaker and it was not significant. For the other pollutants, NO_2, SO_2, CO and PM_{10} were not significantly associated with the occurrence of strokes. Furthermore, no significant associations were found for haemorrhagic stroke. Two-pollutant models were examined to obtain insight into which individual pollutants might influence stroke occurrence independently of the effects of others (table 4). In men, the effect of O_3 remained significant after each of other pollutants was included in the model, in particular with PM_{10} (table 4).

Table 5 shows the ORa obtained according to ischaemic stroke subtypes in men. A significant association was observed for ischaemic strokes of large arteries (ORa 1.140, 95% CI 1.013 to 1.288, p = 0.03) and for transient ischaemic attacks (ORa 1.206, 95% CI 1.038 to 1.401, p = 0.01).

The following ORs were found in men according to age: ⩾80 years, the ORa was 1.138 (95% CI 0.985 to 1.310, p = 0.07, n = 183); 65–79 years, the ORa was 1.140 (95% CI 1.020 to 1.273, p = 0.02, n = 313); 40–64 years, the ORa was 1.121 (95% CI 0.978 to 1.285, p = 0.09, n = 193).

Moreover, we observed a linear exposure-response relation between O_3 exposure with 1-day lag (creating new categorical variable based on the quintiles) and ischaemic stroke occurrence in men over 40 years of age (test for trend, p = 0.01). Using the first quintile as a reference group, we observed to O_3 level: between 9 to 20 μg/m^3, the ORa was 1.226 (95% CI 0.919 to 1.634); between 21 and 32 μg/m^3, the ORa was 1.382 (95% CI 0.996 to 1.918); between 33 to 48 μg/m^3, the ORa was 1.758 (95% CI 1.211 to 2.554); between 48 to 115 μg/m^3, the ORa was 2.070 (95% CI 1.336 to 3.206).

In analyses by subgroup, we observed that the association between ozone exposure and stroke occurrence was stronger in the men's subgroup with at least one cardiovascular risk factor than in the men's subgroup without such risk(s): ORa 1.140 (95% CI 1.052 to 1.235, p = 0.001, n = 584) versus ORa 1.066 (95% CI 0.837 to 1.358, p = 0.60, n = 61). Also, we saw an increase of ORa for subjects with several cardiovascular risk

Table 2 Distribution of air pollution concentrations and meteorological parameters

Variables	Mean	SD	Minimum	Q1	Median	Q3	Maximum
SO_2, μg/m^3	6.9	7.5	0	2	4	9	65
NOx, μg/m^3	39.6	14.3	6	30	39	48	126
O_3, μg/m^3	29.9	21.5	0	12	26	44	115
CO, μg/m^3	683.5	468.8	0	376	590	889	4014
PM_{10}, μg/m^3*	21.1	11.3	2	13	19	27	103
Minimum temperature, °C	6.9	6.4	−14.5	2	7.1	11.9	23.1
Maximum temperature, °C	16	8.5	−6.7	9.6	15.9	22.5	39.3
Maximum humidity, %	93.6	6.8	51	91	96	98	100
Minimum humidity, %	55.9	18.7	12	41	54	70	100

Q1, first quartile value; Q3, third quartile value.
*From 1994 to 2004.

Table 3 Odds ratios between stroke occurrence and concentration of air pollutants, Dijon stroke register (France), from 1994 to 2004

