CO₂ concentration in day care centres is related to wheezing in attending children

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Abstract Poor ventilation at day care centres (DCCs) was already reported, although its effects on attending children are not clear. This study aimed to evaluate the association between wheezing in children and indoor CO₂ (a ventilation surrogate marker) in DCC and to identify behaviours and building characteristics potentially related to CO₂. In phase I, 45 DCCs

from Lisbon and Oporto (Portugal) were selected through a proportional stratified random sampling. In phase II, 3 months later, 19 DCCs were further reassessed after cluster analysis for the greatest difference comparison. In both phases, children's respiratory health was assessed by ISAAC-derived questionnaires. Indoor CO₂ concentrations and building

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What is known—what is new:

Poor ventilation at day care centres (DCC) was already reported. The results of the present study suggest that poor ventilation in DCC could be related to wheezing in children. Even though natural actions could not be sufficient to provide ventilation and reduce CO₂ to acceptable levels, the increment of natural ventilation through effective open window practices and adjusting the number of children in each classroom may be considered.

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characteristics of the DCC were evaluated in both phases, using complementary methods. Mixed effect models were used to analyze the data. In phase I, which included 3,186 children (mean age 3.1 ± 1.5 years), indoor CO₂ concentration in the DCC rooms was associated with reported wheezing in the past 12 months (27.5 %) (adjusted odds ratio (OR) for each increase of 200 ppm 1.04, 95 % CI 1.01 to 1.07). In phase II, the association in the subsample of 1,196 children seen in 19 out of the initial 45 DCCs was not significant (adjusted OR 1.02, 95 % CI 0.96 to 1.08). Indoor CO₂ concentration was inversely associated with the practices of opening windows and internal doors and with higher wind velocity. A positive trend was observed between CO₂ and prevalence of reported asthma (4.7 %). *Conclusion:* Improved ventilation is needed to achieve a healthier indoor environment in DCC.

Keywords Children · Environmental health · Indoor air quality · Kindergarten · Ventilation

Abbreviations

CI	Confidence interval
CO ₂	Carbon dioxide
DCC	Day care centre
ENVIRH	Environment and Health in children day care centres
IAQ	Indoor air quality
OR	Odds ratio
SD	Standard deviation

Introduction

Wheezing is very common in early childhood as almost half of the children suffer at least one wheezing episode before 6 years

of age [12]. Numerous factors have been associated with a higher prevalence of wheezing and asthma, namely atopy, parental history of allergic respiratory disease and exposure to tobacco smoke [22, 17]. Attendance at a day care centre (DCC) also involves a higher risk of infections and wheezing [9, 6]. In fact, during the first years of life, children attending at a DCC have a higher incidence of airway infections and respiratory symptoms than children that stay at home [8].

High concentration of indoor air pollutants and carbon dioxide (CO₂) in DCC for children has been reported, and poor indoor air quality (IAQ) and ventilation conditions were associated with higher levels of CO₂ [21, 3, 15, 4] and with other indoor air pollutants [24].

The type of ventilation at DCC was related to respiratory symptoms [25]. Proper ventilation is the most effective way to reduce indoor air pollutant concentrations [25, 21]. CO₂ is considered an indirect marker of IAQ in the absence of other sources besides the anthropogenic [5]. Its concentration could be highly influenced by the number of occupants in the room [10], human behaviours and building characteristics [4]. A high CO₂ concentration suggests a low air supply which may elicit symptoms due to higher concentrations of air pollutants [21]. On the other hand, it can reflect an excessive number of children in the room [10], which might enhance respiratory infections.

The present study reports results from the Environment and Health in children day care centres (ENVIRH) project conducted in Portugal, with bi-seasonal environment and health assessments. It aimed to evaluate the association between reported wheezing and measured indoor CO₂ and other environmental comfort parameters at DCC for children and to explore potential behaviours and building characteristics which may be related to indoor CO₂ concentrations.

Material and methods

Study sample and design

The ENVIRH project took place in the two main Portuguese cities, Lisbon and Oporto, comprising two cross-sectional phases. The biphasic design aimed to improve both the geographical and demographic representativeness and to enhance the power to find significant associations using limited human and technical resources for accurate IAQ measurements. In phase I (October to December 2010), through proportional stratified random sampling, which considered the proportionality in each stratum (district) of the number of recruited centres to the total number of centres, 45 DCCs (25 in Lisbon and 20 in Oporto) were sampled from the 88 eligible DCCs (48 in Lisbon and 40 in Oporto). The 45 selected DCCs were attended by 5,161 children. A screening questionnaire on child respiratory health addressed to the parents was applied, and environmental air quality and comfort parameters were assessed.

