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Effect of indoor nitrogen dioxide on lung function in urban environment

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ABSTRACT

Background: High levels of indoor NO_2 are associated with increased asthma symptoms and decreased expiratory peak flows in children. We investigated the association of exposure to domestic indoor NO_2, objectively measured in winter and spring, with respiratory symptoms and lung function in a sample of adolescents from a southern Mediterranean area.
Methods: From a large school population sample ($n=2150$) participating in an epidemiological survey in the urban area of the City of Palermo (southern Italy), a sub-sample of 303 adolescents was selected which furnished an enriched sample for cases of current asthma. All subjects were evaluated by a health questionnaire, skin prick tests and spirometry. One-week indoor NO_2 monitoring of their homes was performed by diffusive sampling during spring and again during winter.
Results: We found that about 25% of subjects were exposed to indoor NO_2 levels higher than the 40 $\mu g/m^3$ World Health Organization limit, during both spring and winter. Moreover, subjects exposed to the highest indoor NO_2 concentrations had increased frequency of current asthma ($p=0.005$), wheeze episodes in the last 12 months ($p<0.001$), chronic phlegm ($p=0.013$), and rhinoconjunctivitis ($p=0.008$). Finally, subjects with a personal history of wheeze ever had poorer respiratory function ($FEF_{25-75\%}$, $p=0.01$) when exposed to higher indoor NO_2 concentrations.
Conclusions: Home exposure to high indoor NO_2 levels frequently occurs in adolescents living in a southern Mediterranean urban area and is significantly associated with the risks for increased frequency of both respiratory symptoms and reduced lung function.

1. Introduction

Nitrogen dioxide (NO_2) is a gaseous substance produced during combustion processes using air as an oxidant. Automobile exhaust, emissions from power plants powered by fossil fuels, and refineries are the most common sources of outdoor NO_2. The indoor NO_2 concentration depends on both the outdoor concentration and indoor emissions (Cyrys et al., 2000; Breysse et al., 2010), as well as air exchange rates (Sakai et al., 2004). In indoor environments, NO_2 emission is mainly due to combustion processes, such as unvented combustion appliances (e.g., gas stoves), vented appliances with defective installations, and tobacco smoke. Exposure to low indoor levels of NO_2 is associated with increased frequency of reported respiratory symptoms (van Strien et al., 2004). Moreover, higher levels of indoor NO_2 are associated with increased asthma symptoms and decreased expiratory peak flows in children (Kattan et al., 2007). Despite higher levels of outdoor NO_2 levels found during cold months (January–February) (AMIA SpA, 2007), in a recent study, warmer weather was found to increase the risk of respiratory health effects due to motor vehicle-related NO_2 pollution (Tramuto et al., 2011).

Since the indoor concentration of NO_2 may be even more elevated than outdoor levels (Lee et al., 2002), there has been increased interest in recent decades in indoor NO_2 pollution, and various research programs on the health effects of indoor air quality have been developed worldwide, regarding both school (Simoni et al., 2010, 2011) and domestic (Gillespie-Bennett et al., 2011; Belanger et al., 2013) indoor environments. Children spend most of their time each day in indoor environments (Klepeis et al., 2001), and it appears that exposure to higher home indoor – and

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not outdoor – NO_2 levels is associated with an increased frequency of respiratory symptoms and poorer respiratory function in asthmatic children (Gillespie-Bennett et al., 2011). Nevertheless, in the Mediterranean area of southern Europe, data on the effects of indoor NO_2 – measured in general population samples of indoor residential settlements in urban environments – on respiratory symptoms and pulmonary function are scanty.

Aims of the present study were to investigate the possible association between NO_2 indoor concentrations (objectively measured in spring and winter) and both environmental and home factors; and to investigate the effect of exposure to domestic indoor and outdoor NO_2 on respiratory symptoms and lung function on a sample of adolescents in southern Italy.

2. Materials and methods

2.1. Population sample

In 2005–2006, a cross-sectional epidemiological survey was performed on a population sample of 2150 schoolchildren, aged 10–17 years, from 16 schools in Palermo, a city of 650,000 inhabitants in the Mediterranean area of southern Italy (Cibella et al., 2011). The schoolchildren were investigated by means of a respiratory questionnaire, skin prick tests (SPT), and spirometry. From the original population of 2150 subjects, 323 individuals (14.0%) were selected for cross-sectional NO_2 home monitoring.

Because of the small prevalence of current asthma in the original population (4.2%), all the current asthma cases were asked to participate in the study for home monitoring (independently of the presence of rhinoconjunctivitis), giving rise to an enriched sample for current asthma with respect to non-current asthma cases. The current asthma cases who decided not to participate did not enter the studied sample and the final sample percentage of current asthma cases was 8.9%, versus 4.2% of the original population sample.

A subsample of non-current asthma cases was selected, for entry in the home monitoring study, according to the following procedure: after geolocalization of all subjects' addresses through a Geographical Information System, the whole city area was partitioned into 1 km-square grids, and a subset of the subjects in each square was randomly chosen weighing the probability of being sampled for each subject so as to maintain the rhinoconjunctivitis prevalence in each square close to that of the larger sample in the same square (see Table 1 for more details). In this way, a representative sample of the original population for major respiratory symptoms was obtained. Sampled houses were then grouped into three different areas of the city: Centre, North, Suburbs (Fig. 1). Houses located outside the city beltway were included in the latter group.

We avoided selecting multiple subjects with the same address. A large number of back-up candidates was also selected, in order to have ready replacements for any subjects refusing to participate in the study.

The final sub-sample was studied between April 2007 and February 2009 by questionnaires, spirometry, skin prick tests and indoor and outdoor NO_2 measurements. The current report is based on analyses of 303 cases.