	Ischaemic stroke (women and men), n = 1487		Haemorrhagic stroke (women and men), n = 220		Ischaemic stroke (men), n = 689		Ischaemic stroke (women), n = 798	
Pollutants	**ORa (95% CI)**	**p Value**	**ORa (95% CI)**	**p Value**	**ORa (95% CI)**	**p Value**	**ORa (95% CI)**	**p Value**
SO_2								
D0	0.978 (0.868 to 1.103)	0.72	1.099 (0.815 to 1.483)	0.53	0.900 (0.748 to 1.081)	0.26	1.044 (0.892 to 1.222)	0.58
D-1	0.978 (0.863 to 1.108)	0.73	1.014 (0.747 to 1.376)	0.92	0.981 (0.813 to 1.183)	0.84	0.975 (0.824 to 1.153)	0.77
D-2	1.015 (0.902 to 1.143)	0.79	0.961 (0.712 to 1.297)	0.79	1.109 (0.935 to 1.315)	0.23	0.939 (0.797 to 1.106)	0.45
D-3	1.003 (0.892 to 1.127)	0.95	0.954 (0.729 to 1.248)	0.73	1.033 (0.862 to 1.237)	0.72	0.981 (0.841 to 1.144)	0.80
NO_x								
D0	1.008 (0.956 to 1.062)	0.76	0.974 (0.845 to 1.122)	0.71	1.002 ()0.925 to 1.084	0.95	1.013 (0.943 to 1.087)	0.71
D-1	0.966 (0.915 to 1.019)	0.21	1.015 (0.887 to 1.162)	0.82	0.939 (0.866 to 1.018)	0.12	0.987 (0.919 to 1.061)	0.73
D-2	0.996 (0.945 to 1.050)	0.90	0.975 (0.851 to 1.117)	0.72	1.013 (0.938 to 1.094)	0.73	0.982 (0.913 to 1.055)	0.62
D-3	1.027 (0.975 to 1.082)	0.30	0.936 (0.816 to 1.074)	0.34	1.016 (0.941 to 1.097)	0.67	1.037 (0.966 to 1.114)	0.30
O_3								
D0	1.046 (0.998 to 1.098)	0.059	1.012 (0.902 to 1.158)	0.73	1.058 (0.987 to 1.134)	0.10	1.036 (0.969 to 1.106)	0.29
D-1	1.075 (1.022 to 1.131)	0.005	0.974 (0.978 to 1.122)	0.75	1.133 (1.052 to 1.220)	0.001	1.027 (0.958 to 1.101)	0.44
D-2	1.012 (0.965 to 1.062)	0.60	0.958 (0.842 to 1.090)	0.51	1.020 (0.951 to 1.092)	0.57	1.006 (0.941 to 1.075)	0.85
D-3	0.987 (0.943 to 1.043)	0.60	0.970 (0.856 to 1.098)	0.63	1.000 (0.935 to 1.068)	1.00	0.976 (0.915 to 1.040)	0.46
CO								
D0	0.999 (0.997 to 1.001)	0.43	1.000 (0.996 to 1.004)	0.70	0.998 (0.995 to 1.000)	0.15	1.000 (0.998 to 1.002)	0.84
D-1	0.998 (0.997 to 1.000)	0.17	1.001 (0.997 to 1.005)	0.54	0.998 (0.995 to 1.000)	0.19	0.999 (0.997 to 1.001)	0.70
D-2	0.999 (0.998 to 1.001)	0.82	0.999 (0.995 to 1.004)	0.88	1.000 (0.997 to 1.002)	0.96	0.999 (0.997 to 1.001)	0.70
D-3	1.000 (0.998 to 1.001)	0.93	0.998 (0.994 to 1.002)	0.46	1.000 (0.998 to 1.003)	0.62	0.999 (0.997 to 1.002)	0.72
PM_{10}§								
D0	1.009 (0.930 to 1.094)	0.82	0.901 (0.730 to 1.111)	0.33	0.973 (0.862 to 1.098)	0.66	1.040 (0.933 to 1.160)	0.47
D-1	1.011 (0.998 to 1.094)	0.77	1.014 (0.828 to 1.241)	0.85	0.969 (0.860 to 1.091)	0.60	1.047 (0.942 to 1.163)	0.39
D-2	0.960 (0.889 to 1.036)	0.30	1.100 (0.903 to 1.339)	0.34	0.981 (0.877 to 1.098)	0.74	0.941 (0.847 to 1.045)	0.26
D-3	0.990 (0.919 to 1.066)	0.79	0.991 (0.881 to 1.212)	0.93	0.985 (0.881 to 1.102)	0.80	0.993 (0.899 to 1.096)	0.89

ORa, adjusted odds ratio for minimum temperature (1-day lag), maximum relative humidity (1-day lag), holiday (0-day lag) and influenza epidemics (0-day lag) and for an increase of 10 units ($\mu g/m^3$) of pollutant.
§PM_{10} data from 1999 to 2004: ischaemic stroke (women and men) n = 762, haemorrhagic stroke (women and men) n = 99, ischaemic stroke (men) n = 357, ischaemic stroke (women) n = 412.

factors (smoker, dyslipidemia and hypertension) with ORa at 1.212 (95% CI 0.996 to 1.477, p = 0.056, n = 96).

DISCUSSION

In our study the main finding is a significant association between ozone exposure levels and ischaemic stroke occurrence in men over 40 years of age. This result was observed to 1-day lag (OR 1.133, 95% CI 1.052 to 1.220). For 0-day lag and 2-day lag, the positive association was weaker and not significant: ORa 1.058 and ORa 1.020 respectively. This may suggest a short-term effect of ozone pollution on stroke onset with delayed effect about one day after exposure. We observed that the effect of ozone remained significant after the inclusion of PM_{10} in the logistic model (see table 5). This result also suggests that ozone pollution might influence stroke occurrence independent of PM_{10} effects.

