

Research letters

Relation between airborne pollen concentrations and daily cardiovascular and respiratory-disease mortality

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In a time-series study in the Netherlands, we found a strong association between the day-to-day variation in pollen concentrations and that of deaths due to cardiovascular disease, chronic obstructive pulmonary disease, and pneumonia.

Many studies have investigated associations between daily variations in air pollution and cardiovascular and respiratory deaths.¹ Pollen, a form of biogenic air pollution, has been largely disregarded in such studies. We analysed the association between daily airborne pollen concentrations and daily death rates in the Netherlands.

Numbers of daily deaths were obtained from the Central Bureau of Statistics for 1986–94. Air pollution and meteorological data were obtained from the National Institute of Public Health, which operates a national monitoring network throughout the Netherlands. Airborne pollen concentrations are measured in the west (Leiden) and south (Helmond) on a daily basis in the pollen season.² Distributions of airborne pollen concentrations are highly skewed, since concentrations can be very high on some days but low on others, so we analysed airborne pollen concentrations as categorical variables rather than continuous variables. We analysed the concentrations in air of the most frequently occurring types of pollen in the Netherlands (*Poaceae*, *Betula*, and *Quercus* [90th percentiles of distributions 78, 69, and 13 pollen grains per m³, respectively]). In addition, we analysed *Fraxinus*, *Artemisia*, and *Rumex* pollen. Daily non-accidental mortality was analysed with regard to total mortality and deaths due to cardiovascular diseases (International Classification of Diseases [ICD9] 390–448), chronic obstructive pulmonary disease (COPD; ICD9 490–496), and pneumonia (ICD9 480–486). The relation between daily mortality and airborne pollen concentration was modelled using Poisson regression with generalised additive models. All pollen–mortality associations were adjusted for long-term and seasonal trend, influenza morbidity (based on a sentinel system mediated by general practitioners), ambient temperature (including separate lags for high and low temperature days), humidity, and indicators for day-of-week and holidays. In such analyses, autocorrelation may lead to underestimation of standard errors of regression coefficients when autocorrelation is present in the residuals of the model. After adjusting for seasonal and long-term trends, influenza, and temperature, the first-order autocorrelation coefficient was 0.02. Therefore autocorrelation was not a problem in the present analyses. We analysed airborne pollen concentrations on the same day as

mortality was measured, the previous day, the day before that, and on the 7 days preceding the day of the death counts. Analyses were done with S-plus version 3.3 for Windows. Data were analysed separately for the south and west of the country, and for all of the Netherlands combined. Correlations between daily airborne pollen concentrations from the two stations were 0.92 for *Poaceae*, 0.71 for *Betula*, and 0.75 for *Quercus*. Analyses were restricted to the warm season (April–September) when airborne concentrations of most pollen species are at their highest, and when confounding by respiratory infections is less likely than in winter.

During the study period, the average numbers of daily deaths were 332.5 (total), 141.8 (cardiovascular), 15.8 (COPD), and 9.8 (pneumonia). *Poaceae* pollen concentrations in particular were associated with daily deaths due to COPD and pneumonia. The table shows the results, expressed as estimated relative risks, for different categories of *Poaceae* pollen concentrations as averaged over the 7 days preceding the day mortality was measured; these estimates tended to be the most stable. There was a fairly consistent dose–response relation. Concentrations of other pollen also showed positive associations with mortality, especially *Betula* and *Rumex*. The relative risks for total mortality, comparing the highest to the lowest exposure category, were 1.029 (95% CI 1.005–1.053) and 1.073 (1.023–1.126), respectively. Pollen concentrations were only weakly associated with air pollution, and models in which both pollen and air pollution were included showed that there was no confounding by concentrations of particles smaller than 10 µm in diameter, black smoke, sulphate and nitrate aerosols, nitrogen dioxide, sulphur dioxide, or ozone. Associations between chemical air pollution and mortality were also not confounded by pollen.

Pollen is a well-known trigger of allergies, especially hay fever and asthma. However, deaths related to these conditions are extremely rare, and cannot account for the associations seen in this study. The size of these associations is as large as that between air pollution and mortality, which typically shows a 5–10% increase in mortality on “high pollution” days. If acute asthma deaths cannot account for these associations, what could be the explanation? A cohort study has shown that allergy markers such as peripheral eosinophilia and, in individuals with low forced expiratory volume in 1 s, positivity on skin-prick tests were related to increased all-cause mortality as well as mortality due to cardiovascular diseases and COPD.³ If such allergy markers, which can be found in large segments

	Poaceae pollen concentrations (grains per m ³ air)			
	<22	22–77	78–135	>135
	Relative risk	Relative risk (95% CI)	Relative risk (95% CI)	Relative risk (95% CI)
Cardiovascular disease	1.000	1.015 (1.002–1.029)	1.012 (0.994–1.029)	1.061 (1.038–1.084)
Chronic obstructive pulmonary disease	1.000	1.095 (1.053–1.139)	1.124 (1.069–1.181)	1.150 (1.079–1.225)
Pneumonia	1.000	1.104 (1.049–1.163)	1.093 (1.023–1.168)	1.168 (1.077–1.266)
Total	1.000	1.019 (1.010–1.028)	1.019 (1.008–1.031)	1.043 (1.028–1.058)