2.2. Questionnaire

Subjects answered a questionnaire concerning their personal history of disease and respiratory symptoms. The same two "core" questionnaire modules of ISAAC for 13–14 year olds (wheezing and rhinitis) (Asher et al., 1995) and the criteria for current asthma and rhinoconjunctivitis definitions were the same as those used in the larger survey. In particular, a history of current asthma was defined as a positive answer to the question, "*Have you ever had asthma*" plus at least one wheeze episode in the previous 12 months. Rhinoconjunctivitis was defined as a positive answer to both questions: "*Have you ever had a problem with sneezing, or runny, or blocked nose apart from common cold or flu in the past 12 months*" and "*In the past 12 months, has this nose problem been accompanied by itching and/or watering eyes*" (Cibella et al., 2011).

Table 1
General characteristics of the sample and those relevant to the original population sample.

	Present study	Original population sample	p Value
Total number	303	2150	
Male gender (no., %)	147 (48.5)	1057 (49.2)	0.75[a]
Age, years (mean ± SD)	13.2 (±0.6)	12.6 (±1.0)	<0.0001[b]
Height, cm (mean ± SD)	160.7 (±7.4)	154.2 (±8.4)	<0.001[b]
Weight, kg (mean ± SD)	56.7 (±12.6)	51.0 (±13.0)	<0.001[b]
BMI, kg/m^2 (mean ± SD)	21.8 (±3.9)	21.3 (±4.3)	0.03[b]
Chronic cough (no., %)	18 (5.9)	117 (5.5)	0.76[a]
Wheeze ever (no., %)	93 (30.5)	466 (21.7)	0.0006[a]
Wheeze in the last 12 months (no., %)	41 (13.5)	225 (10.5)	0.12[a]
Rhino-conjunctivitis (no., %)	71 (23.4)	430 (20)	0.26[a]
Current asthma (no., %)	27 (8.9)	90 (4.2)	0.0003[a]
Allergic sensitization (no., %)	165 (54.4)	839 (39.2)	<0.0001[a]
FVC, % of predicted (mean ± SD)	100.7 (±11.7)	96.1 (±11.9)	<0.0001[b]
FEV_1, % of predicted (mean ± SD)	103.4 (±12.2)	99.4 (±11.9)	<0.0001[b]
FEV_1/FVC, % of predicted (mean ± SD)	102.3 (±7.0)	103.1 (±6.7)	0.07[b]
$FEF_{25-75\%}$, % of predicted (mean ± SD)	103.2 (±23.1)	101.5 (±21.6)	0.20[b]

[a] χ^2 Test.
[b] t Test for unpaired comparisons.

The presence of chronic cough (as cough for at least 2 months in the last 12 months), chronic phlegm (as phlegm for at least 2 months in the last 12 months), and wheeze episodes ever in life was also investigated.

Moreover, the technician collected information from an adult member of each family about the presence of gas appliances, second-hand smoke exposure (ETS), mould/dampness exposure at home, the number of hours with open windows, number of rooms, type (detached house/condominium) and floor of residence and the number of years spent in the house. In addition, self-reported traffic exposure was recorded as the frequency of trucks passing on the street of residence on weekdays (*never*/*rare*/*frequent*/*constant*).

Household crowding index, as a proxy for socioeconomic status, was computed. It was defined as the total number of co-residents per household divided by the total number of rooms, excluding kitchen and bathrooms (Melki et al., 2004).

2.3. Respiratory function tests and evaluation of allergic sensitization

Height (in cm) and weight (in kg) were measured in standing position without shoes, using a stadiometer and an electronic digital scale for all the children. Pulmonary function tests were performed through a portable spirometer (MicroLoop, Micro Medical, Chatham Maritime, Kent, UK). Forced expiratory volume in 1 s (FEV_1) and forced vital capacity (FVC) were measured according to ATS/ERS guidelines (Miller et al., 2005): the best FVC and FEV_1 were retained and FEV_1/FVC computed, and $FEF_{25-75\%}$ was selected from the manoeuvre with the largest sum of FEV_1

