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Effects of Ambient Particulate Matter and Fungal Spores on Lung Function in Schoolchildren

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WHAT'S KNOWN ON THIS SUBJECT: Epidemiologic studies examining the combined childhood health effects of both air pollutants and fungal spores have been relatively lacking.

WHAT THIS STUDY ADDS: Exposure to current levels of ambient particulate matter with an aerodynamic diameter of 2.5 μm or less, fungal spores, and O_3 are associated with reduced childhood lung function. These adverse effects were observed in a longitudinal setting, more specifically, in a cohort of schoolchildren that included healthy children.

abstract

OBJECTIVES: Studies examining the combined health effects of both have been relatively lacking. We conducted a longitudinal study to investigate whether exposure to air pollutants and fungal spores might exacerbate childhood respiratory health.

METHODS: Study participants were 100 elementary and middle-school students in Taipei County, Taiwan. A structured respiratory health questionnaire was administered in September 2007, followed by monthly spirometry from October 2007 to June 2008. During the study period, complete daily monitoring data for criteria air pollutants were obtained from the Environmental Protection Administration monitoring station and Aerosol Supersite. Fungal spores were measured from Sunday to Saturday in the week when lung-function measurements were made for each study month. Lung-function measurements were compared with air pollutants and fungal spores using mixed-effects models with 1-day-lag modeling.

RESULTS: The particulate matter with an aerodynamic diameter of 2.5 μm or less level 1 day before the lung function measurements was negatively associated with forced vital capacity. The fungal spore level was negatively associated with both forced expiratory vital capacity and forced expiratory volume in 1 second. O_3 level was negatively associated with forced expiratory flow at 25%, 50%, and 75% of forced vital capacity, and average expiratory flow over the middle half of forced vital capacity.

CONCLUSIONS: This study suggested that exposure to particulate matter with an aerodynamic diameter of 2.5 μm or less and fungal spores might cause adverse effects on the vital capacity of schoolchildren. Exposure to O_3 adversely affected small airway function. *Pediatrics* 2011;127:e690–e698

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KEY WORDS

air pollution, children, particulates, particulate matter with an aerodynamic diameter of 2.5 μm or less, fungal spores, lung function, forced vital capacity

ABBREVIATIONS

PM_{10}—particulate matter with an aerodynamic diameter of 10 μm or less
$PM_{2.5}$—particulate matter with an aerodynamic diameter of 2.5 μm or less
AR—allergic rhinitis
EPA—Taiwan Environmental Protection Administration
FVC—forced vital capacity
FEV_1—forced expiratory volume in 1 second
$FEF_{25\%}$—forced expiratory flow at 25% of FVC
$FEF_{50\%}$—forced expiratory flow at 50% of FVC
$FEF_{75\%}$—forced expiratory flow at 75% of FVC
$FEF_{25\%–75\%}$—average expiratory flow over the middle half of FVC
PM—particulate matter
AR(1)—correlation structure–first order autoregressive correlation structure
CI—confidence interval

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Epidemiologic studies on schoolchildren have shown that ambient particulate matter with aerodynamic diameters of 10 μm or less (PM_{10}) and 2.5 μm or less ($PM_{2.5}$), ozone (O_3), carbon monoxide (CO), nitrogen dioxide (NO_2), and sulfur dioxide (SO_2) were associated with increases in respiratory symptoms,[1–3] emergency-department visits,[4,5] and decreases in lung function.[6–10] Furthermore, $PM_{2.5}$ has been linked to increases in childhood hospital admissions.[11]

There is increasing concern about ambient fungal-spore exposure because fungal species are known sources of outdoor allergens.[12] The effects of fungal spores on morbidities were observed, which include childhood respiratory symptoms,[13] emergency-department visits,[14–16] and lung-function decrements.[17]

Despite many studies on air pollutants and those on fungal spores, epidemiologic studies examining the combined childhood health effects of both have been relatively lacking. We conducted a longitudinal study to investigate whether exposure to air pollutants and fungal spores might exacerbate childhood respiratory health.