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In phase II (March to April 2011), after a cluster analysis (which considered CO₂ concentrations, temperature and humidity at indoors) by a hierarchical model (Ward method), 19 DCCs out of 45 were selected from the more different clusters. Those DCCs were attended by 2,287 children. During this phase, CO₂ concentrations in the DCC were further assessed, and a full health questionnaire was applied to the children.

The ENVIRH project was approved by the Ethics Commission of Faculdade de Ciências Médicas, Universidade Nova de Lisboa. The database was registered in the Portuguese Data Protection Authority (CNPD). Parents were informed on the ENVIRH project, and signed consent was obtained.

Health assessments

In phase I, a short screening questionnaire derived from the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire [1] was distributed to the children that attended the recruited DCC, in order to identify several respiratory symptoms, including wheezing, symptoms in the previous 12 months and the existence of asthma diagnosed by a physician. In phase II, the full Portuguese version of the ISAAC questionnaire [18] was applied to the children studied in phase I who attended the selected DCC. Both questionnaires were filled in by the parents.

The main clinical outcomes in children were reported wheezing in the previous 12 months and reported asthma diagnosis. Reported personal history of atopic dermatitis was used as a marker of atopy in children; parental history of allergic respiratory disease was defined as reported existence of asthma or allergic rhinitis.

CO₂ concentration and comfort assessment

In phase I, an average of three rooms was monitored in each DCC, for a total of 143 rooms. The sampling of rooms allowed conducting the survey in a short period of time, under weather conditions as similar as possible. The sampled rooms were selected for a worst case scenario, through empirical evaluation of the building, considering in each DCC the spaces with a higher density in occupancy, fewer windows and windows that were apparently less permeable to air. For each room, the following data were collected: area and volume, type of windows, window positions at the time of the visit (open or shut), position of internal door (open or shut), number of children present in the room at the time of the survey, and indoor and outdoor air temperature, relative humidity and CO₂ concentrations. CO₂ measurements were performed during a period of DCC activity using a non-dispersive infrared absorption detector (Telaire® 7001, Santa Barbara, USA) after 10 to 15 min of stabilization of the reading in the equipment. A digital thermo-hygrometer

(THGR328N®, Oregon Scientific, Tualatin, OR, USA) was used for the measurement of air temperature and relative humidity.

In phase II, CO₂ was measured in every room of each DCC (total of 125 rooms) using a Photoacoustic Multi-gas Monitor (model 1312, INNOVA AirTech Instruments, Ballerup, Denmark). Sampling periods of 30 min with readings every 75 s were taken. The monitor was placed in the breathing zone of the children near the ground area (0.5–0.7 m) with caution to avoid contamination. Measurements were performed during a period of DCC activity, starting at least 1 h after occupation, in order to obtain CO₂ concentrations more representative of the periods the children spent in the rooms.

In the present analysis, CO₂ (ppm) was considered as a major comfort parameter and as a surrogate of IAQ and ventilation flow rate (13).

Building characteristic assessment

The same assessments were done at the two phases, although in phase II, only modifications since the phase I visit were recorded. Parameters that could influence comfort and IAQ parameters were assessed for each DCC. They included windows and type of shading characteristics, ventilation, heating and air conditioning and ventilation strategy practices. External windows and doors of the rooms were evaluated according to their framing material (aluminium, steel, and wood), opening type (casement, sliding or tilt windows) and type of gaskets (no gaskets, plush gaskets or rubber gaskets). The existence of dampness/moulds in the rooms was assessed by visual inspection.

Statistical analysis

For both phases, an exploratory, descriptive analysis of the variables of interest was carried out. Reported wheezing and asthma diagnosis were both considered as binary variables (“yes” or “no”). Prevalence rates of reported wheezing and related asthma diagnosis were given with 95 % confidence intervals (95 % CI). For phase I, the averages of the measurements of CO₂ concentration, indoor temperature and humidity for selected rooms in each DCC were assumed.

The associations between children’s characteristics and their clinical outcomes were calculated and expressed as crude and adjusted odds ratio (OR). To evaluate the association between IAQ and comfort parameters, including CO₂, indoor temperature and relative humidity at DCC and clinical outcomes, regression models that considered the structure of dependence between individuals within the same institution were used. Parameters of these models were estimated through mixed effect models [23]. Crude and adjusted ORs with 95 % CI were estimated. For the concentration of CO₂, ORs were calculated for each increment of 200 ppm.