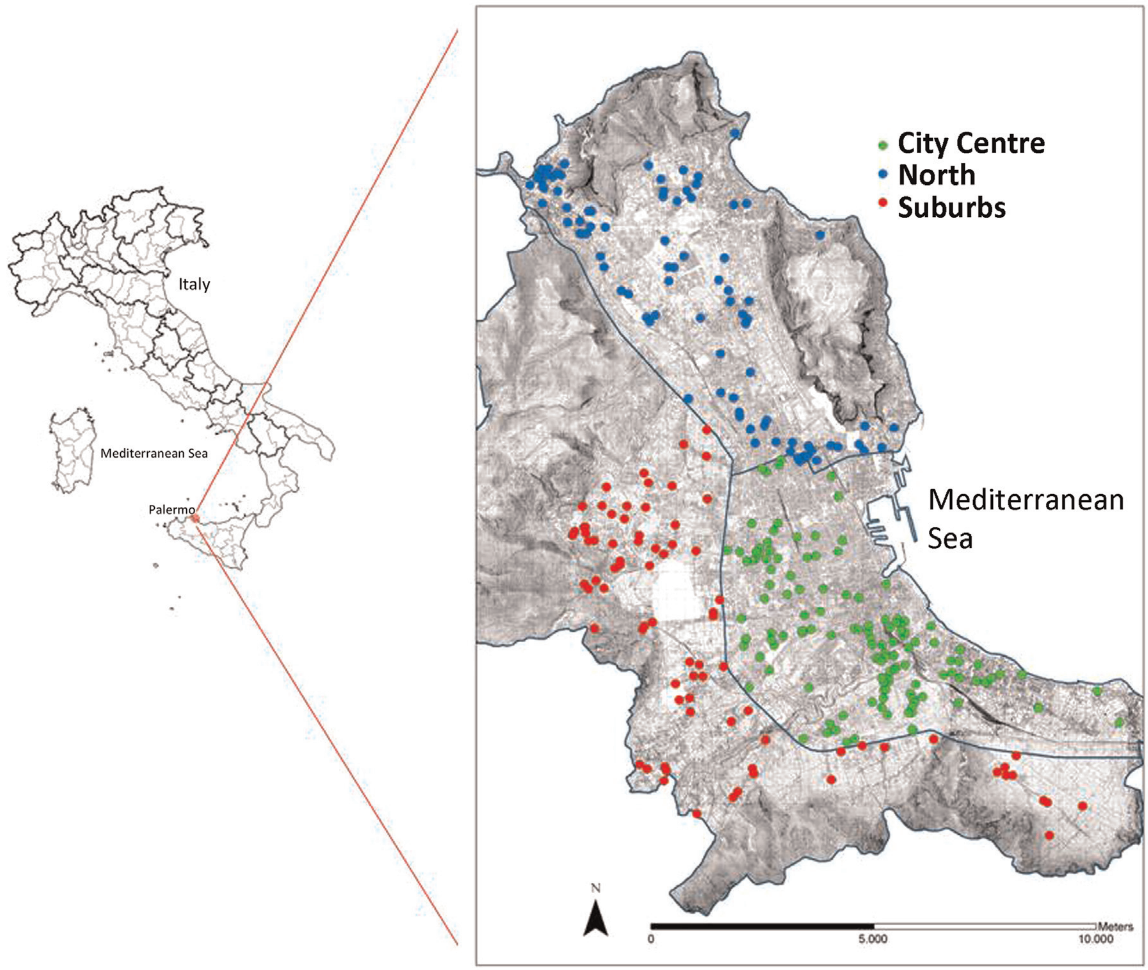

Fig. 1. City map of Palermo, in the Mediterranean area of southern Italy. Houses in which NO_2 measures were performed are depicted separately for different city areas (Centre, North, and Suburbs).

and FVC. Spirometric predicted values were those obtained by the Global Lung Initiative (Quanjer et al., 2012).

Skin prick tests were performed according to EAACI recommendations (The European Academy of Allergology and Clinical Immunology, 1993), with a standard panel including *Dermatophagoides* mix, grass mix, *Parietaria judaica*, olive, dog and cat dander, *Alternaria alternata*, and *Blattella germanica*, plus a positive (histamine 1%) and a negative (saline) control (Stallergènes Italia S. r.l., Milan, Italy). Readings were performed after 15 min: reactions were considered positive if the mean wheal diameter (computed as the maximum diameter plus its orthogonal divided by 2) was 3 mm or greater, after having subtracted the wheal diameter of the reaction to the negative control. Allergic sensitization was defined as the presence of at least one positive skin prick test.

2.4. Monitoring of indoor and outdoor NO_2

Monitoring of indoor and outdoor NO_2 was performed over one week during spring (April–May) and winter months (January–February) in the period indicated above.

We used Radiello® radial diffusive samplers, manufactured by *Fondazione Salvatore Maugeri* (Padua, Italy), consisting of a microporous cylindrical diffusive body (RAD120-1) containing an absorbent polyethylene cartridge coated with triethanolamine (RAD166). The Radiello® for indoor sampling was placed in the main living area (Belanger et al., 2006), the one for outdoor sampling was placed outside (e.g., at a window or on the balcony). The exact times of Radiello® placement and removal were recorded for determining the time of exposure. Once collected, absorbent cartridges were stored in the dark at 4 °C and analysed within 2 months of exposure. Two “blank”, unexposed cartridges were retained for reference for each cartridge batch. The analyses of the absorbent cartridges were performed by *Fondazione Maugeri* laboratories (Padua, Italy). Mean NO_2 concentration was expressed as $\mu g/m^3$.

2.5. Statistical analysis

All continuous variables are reported as mean and standard deviations, categorical variables as absolute number and percentage.

Given the asymmetrical distribution of the NO_2 concentrations, after having tested for distributional normality, the variable was transformed into logarithms and an Analysis of Variance for Repeated Measurements (RMANOVA) was conducted on the log-NO_2 levels to find which environmental factors most affected its concentrations. The variable “Season” (spring and winter) and the variable “Testing Station” (indoor and outdoor) entered the model as within factors; the variables “City Area” (Centre versus Other) and “Traffic Conditions” (questionnaire self-reported evaluation: *never*, *rare*, *frequent*, and *constant*) entered the model as between factors.

A regression analysis was used to determine which home factors could impact indoor NO_2 levels, in spring and in winter separately. Two regression analyses were performed: one with, the other without "outdoor concentrations" as a predictor of "indoor concentrations". Other variables considered were the other home related factors: gas appliances, number of hours of open windows, number of rooms, household crowding index, house type (detached house/condominium), and floor of residence. In each model, a stepwise selection based on the Akaike Information Criterion (AIC) statistics was used, with a search mode operating in both directions (backward and forward).

In the study of the relationship between high level of indoor NO_2 concentration and the onset of current asthma, preliminary analyses were performed to assess the potential impact of confounding factors: a χ^2 test was used to study the possible association between current asthma and other categorical variables (allergic sensitization, parental history for allergy, and City Area), RMANOVA was used to test mean differences of continuous variables (outdoor and indoor concentrations) between levels of the categorical confounding factor.