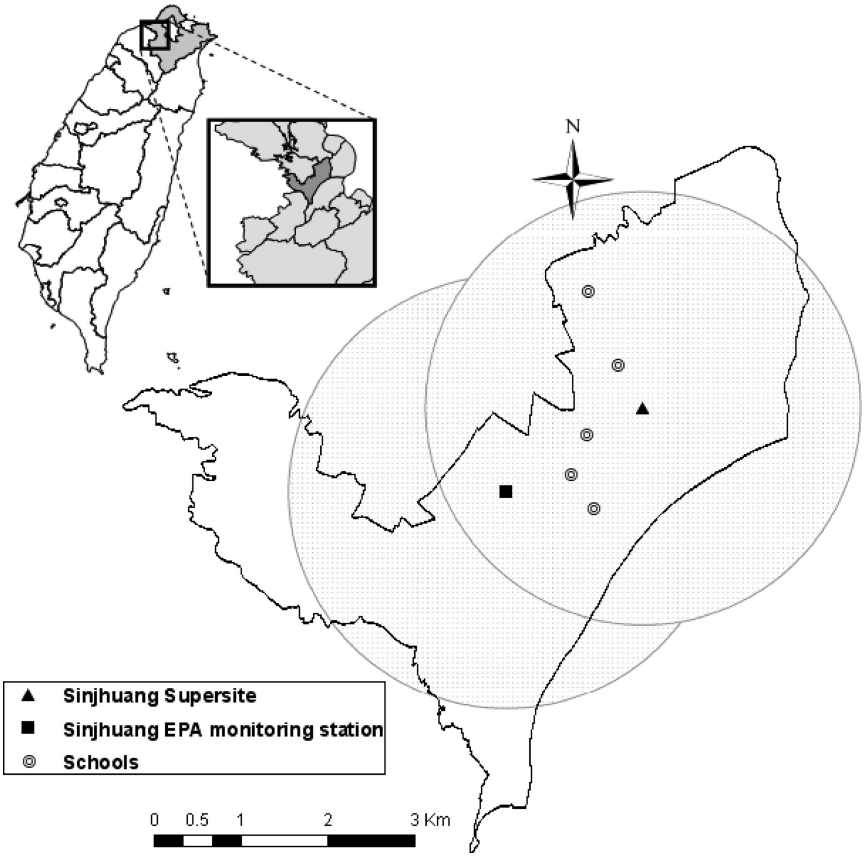

FIGURE 1
A map of the study schools, the Aerosol Supersite, and the EPA monitoring station in Sinjhuang city, Taipei County, Taiwan.

MATERIALS AND METHODS

Study Design

A follow-up study of healthy, asthmatic, and allergic rhinitis (AR) schoolchildren was conducted between September 2007 and June 2008. The children were enrolled in September 2007 after answering an initial questionnaire and were followed for 9 months, excluding January 2008, which was during their winter break. Lung-function tests were conducted on a monthly basis. The study was approved by the interstitial review board of the National Taiwan University Medical Center.

Study Population

The study was conducted in 3 elementary and 2 middle schools located within a 2.5-km radius of the Taiwan Environmental Protection Administration (EPA) monitoring station and Aerosol Supersite in Sinjhuang City, Taipei County (Fig 1). The latter was established to monitor environmental particulate matter continuously. Stratified sampling by grade was performed in each school. A modified Chinese version of the International Study of Asthma and Allergies in Childhood questionnaire was taken home by students and answered by their parents. A total of 4221 children of 5664 candidates completed the questionnaire.

The questionnaire requested information on demographic characteristics, respiratory health history, and indoor environmental factors at home. The validity of the questionnaire has been previously proven.[18] Children were defined as currently having asthma if their parents answered "yes" to all of the following questions: (Question 1) "Has this child ever been diagnosed by a physician as having asthma?" (Question 2) "Has this child ever experienced dyspnea with wheezing in the chest?" and (Question 3) "Has this child ever experienced the above symptoms in the past 12 months?" A "yes" answer from the parents to all 3 of the following questions was set to designate current AR: (Question 4) "Has this child ever been diagnosed by a physician as having AR?" (Question 5) "Has this child ever experienced problems with sneezing or a runny or blocked nose without having a cold or the flu?" and

(Question 6) "Has this child ever experienced the above symptoms in the past 12 months?" Children whose parents answered "no" to Questions 1, 2, 4, and 5 were grouped into the healthy control group. A total of 133, 1059, and 2745 children, respectively, were grouped as having current asthma, having current AR, and being healthy control subjects. Among 133 asthmatic children, 85 also had AR. We randomly invited children to participate in a longitudinal follow-up. Thirty-three currently asthmatic subjects (23 of whom also had AR), 30 currently AR subjects, and 37 healthy children voluntarily participated. The respiratory health conditions of the participants were confirmed by an otolaryngologist at the beginning of the follow-up study in October 2007. Informed consent was obtained from each participant's parents.

Parental atopy was defined as a history of asthma, AR, or atopic eczema in either the father or the mother. Indoor environmental factors at home, including the presence of cockroaches witnessed in the past month, water damage in the past year, wall with visible mold, and environmental tobacco smoke were collected.

Outcome Measurements

Once a month during the follow-up period, medical technologists visited the children's schools and conducted spirometry. Each child was tested with a spirometer while standing (Chestgraph HI-101; CHEST MI, Tokyo, Japan), according to the standardization of spirometry of the American Thoracic Society.[19] Forced vital capacity (FVC), forced expiratory volume in 1 second (FEV_1), forced expiratory flow at 25%, 50%, and 75% of FVC ($FEF_{25\%}$, $FEF_{50\%}$, and $FEF_{75\%}$), and average expiratory flow over the middle half of FVC ($FEF_{25\%–75\%}$) were recorded. Quality control consisted of a 3-L syringe calibration and a leak test before each test day. Ambient temperature and relative humidity were measured during the test. Personal age, height, weight, upper-respiratory infection, and body temperature were recorded in each sampling time. A daily recording card of asthma/AR symptomatic attack and medicinal usage was dispatched to children with current asthma or AR and answered by their parents during the follow-up period.