For phase I, two-level logistic random-intercept models were used to take into account DCC. For phase II, three-level logistic random-intercept models were used, as children were nested in rooms and rooms were nested in DCC. Confounders associated with environmental or health variables ($p < 0.15$) in the univariate analyses were included in the models. They included the following: age (years), gender, personal history of atopic dermatitis, parental history of allergic respiratory disease, parental education (primary or secondary vs high school or university), exposure to parental smoking and existence of older siblings. Other confounders were included for phase II, as birth and gestational weight, descriptives of the home surroundings and dampness and pets at home. Two sensitivity analyses were performed after stratification for children either younger or older than 3 years.

Behaviours and building characteristics associated with CO₂ concentration were identified in univariate and multivariate analyses with CO₂ as the dependent variable, adjusted for confounders. Room volume, number of children in the room, room temperature, outdoor wind velocity, type of window, type of gaskets, and opening of windows and doors were considered. Because CO₂ is a continuous variable, simple and multiple linear regression models were used. Logarithmic transformation of CO₂ values was performed to obtain residuals with a Gaussian distribution. Crude and adjusted regression coefficient estimates were calculated with 95 % CI.

The level of significance $\alpha = 0.05$ was considered, although p values > 0.05 and < 0.1 are reported. Data analysis was performed using STATA (StataCorp LP, Stata Statistical Software: Release 12; TX, USA).

Results

Characteristics of the children and day care centres

In phase I, from the 45 initially selected DCCs, ten refused to participate and were replaced following a new stratified random sampling. A total of 143 out of 310 classrooms were studied (82 in Lisbon and 61 in Oporto). The surveyed sample included 50.5 % of boys and 49.5 % of girls, with a mean age of 3.1 years (SD 1.5 years). The return rate of questionnaires was 62 % (3,186/5,161). For 44.5 % of the 3,186 respondents, at least one wheezing episode was reported, for 27.5 % in the previous 12 months. Asthma diagnosis was reported for 4.6 % and atopic dermatitis for 22.1 %. Parental history of allergic respiratory disease was reported for 33.2 % of the 3,186 respondents. Almost 17 % of the questionnaires reported exposure to tobacco smoke at home. The description of the sample is presented in Table 1.

Data concerning non-respondents were considered confidential for many institutions and were available only for the

Table 1 Socio-demographic characteristics of the sample of children ($n = 3,186$) and reported prevalence of respiratory and atopic symptoms (prevalence and 95 % CI) in phase I

Gender, %	
Boys/girls	50.5/49.5
Age in years, mean (SD)	3.1 (1.5)
Parental education, %	
Primary or secondary	56.1
High school or university	43.9
Exposure to parental smoking at home (95 % CI), %	16.7 (15.5–18.0)
Existence of older siblings (95 % CI), %	53.8 (52.1–55.5)
Wheezing—ever (95 % CI), %	44.5 (42.8–46.2)
Wheezing—in the last 12 months (95 % CI), %	27.5 (26.0–29.1)
Asthma diagnosis (95 % CI), %	4.6 (3.9–5.4)
Atopic dermatitis (95 % CI), %	21.2 (19.8–22.6)
Parental history of asthma or AR (95 % CI), %	33.2 (31.6–34.9)

AR allergic rhinitis, SD standard deviation, CI confidence interval

DCC included in phase II. In the 19 DCCs with available information on missing data, the non-respondent group had a higher proportion of males (55 vs 50 %, $p = 0.034$) and had more children under 3 years old (43 vs 38 %, $p = 0.015$); the DCC with non-respondents had a similar reported prevalence of wheezing in the previous 12 months ($p = 0.198$) than DCC with respondents. No geographic pattern was found for non-respondents.

In phase II, 1,196 children were considered in the final analysis, accounting for 37.5 % of the children that had participated in phase I. The return rate for the second questionnaire was 52.3 %. In this sample, the reported prevalence of wheezing in the previous 12 months and asthma were 29.2 and 4.7 %, respectively. No differences for gender were found between the samples of the two phases.

Half of the DCCs were built in the second half of the twentieth century, although some of the buildings were built in the seventeenth and eighteenth centuries (Table 2).