The relationship between current asthma and indoor NO_2 levels was studied by means of an RMANOVA in which the response variable "indoor NO_2 concentrations" was tested for possible differences between asthmatic subjects and non-asthmatic subjects (between factor). Season was considered as the within factor. A logistic regression model (current asthma as response variable and indoor NO_2 as dependent one) was not used because the enrichment of the sample would have provided artificial estimates of the relative odds ratio. Therefore, the present analysis is focused on a possible correlation between the variables, without regard to any possible causal links between the two variables.

A Receiver Operating Characteristic (ROC) analysis was performed separately for the two seasons to identify those cut-offs of indoor NO_2 concentrations which could possibly represent warning levels for current asthma. A dichotomous variable, representing the exposure to "at risk" NO_2 concentrations was built: subjects were considered exposed to "at risk" NO_2 concentrations if they were exposed to indoor NO_2 values exceeding the spring-cut off or the winter-cut off or both.

The relationship between respiratory symptoms and indoor and outdoor NO_2 concentrations was studied by means of an RMANOVA, as explained above, for the variable current asthma. Preliminarily, a χ^2 test was used to study the association between current asthma and the other respiratory symptoms likely associated with the presence of current asthma. Binomial tests were performed to verify if the subgroups experiencing one of the analysed symptoms distributed differently in the two levels identified by the dichotomous variable representing the exposure to "at risk" NO_2 concentrations. A χ^2 test was not used in order to prevent misleading associations (between the symptom and any other variable) due to the sample enrichment procedure: in fact, the proportion of subjects with current asthma with respect to non-current asthma (as well as the proportions related to all the other respiratory symptoms associated with the presence of current asthma) is not representative of the entire population.

Respiratory function variables (FEV_1, FVC, FEV_1/FVC and $FEF_{25-75\%}$) were considered as percentage of predicted. Correlations between spirometric variables and indoor NO_2 concentrations were computed for both spring and winter.

Respiratory function variables (as percentage of predicted) were also analysed by means of an ANOVA, using the dichotomous variable representing the exposure to "at risk" NO_2 concentrations (as derived by the ROC curve) as well as each health symptom (presence/absence), one at time, as a "between" factor. The analysis was aimed at testing whether respiratory function can be affected by high NO_2 concentrations when in association with each considered health symptom (interaction term).

Any other association between categorical variables was tested performing a χ^2 test, whereas a *t*-test was used to study possible differences of a continuous variable between two levels of a factor.

All analyses were performed in R. A *p*-Value <0.05 was assumed to be statistically significant.

2.6. Ethics statement

The study was approved by the Ethical Committee of the University Hospital of Palermo [01/07]. All parents of the invited children signed a written informed consent. According to the Italian law, the respect of individual privacy concerning clinical data was granted.

3. Results

In Table 1 the general characteristics of the sample (compared to those relevant to the original population sample) are presented. Due to the time shift between the campaign in which the original population sample was obtained (Cibella et al., 2011) and the present indoor monitoring study, significant differences exist in all the anthropometric variables and in those strongly related to lung size (namely, FVC and FEV_1). Moreover, because of the sample enrichment in current asthma, the frequency of all the variables associated with allergic sensitization was significantly higher.

Mean (and SD, μg/m^3) of NO_2 levels during spring were 31.9 (±14.9) indoor and 28.1 (±12.7) outdoor. The same figures during winter were: 32.2 (±16.3) indoor and 28.0 (±14.5) outdoor. In Fig. 2 box plots are presented relevant to the distribution of indoor and outdoor NO_2 concentration in both seasons. During spring and winter, 24.5% and 25.2% of subjects, respectively, were exposed to indoor NO_2 levels higher than the World Health Organization indoor limit of 40 μg/m^3 (WHO, 2010).

3.1. Environmental factors and NO_2 concentrations

Results on NO_2 concentrations from the RMANOVA showed significance for the main factors of Testing Station, City Area, and Traffic Condition ($p<0.001$ in all cases), with higher NO_2 concentrations for indoor station (Fig. 3), centre area and constant traffic; no interaction factor was significant, apart from the interaction between "City Area" and "Testing Station" ($p=0.02$): indoor and outdoor NO_2 concentrations (μg/m^3) did not differ in the city centre, 35.2 (±15.3) versus 32.3 (±12.9) respectively; indoor NO_2 concentrations were higher than outdoor concentrations in both the North (31.4 [±16.3] indoor and 28.0 [±14.1] outdoor) and

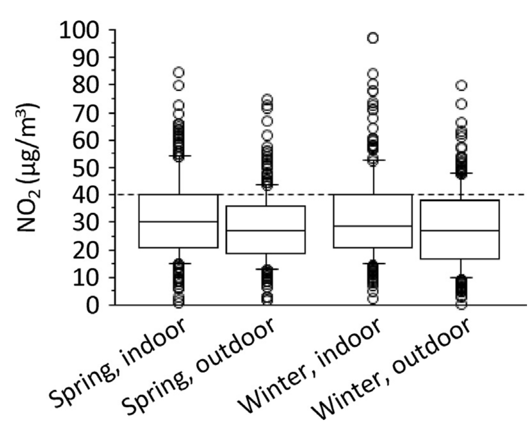

Fig. 2. Distribution of indoor and outdoor nitrogen dioxide (NO_2) values measured during spring and winter. Bars indicate (from the bottom to the top) 10th, 25th, 50th (median), 75th, and 90th percentiles. Values below 10th and above 90th percentiles are plotted as circles. The WHO maximum annual average indoor NO_2 limit (40 μg/m^3) is depicted. Indoor values were significantly higher than outdoor ones (RMANOVA, $p<0.001$), without any effect of season.