Relative risk of mortality associated with average weekly concentrations of airborne pollen in the Netherlands

of the population, are risk factors for reduced long-term survival, acute exacerbations of allergic inflammation associated with high pollen exposures may also precipitate death due to cardiovascular disease, COPD, or pneumonia in patients already suffering from these disorders. High concentrations of pollen allergens have also been shown to occur in thoracic particles ($<10\text{ }\mu\text{m}$ in diameter) and respirable particles ($<2.5\text{ }\mu\text{m}$), and these correlated well in time with airborne pollen concentrations.² This finding means that airborne pollen results in exposure of the lower airways and lung to pollen allergens. The association between air pollution and the number of daily deaths may be related to the inflammatory potential of very small particles,⁴ and our study suggests that particles of biological origin may have similar effects. Our findings require replication, but if substantiated, they suggest that high airborne pollen concentrations, which nowadays are mainly seen as triggers of allergic symptoms, may have far more serious effects than previously thought.

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Zonulin, a newly discovered modulator of intestinal permeability, and its expression in coeliac disease

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We identified zonulin, a novel human protein analogue to the *Vibrio cholerae* derived Zonula occludens toxin, which induces tight junction disassembly and a subsequent increase in intestinal permeability in non-human primate intestinal epithelia. Zonulin expression was raised in intestinal tissues during the acute phase of coeliac disease, a clinical condition in which tight junctions are opened and permeability is increased.

We have shown that zonula occludens toxin (ZOT), a protein elaborated by *Vibrio cholerae*, reversibly regulates the permeability of tight junctions.¹ ZOT interacts with a specific surface receptor¹ with subsequent protein kinase C α -dependent polymerisation of actin microfilaments strategically localised to regulate the paracellular pathway. On the basis of this observation, we investigated whether ZOT might mimic an endogenous modulator of tight junctions. We also postulated that ZOT and its putative eukaryotic analogue could be structurally and immunologically related.

Accordingly, specific anti-ZOT antibodies and an *ex vivo* intestinal permeability assay¹ were used in combination to



Figure 1: Immunoscreening of human intestinal tissues with affinity-purified polyclonal anti-ZOT antibodies

Proteins in tissue lysates of human fetal and adult intestine were subjected to sequential purification steps, resolved by sodium dodecylsulphate polyacrylamide gel electrophoresis, transferred onto polyvinylidene difluoride membranes, and probed with affinity-purified anti-ZOT antibodies. A single protein was purified that migrated with an apparent relative molecular mass of about 47 kDa and immunoreacted with anti-ZOT antibodies.

screen for one or more human intestinal ZOT analogues. Non-primate intestinal tissues were used as an indicator system to identify and purify this analogue. Fetal and adult tissues were obtained from the brain and tissue bank for developmental disorders at the University of Maryland. A single protein (that we named zonulin) with a molecular weight of about 47 kDa was purified to homogeneity from both adult and fetal intestine (figure 1). To establish whether zonulin preparation was biologically active, it was tested on Rhesus monkey intestine with an *ex vivo* assay.¹ Intestinal tissues from the same animal with similar baseline tissue resistances were simultaneously exposed to either zonulin or media alone. Zonulin reversibly increased the monkey intestinal permeability compared with the media control in both jejunum (mean 35.0 [SE 1.8]% *vs* 3.0 [1.5]% permeability increment; $p<0.0001$) and ileum (26.0 [5.6] *vs* 4.9 [1.5] permeability increment), but not in the colon (1.3 [0.6] *vs* 1.1 [0.5] permeability increment, $p=0.37$, Student's *t* test). This increased permeability allowed the transepithelial passage of insulin, a macromolecule normally not absorbed when given orally.²

To establish whether zonulin is perturbed during coeliac disease, a condition in which tight junctions are opened through an as yet undefined mechanism,³ intestinal tissues were obtained from seven patients with active coeliac disease and six controls and probed for zonulin with anti-ZOT antibodies. Immunofluorescence analysis of coeliac disease tissues showed enhanced zonulin expression within the intestinal submucosa with a characteristic reticular pattern that was consistently absent in control tissues. Quantitative immunoblotting of intestinal tissue lysates from patients with active coeliac disease confirmed higher zonulin protein concentrations than in control tissues (figure 2).

Since intestinal zonulin expression was increased during the acute phase of coeliac disease, when tight junctions are opened, this suggests a causal role of this endogenous mediator in

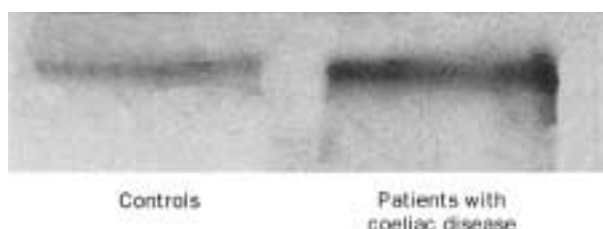


Figure 2: Zonulin protein in intestinal tissues from coeliac disease patients and controls

The increased expression of zonulin in intestinal tissues from coeliac patients was confirmed by western analysis. The amount of zonulin normalised to the total protein content in the tissues analysed was about 3-fold higher in intestinal specimens from patients with coeliac disease than in control tissues. These blots are representative of six specimens.