The median number of children per room was 15 (P_{25} – P_{75} 12–19 children), and the median values for temperature and relative humidity were 19.5 °C (P_{25} – P_{75} 18.1–20.6 °C) and 54.6 % (P_{25} – P_{75} 49.5–66.6 %). Aluminium windows were found in 53 % of the DCC and wood windows in 42 %. Natural ventilation was the most common ventilation type. In fact, only one school had mechanical ventilation. In phase I, the median value of indoor CO₂ was 1,440 ppm (P_{25} – P_{75} 1,085–1,970 ppm). Only 33 % of the DCCs had the windows opened during visits. In phase II, the median value of indoor CO₂ was 1,210 ppm (P_{25} – P_{75} 770–1,536 ppm). CO₂ determinations were moderately correlated between the two phases ($r = 0.49$).

Table 2 Building and environmental characteristics of the activity rooms of the 45 day care centres assessed in phase I

Age of the building (years), median [P_{25} – P_{75}]	50 (20–134)
Area (m ²), median [P_{25} – P_{75}]	36.0 (28.7–40.1)
Volume (m ³), median [P_{25} – P_{75}]	106.5 (77.3–128.0)
Children per room, median number [P_{25} – P_{75}]	15 (12–19)
Temperature—indoor (°C), median [P_{25} – P_{75}]	19.5 (18.1–20.6)
Relative humidity (%), median [P_{25} – P_{75}]	54.6 (49.5–66.6)
Wind velocity (km/h), median [P_{25} – P_{75}]	11.1 (7.4–22.2)
Type of window gaskets, %	
No gaskets	44
Rubber	38
Plush	18
Type of window moving leaf, %	
Casement	50
Sliding	25
Tilting	25
Windows position (open), %	33
CO ₂ (ppm) median [P_{25} – P_{75}]	1,440 (1,085–1,970)
Dampness/moulds %	78

Presented frequencies concern the 143 evaluated rooms. For dampness/mould, frequency concerns the 45 evaluated day care centre

P_{25} 25th percentile, P_{75} 75th percentile

Associations between respiratory health outcomes and characteristics of children and family

The associations between reported clinical outcomes and children's characteristics found in the univariate analysis are shown in Table 3. In phase I, reported wheezing in the last 12 months was inversely associated with female gender and age, while positive associations were found with both higher parental education and parental history of allergic respiratory disease. After adjustment for confounders, odds for wheezing were higher for boys, for younger children, and for those that had suffered from atopic dermatitis or had parental history of allergic respiratory disease (Table 3).

Reported asthma diagnosis presented negative associations with female gender, higher parental education and having older siblings, while positive associations were found with age, atopic dermatitis and parental history of asthma or allergic rhinitis. Except for gender, these associations persisted after adjustment for confounders (Table 3).

Associations of respiratory health outcomes with CO₂ concentration and comfort parameters

In phase I, reported wheezing in the previous 12 months was significantly related in the univariate analysis to the presence of dampness/moulds in the DCC and increasing average

indoor CO₂ concentration (OR 1.03, 95 % CI 1.00 to 1.06, $p=0.032$) (Table 3). The association between CO₂ and wheezing persisted after taking into account factors related to both CO₂ and wheezing (adjusted OR 1.04, 95 % CI 1.01 to 1.07, $p=0.008$) (Table 3). No associations were found between reported diagnosis of asthma and CO₂ and comfort parameters.

In phase II, CO₂ concentration presented a similar but non-significant OR with reported wheezing in the previous 12 months (OR 1.02, 95 % CI 0.96 to 1.08; $p=0.565$). In addition, reported diagnosis of asthma presented a non-significant positive association with CO₂ in the univariate analysis (OR 1.07, 95 % CI 0.98 to 1.16, $p=0.125$) and became significant in the multivariable analysis (adjusted OR 1.10, 95 % CI 1.00 to 1.20, $p=0.041$).

After stratification for age in phase I, the association between reported wheezing in the previous 12 months and CO₂ concentration remained relevant only in children younger than 3 years old (OR 1.02, 95 % CI 1.00 to 1.04, $p=0.075$ in children younger than 3 years old and OR 1.02, 95 % CI 0.99 to 1.05, $p=0.242$ in children older than 3 years).

Factors related to CO₂ concentration

The univariate associations between the characteristics of the building and indoor CO₂ concentration are presented in Table 4. In phase I, positive associations were found with the increasing number of children in each room; negative associations were observed with outdoor wind velocity, having an open window and opening an internal door. These associations remained significant in the multivariable analysis (Table 4).