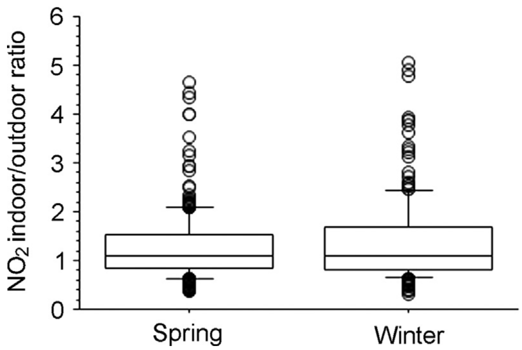

Fig. 3. Distribution of indoor and outdoor NO_2 ratio measured during spring and winter. Indoor NO_2 concentration was significantly higher than outdoor concentration ($p<0.001$ by Analysis of Variance for Repeated Measurements model). Bars indicate (from the bottom to the top) 10th, 25th, 50th (median), 75th, and 90th percentiles. Values below 10th and above 90th percentiles are plotted as circles.

Suburbs (27.6 [$\pm$ 13.7] indoor and 21.1 [$\pm$ 10.9] outdoor) areas. No effect of the "Season" factor was evident.

3.2. Environmental and home factors and NO_2 concentrations

A regression model was built separately for the two seasons: as concerns NO_2 indoor concentrations measured in the spring, the final model included NO_2 outdoor concentration ($p<0.001$), house type ($p=0.036$) and household crowding index ($p=0.093$) as independent variables. In winter, only two variables entered the final model: winter outdoor concentrations ($p<0.001$) and house type ($p=0.11$). In both models, the indoor concentrations increased with an increase of the outdoor concentrations and when the house was of the condominium type. When outdoor concentrations were not considered in the analyses, the only variable included in the final models was the house type, with a *p* Value less than 0.001 in spring and equal to 0.003 in winter.

3.3. Relationship between indoor NO_2 concentrations and current asthma

Because, a priori, the population of non-asthmatic children cannot be representative of the entire population (given the sample-enrichment procedure followed in the present study), preliminary tests were used to verify the possible influence of potential confounding factors in the study of the relationship between high levels of indoor NO_2 and the onset of current asthma. Among the possible confounding factors, allergic sensitization, parental history for allergy and the City Area (Centre, North, Suburbs) were taken into account. The association between allergic sensitization and current asthma, as well as the association between parental history for atopic diseases and current asthma, were both significant ($p<0.001$ and $p=0.001$). In fact, the nearly all (92.6%) subjects with current asthma presented allergic sensitization, and about 37% of asthmatic subjects presented parental atopy, compared to only 12% of non-asthmatic individuals. A χ^2 test on current asthma and City Area was borderline significant ($p=0.05$), given that the majority of subjects with current asthma (67%) live in the city centre where, from a previous analysis, NO_2 concentrations appeared to be significantly higher than in other city areas. Preliminary RMANOVA tests were therefore performed in order to verify if outdoor concentrations were significantly different in the two seasons between subjects with and without current asthma (to take into account the different distribution of subjects in the city), and to test simultaneously: i) the difference in the average indoor NO_2 concentrations in the two levels of the fixed factors, allergic sensitization and parental history for atopy; ii) the possible interaction between the considered factor and the season. These tests would provide a clue to possible systematic errors due to sampling, which would invalidate results related to any relationship between current asthma and indoor NO_2 levels. Outdoor levels to which current asthma and non-current asthma subjects were exposed were not significantly different ($p=0.44$), and no significant interaction appeared between current asthma and Season ($p=0.18$). Moreover, results were not significant in either the simple effects or the interaction terms, when allergic sensitization and parental history for atopy were tested: indoor NO_2 levels did not differ between subjects with or without allergic sensitization ($p=0.23$) or between subjects with or without parental atopy ($p=0.69$).

The RMANOVA, testing the possible difference of indoor NO_2 concentrations between subjects with and without current asthma, showed significance of the current asthma factor ($p=0.005$): indoor NO_2 concentrations were higher for asthmatic subjects both in spring and in winter (36.7 [$\pm$ 11.6] versus 31.5 [$\pm$ 15.1] and 44.3 [$\pm$ 25.3] versus 31.1 [$\pm$ 14.9] $\mu g/m^3$, respectively).

Results from the ROC analyses identified 28.0 and 41.4 $\mu g/m^3$ as optimal cut off values for indoor NO_2 concentrations in spring and winter, respectively. Corresponding to these cut-offs, Sensitivity and Specificity were 81.5% and 50.0% in spring, and 60.0% and