In men, the ORs according to age were of a similar strength among three groups (ORa 1.138 to ORa 1.121) and did not increase compared to our main finding (ORa 1.133). The statistically significant association in the second group was explained by the lack of patients in the other groups. Therefore, our analysis did not reveal that some age groups were more sensitive to ozone effects.

Furthermore, we found a sex difference—the strength of association between ozone exposure and stroke occurrence being weaker among women than men (women, ORa 1.036, 1.027 and 1.006 for respectively 0-day lag, 1-day lag and 2-day lag). The incidence of ischaemic stroke was higher among women than men (798 cases vs 689 cases, respectively), therefore this cannot be explained by a lack of statistical power. This suggests that women might be less susceptible to the harmful effects of ozone exposure than men. The reasons for weaker associations in women are not clear. Iemolo *et al* studied sex difference in carotid plaque and stenosis and have reported that stenotic lesions were more common in women while plaque was more common in men.[32] Moreover, plaque areas were a stronger predictor of outcome (stroke, myocardial infarctus, overall mortality) than stenosis.[32] Therefore, the

Table 4 Odds ratios between ischaemic stroke occurrence and ozone concentration from 1-day lag, in 2-pollutant models, Dijon stroke register (France), from 1994 to 2004

	Adjusted for PM_{10}*		Adjusted for SO_2		Adjusted for NO_2		Adjusted for CO	
Pollutant	**ORa (95% CI)**	**p Value**	**ORa (95% CI)**	**p Value**	**ORa (95% CI)**	**p Value**	**ORa (95% CI)**	**p Value**
O_3	1.129 (1.025 to 1.244)	0.013	1.135 (1.053 to 1.222)	0.001	1.125 (1.044 to 1.218)	0.002	1.128 (1.045 to 1.217)	0.002
PM_{10}*	0.988 (0.877 to 1.112)	0.84	–		–		–	
SO_2	–		1.027 (0.846 to 1.239)	0.77	–		–	
NO_2	–		–		0.963 (0.888 to 1.044)	0..36	–	
CO	–		–		–		0.999 (0.996 to 1.002)	0.630

ORa, adjusted odds ratio for minimum temperature (1-day lag), maximum relative humidity (1-day lag), holidays (0-day lag), influenza epidemics (0-day lag) and for an increase of 10 $\mu g/m^3$ of pollutant.
*From 1999 to 2004: n = 357.

Table 5 Odds ratios between ischaemic stroke subtypes (TOAST classification) and pollutant concentrations from 1-day lag, Dijon stroke register (France), from 1994 to 2004

	Ischaemic stroke: large arteries (n = 262)†		Ischaemic stroke: lacunar (n = 193)‡		Ischaemic stroke: cardioembolic (n = 208)§		Ischaemic stroke: transient ischaemic attacks (n = 172)¶	
Pollutant	**ORa (95% CI)**	**p Value**	**ORa (95% CI)**	**p Value**	**ORa (95% CI)**	**p Value**	**ORa (95% CI)**	**p Value**
SO_2	1.088 (0.785 to 1.508)	0.61	0.831 (0.605 to 1.412)	0.25	0.974 (0.707 to 1.344)	0.87	0.966 (0.659 to 1.416	0.86
NO_x	0.988 (0.863 to 1.130)	0.20	0.896 (0.771 to 1.042)	0.15	1.028 (0.888 to 1.190)	0.70	0.860 (0.742 to 1.022)	0.09
O_3	1.140 (1.013 to 1.288)	0.03	1.077 (0.931 to 1.245)	0.31	1.113 (0.970 to 1.278)	0.12	1.206 (1.038 to 1.401)	0.01
CO	0.998 (0.994 to 1.003)	0.64	0.997 (0.992 to 1.001)	0.24	0.999 (0.995 to 1.004)	0.91	0.999 (0.995 to 1.004)	0.91
PM_{10}*	0.938 (0.769 to 1.144)	0.52	0.881 (0.698 to 1.111)	0.28	1.101 (0.895 to 1.354)	0.36	1.095 (0.833 to 1.432)	0.50

ORa, adjusted odds ratio for minimum temperature (1-day lag), maximum relative humidity (1-day lag), holidays (0-day lag), influenza epidemics (0-day lag) and for an increase of 10 $\mu g/m^3$ of pollutant.
*PM_{10} data from 1999 to 2004: †n = 145; ‡n = 93; §n = 92; ¶n = 80.

association between O_3 and stroke in men only suggests that carotid plaque might be more sensitive to pollutants than stenotic lesions, explaining the relation in men observed in the present study. Another explanation might be the lower proportion of women smokers (9.3% vs 67.4%, see table 1). The smoking subjects might be more susceptible to ozone exposure effect.