Exposure Assessment

Particulate Matter

The monitoring data for PM_{10} and $PM_{2.5}$ were obtained from the Aerosol Supersite. PM_{10} and $PM_{2.5}$ were measured by a tapered element oscillating microbalance, as previously detailed.[20] $PM_{2.5–10}$ was derived by subtracting $PM_{2.5}$ from PM_{10}.

Criteria Air Pollutant

The data for O_3, CO, NO_2, and SO_2 were obtained from the EPA monitoring station. O_3 was measured by ultraviolet absorption, CO by nondispersive infrared absorption, NO_2 by chemiluminescence, and SO_2 by ultraviolet fluorescence. The concentrations of PM and criteria air pollutant were measured continuously and reported hourly. Daily mean levels were calculated by averaging their hourly concentrations.

Fungal Spores

During the week of lung-function measurements in each study month, a continuous fungal-spore sampling was conducted from Sunday to Saturday (an entire week) using a Burkard Seven-Day Recording Volumetric Spore Trap (Burkard Manufacturing, Rickmansworth, England) at the Aerosol Supersite. Collected 7-day tapes stuck to fungal spores were cut into 7 24-hour segments. The concentrations of total fungal spores were identified via glycerin-jelly stain and microscopy[21] and were reported daily in spores per m^3. Individual exposure was assumed to follow the outdoor and daily average data, as described above.

Statistical Analysis

Correlations among air pollutants and fungal spores were analyzed via the Pearson correlation. When analyzing the association between lung function and exposure to each of the air pollutants and fungal spores, a mixed-effects model was used with repeated measurements to account for the between-subject and within-subject variations. Unstructured and first-order autoregressive correlation structures [AR(1)] were chosen as the assumption of the correlations between repeated measurements. The exposure measurements were obtained on the day of and 1, 2, and 3 days before the lung-function measurements (ie, using 0-, 1-, 2-, and 3-day-lag assumptions). Models were adjusted for every child's age, square of age, height, square of height, interaction term of age and height, upper-respiratory infection, asthma/AR symptomatic attack, asthmatic/AR medicinal usage, ambient temperature, ambient relative humidity, and the day-of-week for each sampling time. Factors that remained constant during the follow-up period, such as gender, school, parental education, parental atopy, and home environmental tobacco smoke also were adjusted. The results were expressed as changes in lung function per interquartile range concentration increase for each pollutant.

Two-pollutant models were used to adjust for the potential confounding effects of copollutants. In addition, an interaction term of 2 pollutants was added to the model to examine whether effect modification (or interaction) existed.

TABLE 1 Characteristics of the Study Population

	Participants in the Follow-up Study[a]				Nonparticipants in the Follow-up Study[a]			
	All, $n = 100$	Asthma, $n = 33$	AR, $n = 30$	Healthy Control Subjects, $n = 37$	All, $n = 3837$	Asthma, $n = 100$	AR, $n = 1029$	Healthy Control Subjects, $n = 2708$
Gender ratio (boy/girl)	1.6	2.0	1.7	1.3	1.4	2.1	1.7	1.2
Age, y	10.6 ± 2.5	10.1 ± 2.6	11.9 ± 2.1	9.8 ± 2.4	10.7 ± 2.5	10.2 ± 2.6	10.7 ± 2.5	10.8 ± 2.5
Height, cm	143.1 ± 14.4	139.3 ± 15.5	151.2 ± 10.7	139.6 ± 13.6	144.4 ± 16.1	140.0 ± 16.0	149.2 ± 16.3	144.9 ± 16.0
Weight, kg	41.8 ± 13.8	40.9 ± 14.8	46.7 ± 10.8	38.4 ± 14.3	41.6 ± 14.1	40.5 ± 14.1	45.2 ± 14.4	40.3 ± 14.0
BMI, kg/m^2	19.9 ± 3.7	20.4 ± 4.1	20.2 ± 3.0	19.1 ± 3.9	18.8 ± 3.7	19.3 ± 3.5	18.8 ± 3.7	18.7 ± 3.7
Highest parental education ≥13 y, %	50.5	50.0	50.0	51.4	50.4	48.3	48.6	51.2
Parental atopy, %	52.0	64.3	66.7	30.1	39.2	63.1	67.0	27.7
Asthma medicinal usage, %	25.0	75.8	0.0	0.0	2.0	74.7	0.0	0.0
AR medicinal usage, %	36.0	54.5	60.0	0.0	18.3	55.1	63.0	0.0
Indoor environmental factors								
Cockroaches seen monthly at home >6 times per mo, %	30.9	43.8	32.1	17.7	29.4	40.0	30.1	28.8
Water damage at home >1 mo/y, %	1.0	3.0	0.0	0.0	0.4	2.0	0.0	0.4
>2 wall with visible mold at home, %	7.2	6.3	3.5	11.1	8.5	7.6	4.2	10.2
Environmental tobacco smoke, %	36.2	36.7	32.1	38.9	37.3	35.0	34.0	38.7

[a] There were 3937 eligible children (who completed the questionnaire and had a clear definition of respiratory health) in this study; 100 children voluntarily participated, but 3837 children did not participate in a longitudinal follow-up.