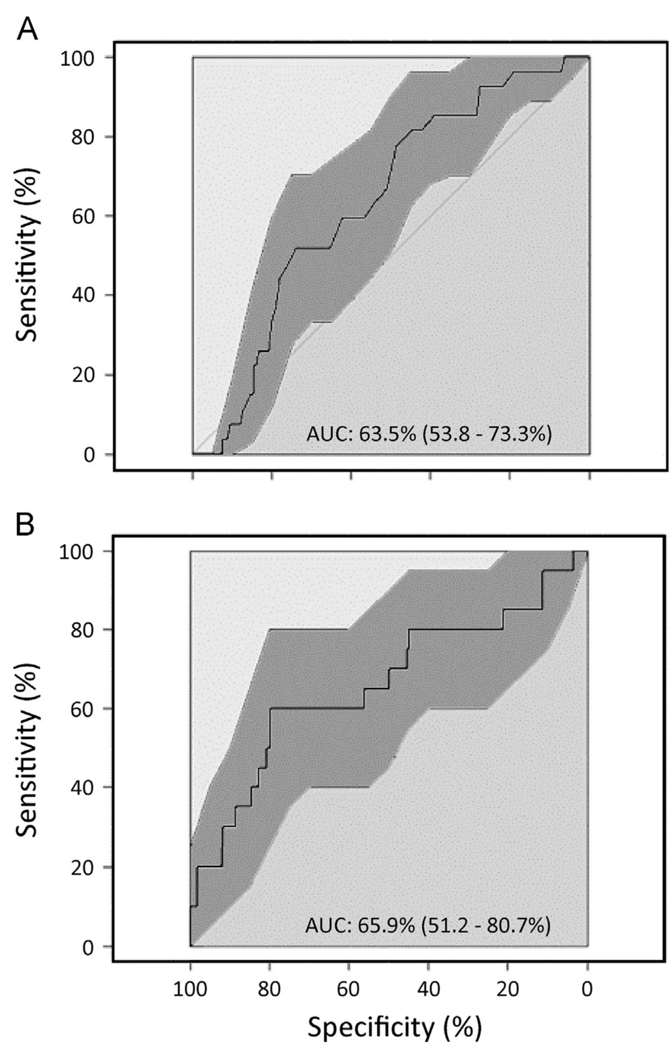

Fig. 4. Receiver operating characteristic curves. Sensitivity and Specificity, related to "at risk" cut-off values for current asthma, of indoor NO_2 concentrations in spring (panel A) and in winter (panel B).

79.7% in winter. The areas under the curves were 63.5% (CI: 53.8–73.3%, $p=0.02$) in spring and 65.9% (CI: 53.8–73.3%, $p=0.018$) in winter. In Fig. 4, panels A and B report the two ROC curves in spring and winter.

3.4. Relationship between high levels of indoor NO_2 concentrations and respiratory symptoms

The variables wheeze in the past 12 months, rhinoconjunctivitis and allergic sensitization were strongly associated with current asthma ($p<0.001$ from χ^2 test). In fact, all subjects with current asthma, due to its definition, presented wheeze in the past 12 months; moreover, 74% of current asthma individuals had rhinoconjunctivitis and 92.6% presented allergic sensitization). Thus, when studying the relationships existing among these health conditions (i.e., wheeze in the past 12 months, rhinoconjunctivitis, and allergic sensitization) and indoor NO_2 levels, the results may depend on the interaction between the considered symptom and current asthma. The other studied health symptoms were chronic cough and chronic phlegm. When an RMANOVA was used, apart from the expected significant differences in the level of indoor NO_2 concentrations between subjects with wheeze in the past 12 months or with rhinoconjunctivitis and subjects without symptoms ($p=0.003$ and $p=0.02$, respectively), results showed that subjects with chronic phlegm were exposed to significantly ($p=0.032$) higher levels of indoor NO_2 concentrations than subjects without chronic phlegm: 41.1 [±20.5] versus 31.5 [±15.0] $\mu g/m^3$, independent of the Season. Table 2A and B report the mean values of indoor and outdoor NO_2 concentrations respectively, in subjects with and without respiratory symptoms, in spring and in winter. The *p* Values from RMANOVA related to the symptom are also reported.

By means of the cut-off values identified by ROC analysis (28.0 and 41.4 $\mu g/m^3$ in spring and in winter, respectively), each subject could be categorized as exposed or not to indoor NO_2 concentrations at risk for current asthma. According to this classification, 174 subjects (57.4%) out of 303 were determined to be exposed to “at risk” indoor NO_2 concentrations. For each health symptom, a binomial test was performed to verify if, within the subgroup where one of the symptoms was present, there was a significant difference in the proportion of subjects belonging to the two levels of the dichotomous variable. Again, tests were significant for current asthma ($p<0.001$), wheeze in the past 12 months ($p<0.001$), rhinoconjunctivitis ($p=0.008$), allergic sensitization ($p=0.019$) and chronic phlegm ($p=0.013$). Table 3 reports the distributions of the subjects with the symptoms in each level of the cut-off variable. In Table 4 the distribution of current asthma is presented for each combination of exposure to “at risk” indoor NO_2 concentration and of allergic sensitization: almost one fourth of subjects presented current asthma if allergic sensitization was present along with the exposure to “at risk” indoor NO_2 concentration.

3.5. Relationship between high levels of indoor NO_2 concentrations and respiratory function

Correlation between both indoor and outdoor NO_2 concentration and the variables relevant to the respiratory function were all non-significant. Conversely, the ANOVA analyses performed on respiratory function, conducted using the exposure to “at risk” NO_2 concentrations and each health symptom as “between” factors, showed significant interaction only between $FEF_{25-75\%}$ and wheeze ever. In fact, $FEF_{25-75\%}$, when expressed as percent of predicted, was significantly smaller in subjects with wheeze ever exposed to at risk indoor NO_2 concentration levels: 95.2 ± 20.0 versus 110.3 ± 22.8 ($p=0.01$) in the presence and absence of wheeze ever, respectively; on the contrary, values were very similar when subjects were exposed to low NO_2 concentrations: 101.1 ± 23.0 and 101.1 ± 23.4 in the presence and absence of wheeze ever, respectively.

4. Discussion

In the present cross-sectional study, more than 300 children were evaluated by a self-administered questionnaire: in addition, objective measures of lung function (spirometry) and individual allergic sensitization (skin prick tests) were performed. At the same time, all their residences were investigated for NO_2 environmental pollution (by Radiello® passive samplers) in order to objectively assess the association between environmental factors and individual's health status.