Discussion

In this biphasic cross-sectional study of children attending DCC, reported wheezing in the previous 12 months was associated with CO₂ concentration in the attended DCC. Wheezing is a common symptom in early childhood, and it could result from a combination of different factors. DCC attendance has been related to increased risk of wheezing [8], although which DCC characteristics can influence children's health remains unclear.

This study aimed to provide insights about the relation between CO₂ concentration, other comfort parameters and respiratory diseases. Concentration of CO₂, temperature and relative humidity determinations were taken, as they are easy to measure and reliable indicators of indoor air contamination due to human presence (CO₂) and indoor comfort. The association between CO₂ concentration and respiratory illnesses in school aged children has been reported [20, 7]. In addition, the

Table 3 Relationships between children characteristics, environmental assessments and wheezing in the last 12 months and diagnosed asthma in phase I

	Crude odds ratio		Adjusted odds ratio	
	(95 % CI)		(95 % CI)	
	Wheezing in the last 12 months	Asthma diagnosis	Wheezing in the last 12 months ^a	Asthma diagnosis ^b
Gender (female)	0.85 (0.73 to 0.99) <i>p</i> =0.039	0.74 (0.55 to 1.00) <i>p</i> =0.054	0.86 (0.73 to 1.01) <i>p</i> =0.066	0.78 (0.57 to 1.06) <i>p</i> =0.112
Age (years)	0.76 (0.72 to 0.78) <i>p</i> <0.001	1.26 (1.12 to 1.42) <i>p</i> <0.001	0.74 (0.70 to 0.78) <i>p</i> <0.001	1.21 (1.08 to 1.37) <i>p</i> <0.001
Parental education	1.18 (1.03 to 1.35) <i>p</i> =0.016	0.68 (0.46 to 0.99) <i>p</i> =0.048	* <i>p</i> =0.048	0.59 (0.40 to 0.87) <i>p</i> =0.007
Parental smoking	0.96 (0.85 to 1.07) <i>p</i> =0.454	1.08 (0.90 to 1.30) <i>p</i> =0.425	* <i>p</i> =0.425	* <i>p</i> =0.425
Having older siblings	1.04 (0.95 to 1.13) <i>p</i> =0.439	0.61 (0.41 to 0.90) <i>p</i> =0.013	* <i>p</i> =0.013	0.61 (0.41 to 0.92) <i>p</i> =0.018
Atopic dermatitis	1.43 (1.21 to 1.69) <i>p</i> <0.001	2.35 (1.69 to 3.26) <i>p</i> <0.001	1.35 (1.14 to 1.60) <i>p</i> <0.001	2.07 (1.42 to 3.01) <i>p</i> <0.001
Parental history of asthma or AR	2.01 (1.71 to 2.37) <i>p</i> <0.001	1.80 (1.36 to 2.36) <i>p</i> <0.001	2.00 (1.67 to 2.39) <i>p</i> <0.001	1.79 (1.31 to 2.44) <i>p</i> <0.001
Number of children	1.01 (0.99 to 1.02) <i>p</i> =0.080	0.99 (0.99 to 1.00) <i>p</i> =0.650	* <i>p</i> =0.650	* <i>p</i> =0.650
CO ₂	1.03 (1.00 to 1.06) <i>p</i> =0.032	0.99 (0.99 to 1.00) <i>p</i> =0.112	1.04 (1.01 to 1.07) <i>p</i> =0.008	* <i>p</i> =0.008
Temperature—indoors	1.01 (0.96 to 1.06) <i>p</i> =0.713	0.95 (0.87 to 1.03) <i>p</i> =0.175	* <i>p</i> =0.175	* <i>p</i> =0.175
Relative humidity—indoors	0.99 (0.99 to 1.00) <i>p</i> =0.221	0.99 (0.97 to 1.02) <i>p</i> =0.588	* <i>p</i> =0.588	* <i>p</i> =0.588
Dampness/moulds in the day care centre	1.22 (1.02 to 1.46) <i>p</i> =0.031	0.81 (0.53 to 1.25) <i>p</i> =0.346	* <i>p</i> =0.346	* <i>p</i> =0.346
Age of the building	1.00 (0.99 to 1.00) <i>p</i> =0.232	0.99 (0.99 to 1.00) <i>p</i> =0.562	* <i>p</i> =0.562	* <i>p</i> =0.562