Our main finding is consistent with the results of other studies reporting an association between O_3 pollution and stroke occurrence.[5 12 13 16] In Seoul, Hong *et al* observed an estimated increase of 2.9% (95% CI 0.3 to 5.5%) and 6% (95% CI 2 to 10%) in stroke mortality for each interquartile range increase in O_3 concentrations respectively with 0-day lag and 3-day lag.[12 13] With a case-crossover approach, Tsaï *et al* reported a positive association between ischaemic stroke hospital admissions and O_3 (OR 1.15, 95% CI 1.07 to 1.23) in the single-pollutant models on warm days (>20°C).[16] In Taipei (Taiwan) emergency admissions for cerebrovascular diseases among adults were positively associated with increasing air pollution levels of O_3 lagged 0 days.[33] Also, a French study reports an association between atherosclerotic disease (myocardial infarction) and O_3 pollution with 0-day lag and 1-day lag.[10]

However, these results are not completely comparable with other studies. In the more precise studies, associations were observed with several pollutants: SO_2, NO_2, CO, PM_{10}.[13 15 16 18 33] On the one hand, the moderate levels of air concentration pollutants in Dijon might explain this lack of association. The mean values for some pollutants in Dijon (PM_{10} 21.14 $\mu g/m^3$, SO_2 6.88 $\mu g/m^3$) are 3–5-fold lower than in Asian cities (Seoul[13] PM_{10} 71.1 $\mu g/m^3$, SO_2 24.2 $\mu g/m^3$; Kaohsiung[16] PM_{10} 77.63 $\mu g/m^3$, SO_2 19.52 $\mu g/m^3$; Shanghai[15] PM_{10} 97 $\mu g/m^3$, SO_2 48.4 $\mu g/m^3$). The studies conducted in Asia were carried out in cities with rapid development and urbanisation and severe pollution.[13 15] On the other hand, if Dijon is not considered a particularly polluted town, the same is not true for ozone pollution. Since 1994, there has been a reduction in the mean annual levels for SO_2, NO_x, CO but not O_3, for which levels rose: 18.8 $\mu g/m^3$ in 1994, 31.9 $\mu g/m^3$ in 1997, 33.7 $\mu g/m^3$ in 2000, 40.1 $\mu g/m^3$ in 2003 and 35.7 $\mu g/m^3$ in 2004. The progression of motor vehicle pollution and ozone pollution displaced from other regions would explain this situation. Therefore, our finding might be consistent with the features of urban pollution in Dijon.

Using incident morbidity data from a population-based register, in contrast with other studies which used stroke mortality or hospital admission indicators for stroke, there may be another explanation.[5 6 13 15–17] These indicators (mortality, hospital admission) would be less reliable for stroke epidemiology and associations might be overestimated from differential classification bias.[21 34] For example with mortality indicators, we do not know whether air pollution is a causative factor of stroke occurrence or only a precipitating factor of death after stroke. In the present study, the diagnosis of stroke occurrence is more precise with 98% of the patients having undergone a CT scan. Moreover, the recording of all cases of incident stroke in Dijon at the population level enables the risk reduction of potential selection bias.[34] The outpatients accounted for 34% of the cases of studied stroke from the Dijon register.

Globally, the conflicting results could be due to different features and levels of pollution linked to each town and the multiplicity of the used outcome. But this might also be explained by some limitations of our study. First, the case-crossover design is a reliable method in order to evaluate the short-term health effects of exposure to air pollution.[26] This design controls the potential biases (seasonality and long-term trends) if the reference periods are used judiciously.[7 26] Since case-crossover design uses each subject as his own control, the methodology enables control of other confounding factors related to individual subject characteristics. But as several authors have pointed out, this design might present a loss of statistical power compared to approaches using time series analysis.[7 9] Moreover, the pollutant exposures of the studied subjects were evaluated using the arithmetic mean from reports generated by an urban monitoring station.[35] However exposures were not homogenous over the entire studied area, with exposure misclassification for some individuals. The errors would be non-differential and therefore underestimate of the strength of an association.[35] The absence of an association with some pollutants in this study might be the result of these methodological disadvantages. Our study, carried out in medium-size town (about 150 000 inhabitants), might present a lack of statistical power in global terms. This might explain, for example, that the magnitude of the association between ischaemic stroke in men and SO_2 2-day lag (ORa 1.109, 95% CI 0.935 to 1.315) or NOx 2-day lag (ORa 1.016, 95% CI 0.941 to 1.097) are not statistically significant. Besides, we cannot rule out the possibly of real associations between stroke occurrence and other pollutants. Lastly, our findings could be explained by the role of chance in relation to multiple testing. Thus to accept them, they must be confirmed by other similar studies and our results still need to be replicated in other areas.