RESULTS

The demographics of the study population are shown in Table 1. Demographic characteristics and indoor environmental factors between the participants and nonparticipants were similar in each respiratory health condition group. At the beginning of the follow-up study in October 2007, average FVC, FEV_1, $FEF_{25\%}$, $FEF_{50\%}$, $FEF_{75\%}$, and $FEF_{25\%–75\%}$ were 1.98 L, 1.79 L, 3.70 L per second, 2.67 L per second, 1.38 L per second, and 2.40 L per second, respectively.

The distributions of ambient air pollutants and fungal spores during the follow-up period are shown in Fig 2. The mean levels of PM_{10}, $PM_{2.5}$, total fungal spores, O_3, CO, NO_2, and SO_2 across the follow-up period were 40.7 $\mu g/m^3$, 28.2 $\mu g/m^3$, 1548.4 spores per m^3, 29.4 ppb (8-hourly standard: 60 ppb in Taiwan), 0.6 ppm (8-hourly standard: 9 ppm), 24.2 ppb (annual standard: 50 ppb), and 5.9 ppb (annual standard: 30 ppb), respectively. The highest weekly means of PM_{10} (55.8 $\mu g/m^3$), $PM_{2.5}$ (38.9 $\mu g/m^3$), and total fungal spores (3567.1 spores per m^3) occurred in February 2008, February 2008, and May 2008, respectively. Pearson correlation coefficients of the air pollutants and fungal spores are shown in Table 2. $PM_{2.5}$ was associated with $PM_{2.5–10}$, CO, NO_2, and SO_2. Total fungal spores were associated with CO.

There were 682 observations available for analysis of the expected 800 measurements (from the total monthly data for 8 months in 100 children). Missing data from the measurements were results of refusals, transfers, or sick leaves of the participants.

Table 3 presents changes in lung function in relation to increases in interquartile range concentrations of exposure in the single-pollutant model. Running unstructured correlation structure required various parameters. The sample size of this study did not provide adequate statistical power. We fit an AR(1) with the production of lower-level Akaike's information criterion than unstructured correlation structure did. When 0-, 1-, 2-, and 3-day-lag assumptions were examined, 1-day lag gave the most stable relationship between decreased lung function and exposure to air pollutants and fungal spores. We therefore continued the data analysis by using the 1-day-lag assumption. After adjusting for potential confounders, an increase of 9.9 $\mu g/m^3$ in 1-day-lag $PM_{2.5}$ level was associated with a 0.16-L decrease (95% confidence interval [CI]: −0.23 to −0.08) in FVC and a 0.12-L decrease (95% CI: −0.20 to −0.05) in FEV_1. Total fungal spores were associated with decrements in FVC and FEV_1. Both $PM_{2.5–10}$ and O_3 were negatively associated with FVC, FEV_1, $FEF_{25\%}$, $FEF_{50\%}$, $FEF_{75\%}$, and $FEF_{25\%–75\%}$.

Two-pollutant models were used to adjust for the potential confounding effects of copollutants (Table 4). For total fungal spores, the 2-pollutant model yielded the coefficients and statistical significance to be essentially unchanged from those via the 1-pollutant model. The effects of $PM_{2.5}$ on FVC and O_3 on $FEF_{25\%}$, $FEF_{50\%}$, $FEF_{75\%}$, and $FEF_{25\%–75\%}$ remained significant. However, the effects of $PM_{2.5}$ on FEV_1 and O_3 on FVC and FEV_1 became insignificant when other copollutants were included in the analytical models. The effects of $PM_{2.5–10}$ on each lung-function parameter became insignificant when total fungal spores were included in the models. Furthermore, the interaction term between 2 pollutants proved to be statistically insignificant.