With regard to the recent recommendation of the World Health Organization of a maximum annual average indoor NO_2 limit of 40 $\mu g/m^3$ (WHO, 2010), one of the findings of the present study is that about 25% of the subjects living in the investigated houses appear to be exposed to indoor NO_2 levels higher than this limit, during both spring and winter. To our knowledge, this is the first study estimating indoor exposure to NO_2 in domestic environments of a general population in relation to the WHO limit. Moreover, we found that: (1) a positive significant relationship exists between indoor and outdoor NO_2 levels during both spring and winter, without any significant effect of season; 2) NO_2 levels were higher in the residences located in the city centre than in suburban area; 3) both outdoor and indoor NO_2 levels were significantly higher in the residences of subjects self-reporting high exposure to truck traffic; 4) exposure to the highest levels of indoor NO_2 concentration was associated with an increased prevalence of chronic phlegm, wheeze in the past 12 months, and current asthma; and 5) subjects with a personal history of wheeze ever had poorer respiratory function when exposed to higher indoor NO_2 concentration.

In accordance with a previous French study using a similar methodological approach, we found that indoor NO_2 level is

Table 2A
Indoor NO_2 concentrations ($\mu g/m^3$, mean and SD) in the two Seasons in subjects with or without respiratory symptoms.

	Indoor NO_2 concentrations ($\mu g/m^3$)				*p* Value[a]
	Spring Yes	Winter	Spring No	Winter	
Rhino-conjunctivitis	34.6 (±14.0)	35.5 (±17.1)	31.1 (±15.1)	31.4 (±16.1)	0.02
Chronic phlegm	42.4 (±17.6)	39.4 (±24.2)	31.3 (±14.5)	31.8 (±15.7)	0.032
Chronic cough	34.7 (±12.2)	32.3 (±16.3)	31.8 (±15.0)	32.2 (±16.3)	0.46
Wheeze in the last 12 months	36.7 (±14.1)	40.4 (±22.2)	31.2 (±14.9)	31.1 (±15.1)	0.003
Current asthma	36.7 (±11.6)	44.3 (±25.3)	31.5 (±15.1)	31.1 (±14.9)	0.005
Allergic sensitization	32.4 (±14.7)	32.4 (16.4)	31.4 (±15.1)	32.0 (±16.3)	0.69

[a] *p* from RMANOVA.

Table 2B
Outdoor NO_2 concentrations (μg/m^3, mean and SD) in the two Seasons in subjects with or without respiratory symptoms.

	Outdoor NO_2 concentrations (μg/m^3)				p Value[a]
	Spring Yes	Winter	Spring No	Winter	
Rhino-conjunctivitis	29.2 (±12.6)	29.9 (±15.4)	27.7 (±12.7)	27.5 (±14.3)	0.28
Chronic phlegm	29.9 (±14.2)	30.3(±17.1)	28.0 (±12.6)	27.9 (±14.4)	0.54
Chronic cough	26.6 (±11.3)	25.3 (±14.4)	28.2 (±12.7)	28.2 (±14.6)	0.59
Wheeze in the last 12 months	29.1 (±10.2)	30.8 (±17.6)	27.9 (±13.0)	27.6 (±14.1)	0.24
Current asthma	29.8 (±11.3)	33.7 (±19.1)	27.9 (±12.8)	27.5 (±14.0)	0.19
Allergic sensitization	29.1 (±12.5)	29.6 (15.8)	26.8 (±12.8)	26.2 (±12.9)	0.66

[a] p from RMANOVA.

Table 3
Distribution of the subjects with respiratory symptoms exposed to "at risk" indoor NO_2 concentrations, as evaluated through ROC curve cut-off values.

	Number of subjects with symptoms exposed to "at risk" indoor NO_2 concentrations/ number of subject with symptoms (%)	p[a]
Chronic phlegm	14/17 (82%)	0.013
Chronic cough	13/18 (72%)	0.096
Allergic sensitization	98/165 (59%)	0.019
Wheeze in the last 12 months	32/41 (78%)	<0.001
Rhino-conjunctivitis	47/71 (66%)	0.008
Current asthma	24/27 (89%)	<0.001

[a] p from binomial tests.

Table 4
Prevalence of current asthma for each combination of exposure to "at risk" indoor NO_2 concentration and allergic sensitization. The prevalence of each combination is also displayed.

No. (%)[a]	Exposure to "at risk" of indoor NO_2 concentration	Allergic sensitization	Current asthma (%)
62 (20.5%)	No	No	0.0
76 (25.1%)	Yes	No	2.6
67 (22.1%)	No	Yes	4.5
98 (32.3%)	Yes	Yes	22.4

χ^2 for frequency distribution of current asthma was significant ($p<0.001$).

[a] Absolute count (and prevalence) of each combination of exposure to "at risk" indoor NO_2 concentration and of allergic sensitization.

correlated to outdoor NO_2 (Flamant-Hulin et al., 2010). In addition, similarly to previous results (Hansel et al., 2008), we found that indoor NO_2 levels are higher than outdoor levels, possibly due to the presence of indoor NO_2 sources (Cyrys et al., 2000). Historical data from urban pollution monitoring stations in Palermo show that an increase of outdoor NO_2 levels is usually detected during January and February; lower values are usually recorded during spring. Despite these data, we did not find any significant difference between winter and spring in indoor NO_2 measures. Thus, elevated indoor NO_2 levels may persist without significant seasonal changes: relatively mild winters, typical of the Mediterranean area, with small changes in temperature between summer and winter, might explain this result. Moreover, it must be remembered that Radiello® outdoor measures were performed in the immediate outdoor area of each house, i.e., in a location largely different from that used for environmental monitoring stations.