Nevertheless, in our study several complementary results argue for a real association between ozone exposure and stroke occurrence. First, our analyses using ischaemic stroke subtypes (TOAST classification) confirmed a consistent association between ozone exposure and two stroke subtypes: large artery ischaemic stroke and transient ischaemic attack. Risk factors differ between the different subtypes of stroke and these results might be explained by the epidemiology of the stroke.[21] We can see an association between ozone exposure and stroke subtypes (large artery ischaemic stroke and transient ischaemic attack) in which the complications of atherosclerosis (plaque rupture, thrombosis) play an important role.[21 34] Secondly, we found a

lack of association between ozone exposure and haemorrhagic strokes (see table 3). This was found in other studies too.[12 18] We know that a risk factor for haemorrhagic stroke is not atherosclerosis but often appeared to be hypertension or high alcohol intake.[21 34] Thirdly, in our study among subjects with several cardiovascular risk factors (smoker, hypertension, dyslipidemia), we found an increase in the strength of association between ozone exposure and stroke onset (ORa 1.050 vs ORa 1.212). This suggests that men at highest cardiovascular risk would be more susceptible to the harmful effects of ozone exposure. Therefore, our sensitivity analyses results present an interesting coherence with the pathophysiological mechanisms explaining stroke subtype onset, and provide arguments in favour of a real association. Furthermore, we show a linear exposure-response relation between ozone levels and ischaemic stroke occurrence (test for trend, $p = 0.012$). The existence of an exposure-response relation is one of the central criteria identified by Hill for the establishment of a causal relation between one agent and one outcome.[35]

Some pathophysiological hypotheses can also be advanced to explain our finding. Several authors have been suggested that there are possible interactions between atherosclerotic disease and pollutants.[11] Concerning cardiovascular effects, experimental data might explain our observations.[32] Brook *et al* showed that short-term inhalation of O_3 and $PM_{2,5}$ at concentrations that occur in the urban environment causes acute artery vasoconstriction in healthy adults.[36] The vasoconstrictor effects might induce obstruction in an stenotic artery, giving rise to ischaemia, or trigger rupture of unstable atherosclerosis plaque. However the existence of a 1-day lag time between exposure and effect also argues for a delayed effect. Experimental data show ozone inhalation induces epithelial injury with concomitant acute inflammatory response in upper and lower airways.[37 38] Oxidative stress exists when there is an excess of free radicals over antioxidant defences. As a consequence, free radicals attack and oxidise other cell components such as lipids (particularly polyunsaturated lipids with formation of aldehydes), proteins and nucleic acid.[37] This leads to tissue injury and, in some cases, influx of inflammatory cells to the sites of injury.[37] Several authors also suggest that, following pollutant exposure, delayed indirect effects (several hours to days) mediated through pulmonary oxidative stress and systemic inflammatory response may occur.[11 37] Moreover following activation of those biological processes, delayed indirect effects occur, which may also ultimately activate a thrombotic phenomenon or induce rupture of unstable atherosclerosis plaque.[11] However this is yet to be be demonstrated.[39]

In conclusion, our observational data argue for an association between ischaemic stroke occurrence and O_3 pollution levels. The ischaemic stroke occurrence only in men over 40 years of age suggests that atherosclerosis plaques in men are more sensitive to ozone pollution. The inflammatory process may be the link between ozone exposure and ischaemic stroke by way of plaque instability. We found that the risk of stroke occurs at low concentrations and this highlights the need to reduce O_3 air pollution in urban areas. However, these underlying biological mechanisms still need to be further explored and other studies must confirm our findings.[40]

Main messages

- We used bi-directional case-crossover analyses from a 10-year population-based register.
- We found a positive relation between ozone exposure and ischaemic stroke occurrence in men over 40 years of age.
- The relation between O_3 and stroke remained significant after each of the other pollutants was included in the model, in particular with PM_{10}.
- Among aetiological subtypes, we observed an association between ozone exposure and ischaemic stroke of large arteries and transient ischaemic attacks.

Policy implications

- These results contribute to a growing body of scientific evidence demonstrating the effects of ozone pollution on cardiovascular outcomes.
- The risk of stroke occurs at low concentrations, suggesting the need to reduce O_3 air pollution in urban areas.

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Competing interests: None declared.

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