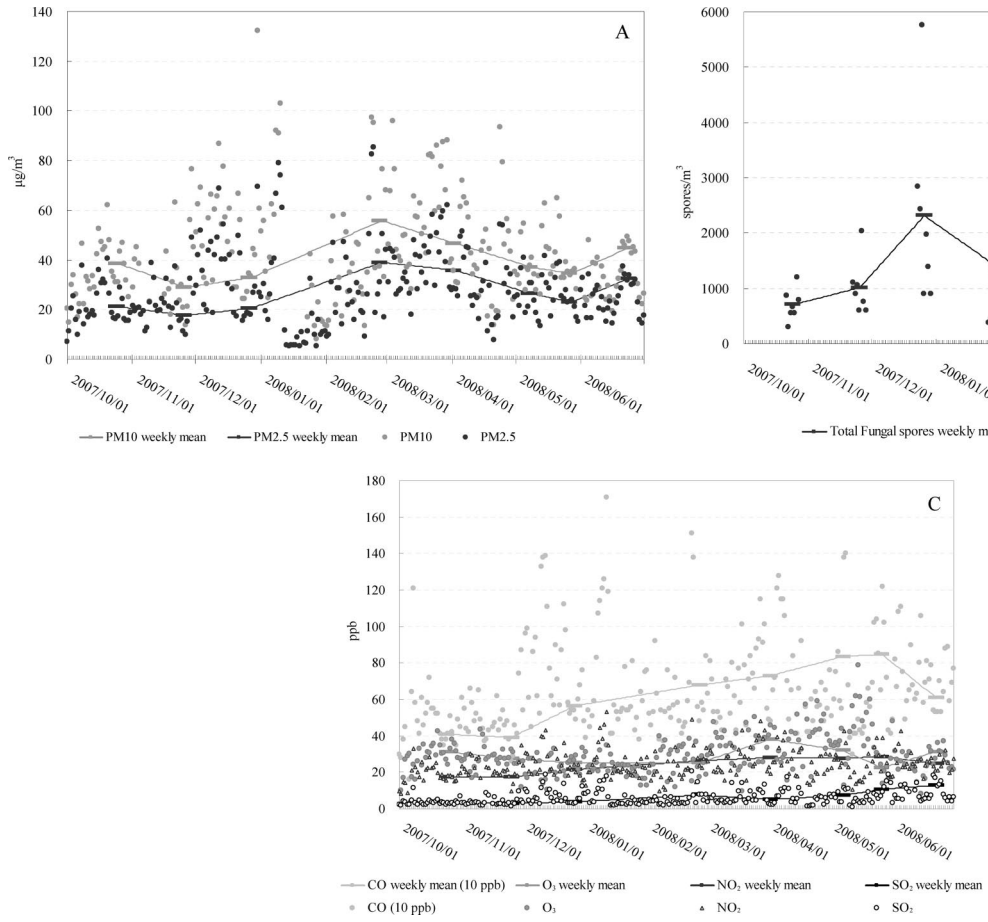

FIGURE 2
Ambient air pollutants and fungal spores in Sinjhuang city, Taipei County, Taiwan, from October 2007 to June 2008. Dots represent daily means and horizontal bars represent weekly means in the week of lung-function measurements in each study month. A, particulate matter. B, fungal spores. C, criteria air pollutants.

TABLE 2 Pearson Correlation Coefficients of Air Pollutants, October 2007 to June 2008

Variable	$PM_{2.5-10}$	Total Fungal Spores	O_3	CO	NO_2	SO_2
$PM_{2.5}$	0.50[a]	0.00	0.13[a]	0.64[a]	0.57[a]	0.56[a]
$PM_{2.5-10}$		−0.20	0.30[a]	0.07	0.06	0.24[a]
Total fungal spores			−0.04	0.37[a]	0.24	0.15
O_3				−0.33[a]	−0.32[a]	−0.22[a]
CO					0.88[a]	0.58[a]
NO_2						0.58[a]

[a] $P < .05$.

DISCUSSION

This study used a longitudinal follow-up approach to examine the effects of air pollutants and fungal spores on childhood lung functions. $PM_{2.5}$ and fungal spores adversely affected the FVC 1 day after the exposure. Such effects were not altered when copollutants were included in the models, suggesting that the vital capacity effects of $PM_{2.5}$ and fungal spores were independent of other pollutants. The effect of O_3 on FVC and FEV_1 became insignificant, whereas the effects on $FEF_{25\%}$, $FEF_{50\%}$, $FEF_{75\%}$, and $FEF_{25\%-75\%}$ were not altered when copollutants were included, suggesting that O_3 independently affected the small airways. The lung-function effects of $PM_{2.5-10}$ became insignificant when fungal spores were included in the models, suggesting that the observed effects of $PM_{2.5-10}$ were mainly contributed by the fungal spores.

In cross-sectional studies, the effects of $PM_{2.5}$ and O_3 on childhood lung function have been found.[6] In longitudinal studies, increased exposure to $PM_{2.5}$ was associated with reductions in FEV_1,[7,10] $FEF_{25\%-75\%}$,[7] lowest daily values of FEV_1,[9] as well as with an induction in the diurnal variety of FEV_1[9] in asthmatic children. Exposure to $PM_{2.5}$ also resulted in FVC deficits in both asthmatic and nonasthmatic children.[8] Human experimental studies found that acute O_3 exposure caused more protracted changes in small-

TABLE 3 Association Between 1-Day-Lag Ambient Air-Pollutant Concentrations and Childhood Lung Functions, Single-Pollutant Models