As concerns outdoor NO_2, in the absence of power plants or petroleum refineries close to Palermo, the main sources of fossil fuel pollution are vehicular traffic and ship transportation. Accordingly, we found that outdoor NO_2 showed its highest values in the city centre, both in spring and winter, while suburbs presented significantly lower NO_2 levels. In view of the correlation between indoor and outdoor NO_2 concentrations, residences located in the central urban area also presented higher values for indoor NO_2. Thus, living in the central area of the city and in condominiums were determinants for the highest indoor NO_2 levels. Conversely, we did not find a significant effect of the presence of gas appliances, number of hours of window opening, number of rooms, household crowding index, and floor of residence on indoor NO_2 levels. We did not take into account the presence of "gas fires", but only the presence of indoor sources of gas for the production of hot water and domestic heating: in fact, domestic habits, in Palermo, exclude the use of electric stoves for cooking.

Although the highest NO_2 levels are usually found in the kitchen (Simoni et al., 2002), we placed Radiello® passive monitors in the living room because children spend most of their time in this room during daily life at home. Moreover, boys may spend their time in the kitchen differently from girls (Jarvis et al., 1996).

We found a significantly increased indoor NO_2 level measured in the houses of subjects reporting high truck traffic near their residence. This result supports the validity of self-reported exposure to vehicular traffic collected by questionnaires. Despite the fact that self-reported air pollution in residential areas appeared to be significantly associated with increased levels of GIS-modelled air pollution, subjects with chronic disease tend to report more air pollution in the residential area (Piro et al., 2008). In the present paper, the use of direct indoor and outdoor measures of a specific pollutant made possible the estimation of exposure/effect relationships, avoiding any reporting bias that might alter the association between reported exposure to road traffic and disease (Kuehni et al., 2006), and supports the utility of questionnaire-based exposure information (see also Nuvolone et al., 2011).

We investigated whether poor indoor air quality significantly increases respiratory symptoms using the well-validated questionnaire-based definitions of the ISAAC Study (Asher et al., 1995). Cut-off values for higher NO_2 indoor concentration were computed by ROC curves (Fig. 4) to identify, separately for spring and winter, NO_2 levels representing a possible risk for respiratory symptoms. Our results support an association between indoor NO_2 concentration and respiratory health in adolescents: conversely, as previously reported (Gillespie-Bennett et al., 2011), no significant association with outdoor NO_2 concentration was found. In bivariate models, we found that 82% of children reporting chronic phlegm and 78% of those with wheeze in the past 12 months were exposed to higher indoor NO_2 levels. Also, the presence of current asthma was strongly associated with higher indoor NO_2 concentrations. Thus, our results support other studies finding that home exposure to higher indoor NO_2 levels is associated with an increased prevalence of lower respiratory symptoms, previously shown both in children from a general population sample (Neas et al., 1991) and in asthmatic children (Kattan et al., 2007; Belanger et al., 2006). A recent meta-analysis has also shown that NO_2 increases the risk of current wheeze in children (Lin et al., 2013). It is of interest that our results, based on the objective evaluation of i) allergic sensitization and ii) exposure to "at risk" NO_2 indoor concentration, show that children presenting both conditions (i.e., allergic sensitization and exposure to high indoor NO_2 levels) had a striking increase of the risk for current asthma, up to 22.4% prevalence (while none of the subjects without allergic sensitization and exposed to lower indoor NO_2 levels presented current asthma). Previous reports exist on the possible synergistic action between allergic sensitization and exposure to indoor air pollutants such as NO_2 (Flamant-Hulin et al., 2010), even though controversial results exist: in fact, in a previous paper increasing levels of NO_2 produced only a small and non-significant increase in the risk of respiratory symptoms in atopic children (Garrett et al., 1998). Interestingly, we found clear evidence that in allergic adolescents with personal history of bronchial asthma the exposure to indoor NO_2 is associated with an increased risk of wheeze episodes indicating a possible gene–environment interaction.

Finally, in children presenting both a personal history of wheeze ever and an exposure to higher indoor NO_2 concentration we found a significantly lower $FEF_{25-75\%}$ with respect to those exposed to lower indoor NO_2 levels. This result is in agreement with a previous paper by Gillespie-Bennett et al. (2011) showing the effect of indoor NO_2 exposure on asthmatic children in New Zealand. In this paper, NO_2 exposure was associated with increased frequency of respiratory tract symptoms and decreased FEV_1. Similarly, exposure to environmental NO_2 – estimated by means of a land use regression model – was shown to be associated with reduced expiratory flows in schoolchildren (Rosenlund et al., 2009). These results strongly suggest that home exposure to indoor NO_2 not only increases the frequency of respiratory symptoms (subjective data), but also impairs lung function (objective data) in children.

5. Conclusions

To our knowledge, this is the first study performed in the Mediterranean area evaluating the association between indoor NO_2 pollution and respiratory health in adolescents, thus assessing the interaction between individual risk factors and home environments in a warm climate characterized by hot summers with low rainfall and mild winters. In this area, populations have largely different standards of living and lifestyles. Because these differences may act as effect modifiers in the relationship between indoor pollutants and respiratory health, our findings warrant being confirmed in different local settings.

Exposure to high indoor NO_2 levels frequently occurs in the residences evaluated in the present survey, and this exposure significantly increases the risk for both respiratory symptoms and reduced lung function. Of particular interest is the interaction between allergic sensitization and environmental NO_2 exposure, which yields a very high risk for respiratory morbidity in adolescents. Public health policies aimed at specifically addressing the issue of air pollution in indoor environments should be promoted with a specific focus on possible domestic emissions.

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