	$PM_{2.5}$, β (95% CI), $\mu g/m^3$	$PM_{2.5-10}$, β (95% CI), $\mu g/m^3$	Total Fungus Spore, β (95% CI), Log Spores per m^3	O_3, ppb, β (95% CI)
FVC, L	−0.16 (−0.23 to −0.08)	−0.19 (−0.28 to −0.10)	−0.12 (−0.21 to −0.03)	−0.11 (−0.17 to −0.05)
FEV_1, L	−0.12 (−0.20 to −0.05)	−0.17 (−0.25 to −0.08)	−0.10 (−0.17 to −0.02)	−0.13 (−0.18 to −0.07)
$FEF_{25\%}$, L/s	−0.06 (−0.25 to 0.12)	−0.29 (−0.50 to −0.08)	−0.07 (−0.26 to 0.12)	−0.39 (−0.51 to −0.27)
$FEF_{50\%}$, L/s	−0.08 (−0.22 to 0.05)	−0.26 (−0.42 to −0.10)	0.02 (−0.11 to 0.16)	−0.32 (−0.41 to −0.24)
$FEF_{75\%}$, L/s	0.00 (−0.09 to 0.08)	−0.13 (−0.23 to −0.03)	0.05 (−0.03 to 0.13)	−0.21 (−0.26 to −0.15)
$FEF_{25\%-75\%}$, L/s	−0.06 (−0.18 to 0.07)	−0.23 (−0.37 to −0.08)	0.05 (−0.08 to 0.17)	−0.30 (−0.38 to −0.22)

The coefficient was calculated for an interquartile range of pollutant: 9.9 $\mu g/m^3$ for $PM_{2.5}$, 8.1 $\mu g/m^3$ for $PM_{2.5-10}$, 1.3 log spores per m^3 for total fungal spores, and 9.5 ppb for O_3. Adjusted for age, square of age, height, square of height, interaction term of age and height, upper-respiratory infection, asthma/AR symptomatic attack, asthmatic/AR medicinal usage, ambient temperature, ambient relative humidity, day-of-week, gender, school, parental education, parental atopy, and home environmental tobacco smoke.

TABLE 4 Association Between 1-Day-Lag Ambient Air-Pollutant Concentrations and Childhood Lung Functions, 2-Pollutant Models

	$PM_{2.5}$, β (95% CI), $\mu g/m^3$	$PM_{2.5-10}$, β (95% CI), $\mu g/m^3$	Total Fungus Spore, β (95% CI), Log Spores per m^3	O_3, ppb, β (95% CI)
FVC, L				
With $PM_{2.5}$	—	−0.14 (−0.24 to −0.04)	−0.14 (−0.23 to −0.05)	−0.11 (−0.17 to −0.06)
With $PM_{2.5-10}$	−0.10 (−0.19 to −0.02)	—	−0.16 (−0.25 to −0.07)	−0.07 (−0.15 to 0.01)
With total fungal spores	−0.10 (−0.18 to −0.03)	−0.12 (−0.25 to 0.00)	—	−0.06 (−0.14 to 0.02)
With O_3	−0.16 (−0.24 to −0.09)	−0.18 (−0.28 to −0.07)	−0.19 (−0.28 to −0.10)	—
FEV_1, L				
With $PM_{2.5}$	—	−0.13 (−0.23 to −0.04)	−0.11 (−0.19 to −0.03)	−0.12 (−0.18 to −0.07)
With $PM_{2.5-10}$	−0.07 (−0.15 to 0.01)	—	−0.12 (−0.21 to −0.04)	−0.09 (−0.18 to 0.01)
With total fungal spores	−0.07 (−0.17 to 0.03)	−0.09 (−0.20 to 0.02)	—	−0.08 (−0.16 to 0.00)
With O_3	−0.13 (−0.20 to −0.06)	−0.15 (−0.25 to −0.06)	−0.15 (−0.23 to −0.08)	—
$FEF_{25\%}$, L/s				
With $PM_{2.5}$	—	−0.32 (−0.55 to −0.08)	−0.11 (−0.30 to 0.09)	−0.39 (−0.51 to −0.27)
With $PM_{2.5-10}$	0.06 (−0.15 to 0.26)	—	−0.11 (−0.32 to 0.09)	−0.37 (−0.49 to −0.24)
With total fungal spores	−0.14 (−0.40 to 0.11)	−0.15 (−0.42 to 0.13)	—	−0.32 (−0.48 to −0.16)
With O_3	−0.07 (−0.24 to 0.10)	−0.15 (−0.38 to 0.07)	−0.21 (−0.39 to −0.02)	—
$FEF_{50\%}$, L/s				
With $PM_{2.5}$	—	−0.27 (−0.45 to −0.10)	0.00 (−0.14 to 0.14)	−0.32 (−0.41 to −0.24)
With $PM_{2.5-10}$	0.02 (−0.13 to 0.17)	—	−0.01 (−0.15 to 0.14)	−0.29 (−0.38 to −0.20)
With total fungal spores	−0.09 (−0.28 to 0.09)	−0.10 (−0.30 to 0.10)	—	−0.24 (−0.35 to −0.13)
With O_3	−0.09 (−0.22 to 0.03)	−0.18 (−0.34 to −0.02)	−0.07 (−0.20 to 0.05)	—
$FEF_{75\%}$, L/s)				
With $PM_{2.5}$	—	−0.16 (−0.27 to −0.05)	0.04 (−0.05 to 0.12)	−0.21 (−0.26 to −0.15)
With $PM_{2.5-10}$	0.06 (−0.04 to 0.15)	—	0.05 (−0.04 to 0.14)	−0.19 (−0.25 to −0.13)
With total fungal spores	−0.05 (−0.16 to 0.06)	0.01 (−0.11 to 0.13)	—	−0.17 (−0.24 to −0.10)
With O_3	−0.02 (−0.10 to 0.06)	−0.11 (−0.21 to −0.01)	0.04 (−0.05 to 0.12)	—
$FEF_{25\%-75\%}$, L/s				
With $PM_{2.5}$	—	−0.25 (−0.41 to −0.09)	0.02 (−0.10 to 0.15)	−0.30 (−0.38 to −0.22)
With $PM_{2.5-10}$	0.04 (−0.10 to 0.18)	—	0.02 (−0.11 to 0.16)	−0.28 (−0.36 to −0.19)
With total fungal spores	−0.09 (−0.26 to 0.08)	−0.08 (−0.26 to 0.10)	—	−0.25 (−0.35 to −0.16)
With O_3	−0.07 (−0.18 to 0.04)	−0.16 (−0.30 to −0.01)	−0.04 (−0.15 to 0.08)	—

The coefficient was calculated for an interquartile range of the pollutant: 9.9 $\mu g/m^3$ for $PM_{2.5}$, 8.1 $\mu g/m^3$ for $PM_{2.5-10}$, 1.3 log spores per m^3 for total fungal spores, and 9.5 ppb for O_3. Adjusted for age, square of age, height, square of height, interaction term of age and height, upper-respiratory infection, asthma/AR symptomatic attack, asthmatic/AR medicinal usage, ambient temperature, ambient relative humidity, day-of-week, gender, school, parental education, parental atopy, home environmental tobacco smoke, and interaction term of 2-pollutant. Examination of SO_2, CO, and NO_2 effects on lung function by adding these pollutants in the 2-pollutant models did not change the negative effects of $PM_{2.5}$, $PM_{2.5-10}$, fungal spores, and O_3.

airway function than changes in FVC or FEV_1.[22,23]

In this study, we observed that FVC, but not FEV_1, was associated with interquartile range differences (18.3–28.3 $\mu g/m^3$) in $PM_{2.5}$. It was comparable with previous follow-up studies that showed significant reductions of FVC, but not FEV_1, in asthmatic and nonasthmatic children exposed to $PM_{2.5}$.[8] Under the exposure range in this study, increases in asthma attacks were not observed. Such findings might suggest that observable adverse effects on FVC occurred at a dose range lower than that reported to induce asthma attacks. Additional investigations on potential mechanisms mediating such effects are warranted.

In humans, exposure to ambient air pollutants with strong oxidative potential may result in adverse airway cellular effects through mechanisms such as cytotoxicity, mutagenicity, and secretions of cytokine, chemokines, and adhesion molecules.[24] PM has been

demonstrated to cause the formation of excessive amounts of reactive oxygen species in respiratory systems of experimental animals, leading to tissue inflammation and cell death.[25] It is believed that the smaller the size of PM, the higher the toxicity through mechanisms of oxidative stress and inflammation.[26] O_3 has a strong oxidative potential, and exposure to it is likely to result in increases of both reactive oxygen and nitrogen species. One longitudinal study[7] found that $PM_{2.5}$ was associated with decrements in FEV_1 and $FEF_{25\%–75\%}$, as well as with increases in thiobarbituric acid reactive substances in the breath condensation of asthmatic children. These findings suggested a coherent outcome for lung-function decrement and oxidative stress. Supportive evidence has shown that in asthmatic children exposed to $PM_{2.5}$ and O_3, the interleukin-8 in nasal lavage and nitric oxide in exhaled air increased, indicating local inflammation of the respiratory tract.[8] Results from an experimental study[27] suggested that after exposure to O_3, a reduction in $FEF_{25\%–75\%}$ was related to the increase in the fibrinogen levels in the bronchoalveolar lavage fluid. However, given the complexity of the inflammatory response to air pollution, these results should be interpreted with caution.

Epidemiologic evidence suggested that fungal-spore exposure was associated with a deficit in childhood morning peak expiratory flow rates.[17] In this study, elevated fungal spores were found to cause adverse effects on FVC and FEV_1. Little is known about the pathologic mechanisms behind this relationship. Animal studies have indicated that fungal spores might induce an inflammatory response (production of nitric oxide, interleukin-1β, interferon-α, interleukin-6, and interleukin-10) and cytology in macrophages.[28,29] Inflammatory responses toward specific structural components or metabolic products of microbes might have contributed to the observed respiratory effects measured by elevated fungal spore counts in this study.

As previously documented, air pollutants were not independent of each other. Therefore, we used 2-pollutant models to delineate whether the observed effect of a single pollutant was actually caused by the copollutants. This 2-pollutant model has been an approach used by several other articles studying the health effects of air pollutants.[9,10] Asthma/AR symptomatic attack, Asthma/AR medicinal usage, and upper-respiratory infection in children served as potential confounding factors and were adjusted in the models. In addition, when these variables were compared with 1-day-lag air-pollutant and fungal-spore exposure, no significant relationship was observed. This could be because of the relatively small sample size of the study population. On the other hand, our findings suggested that before causing observable effects on symptomatic attack and medicinal usage, air- pollutant and fungal-spore exposures could have affected lung function. This study was designed to examine the effects of the temporal variation in the ambient air pollutants and fungal spores but not on spatial variation. An assumption of this study was that the participating schools and their students were exposed to similar levels of air pollutants and fungal spores. Although the variable "school" was added to the analytical model to avoid potential confounding factors caused by unforeseen differences, we did not believe that major differences in air pollutants and fungal spores existed among the schools. The analytical results actually showed that school did not affect lung function, nor did it interact with the effects caused by exposure to air pollutants and fungal spores. We tried to limit potential confounding effects from socioeconomic status by recruiting our subjects from the same public-school system as a proxy of socioeconomic status and by adjusting for parental education. The ethnicities of our subjects were rather homogeneous (ie, among 100 participants, the paternal ethnicities were 99 Taiwanese and 1 African, whereas the maternal ethnicities were 99 Taiwanese and 1 Vietnamese).

The strengths of our study included (1) longitudinal measurements of air pollutants, fungal spores, and lung functions (In such a design, lung-function measurements were consistently done after measurements of environmental exposure, and they were compared among individual children using essentially the same methods of data collection. In addition, other personal factors affecting lung function were self-adjusted.); (2) the ability to examine the simultaneous effects of air pollutants and fungal spores; and (3) the uses of various lung-function parameters as outcome measurements. The data presented here are based on 100 children from the original 3937 eligible children with completed questionnaire information. It seems unlikely that selection bias was a factor, given that (1) in each respiratory health condition group, demographic characteristics between subjects and nonparticipants were similar; (2) the basis of subject selection was not associated with the magnitude of respiratory health responses to air pollution; and (3) in this longitudinal design, each subject served as his or her own control, meaning that a different distribution of unmeasured covariates was less likely to affect the association between air pollutants and respiratory health.

There are limitations to this study. First, the exposure assessment used

urban-scale air pollutants as surrogates for personal exposure. A report from the EPA examined the representativeness of the monitoring stations by measuring the levels of pollutants in the surrounding areas. Using a criterion with the correlation coefficient set at 0.9, the coverage of the monitoring station was 3.3 km in radius for the PM, 3.7 km for O_3, 1.4 km for CO, 3.3 km for NO_2, and 2.1 km for SO_2.[30] In our study, almost all children attended schools within 1 km of their homes. Monitoring stations located near the schools (within a 2.5-km radius) also were likely to be near the children's homes. Therefore, measurements by the monitoring stations provided reasonably good indicators of both school and home exposure. Second, the temporal variations of individual air-pollutant exposure were assumed to follow the outdoor and daily average data of the monitoring station. Because of the lack of time-activity data for each child, we could not specify the time spent outdoors, indoors, or exercising, which might have modified exposure to air pollutants in individuals. We assumed that the time-activity among schoolchildren was similar because of the compulsory school timetable. All classrooms in the participating schools used an open environment without air-conditioning facilities; therefore, the children were mostly directly exposed to outdoor air in the school. When the children went home, they were mostly indoors, and the exposure to air pollutants was probably lower. It would cause a nondifferential exposure misclassification if the proportion of time spent indoors was unrelated to the air pollution level. It also would cause a bias toward observing less pollutant effects if the children chose to stay indoors when the air pollution levels were higher. In any case, it was unlikely that the observed effects were overestimated. Third, because of the constraint that pollen levels were not measured, the potential confounding effects of pollen on the observed lung function effects caused by air pollutants and fungal spores could not be totally ruled out. However, the main effects of pollen would have been obstructive ventilator defects of the lung[31] and not the restrictive pattern we observed to be associated with air pollutants and fungal spores. In addition, previous reports from Taipei, Taiwan, showed different temporal distributions of allergenic pollens, air pollutants, and fungal levels.[32,33] Foreign reports[34,35] found different temporal distributions between allergenic pollens and fungal spores as well. Therefore, although we could not completely rule out the confounding effects of pollens in this study, they should be minimal. Finally, the sick leaves of participants in each sampling time could have resulted from respiratory health conditions, which would have caused an underestimation of the health effects of air pollutants and fungal spores.

CONCLUSIONS

The results from our study provide evidence that exposure to current levels of ambient $PM_{2.5}$, fungal spores, and O_3 are associated with reduced childhood lung function. These adverse effects were observed in a longitudinal setting, more specifically in a cohort of schoolchildren that included healthy children. Our results emphasize the continued need to examine the existing standards by documenting potential human adverse effects.

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Effects of Ambient Particulate Matter and Fungal Spores on Lung Function in Schoolchildren

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