AR allergic rhinitis, CI confidence interval

*Corresponding factor not selected for the multivariable analysis (univariate *p* value >0.15) or with *p* value >0.15 in the final model; CO₂, odds ratio represent the mean change in outcomes for increments of 200 ppm

^a Gender, age, parental education, atopic dermatitis, parental history of asthma or AR, number of children in the day care centre, CO₂ concentration (200-ppm increments) and dampness/moulds in the day care centre were included in the final model

^b Gender, age, parental education, older siblings, atopic dermatitis and parental history of asthma or AR were included in the final model

association between respiratory health and IAQ in DCC was reported and related to the ventilation strategy [24, 25].

The major strengths of this study are the inclusion of a carefully selected sample of DCC, the detailed characterization of each DCC concerning the building characteristics and the statistical analysis approach that took in consideration the correlation structure of the observations for each DCC.

A major drawback of this study is the use of point in time determinations of CO₂ instead of continuous measurements, although these short-term measurements have been reported as ventilation surrogates in studies conducted at schools [19].

In order to overcome it, measurements were systematically obtained during periods of typical activities in the rooms after an extended period of steady occupancy. Another limitation is the sampling of rooms for environmental measurements instead of assessing all the classrooms in phase I. This was due to seasonal time constraints.

The purpose of phase I was to collect data for sampling DCC, based on a cluster analysis, for more extreme measurements in phase II. Recruiting DCC from the more different clusters was supposed to provide a higher power for the analysis with a smaller sample size, due to expected bigger differences in both environmental exposures and clinical

Table 4 Associations between characteristics of the day care centre and CO₂ concentration in phase I

	Crude regression coefficients (95 % CI) Log CO ₂ concentration	Adjusted regression coefficients ^a (95 % CI) Log CO ₂ concentration
Area (m ²)	−0.002 (−0.008 to 0.003) <i>p</i> =0.408	*
Volume (m ³)	0.001 (−0.002 to 0.004) <i>p</i> =0.549	*
Number of children	0.013 (0.003 to 0.022) <i>p</i> =0.012	0.018 (0.009 to 0.026) <i>p</i> <0.001
Temperature—indoor (°C)	−0.002 (−0.047 to 0.043) <i>p</i> =0.930	*
Wind velocity (km/h)	−0.014 (−0.024 to −0.004) <i>p</i> =0.005	−0.009 (−0.018 to −0.003) <i>p</i> =0.043
Type of window moving leaf ^b		
Sliding	−0.041 (−0.261 to 0.179) <i>p</i> =0.713	*
Tilting	−0.146 (−0.346 to 0.054) <i>p</i> =0.153	*
Open window practice	−0.306 (−0.438 to −0.173) <i>p</i> <0.001	0.362 (−0.485 to −0.240) <i>p</i> <0.001
Open an internal door	−0.146 (−0.286 to −0.006) <i>p</i> =0.041	−0.189 (−0.310 to −0.069) <i>p</i> =0.002

Results are expressed as regression coefficients with 95 % confidence interval (95 % CI)

Log logarithm

*Corresponding factor not selected for the multivariable analysis (univariate *p* value >0.15) or with *p* value >0.15 in the final model

^aNumber of children in the room, wind velocity, open window practice and open an internal door were included in the final model

^bReference: casement windows

outcomes, thus complying with time and budget constraints. In phase II, every classroom in each recruited DCC was assessed.

Reported wheezing in the previous 12 months was significantly related to CO₂ concentration, the number of children attending the DCC and the presence of dampness/moulds assessed in phase I but not to CO₂ concentration in phase II. Unexpectedly, this phase was unable to confirm one of the most significant associations highlighted by phase I. This was probably due to smaller ranges of CO₂ in phase II, consequence of better ventilation practices in this period (the transition from winter to spring) and to a much smaller sample of DCC and children.

The findings suggest that DCC environment may influence children's wheezing. CO₂ concentration remained highly associated with wheezing in the previous 12 months in the multivariable analysis. The significant odds for each increase of 200 ppm of CO₂ reflect the effect of even small variations. Although this effect might be smaller than other risk factors, it has a high potential for modification through simple ventilation strategies. In fact, in 90 % of the measurements in our

sample, the recommended limit of 1,000 ppm (1,800 mg/m³) [2] was exceeded as previously reported in schools [20], reinforcing the importance of the dissemination of better ventilation practices. Improving ventilation rates was recently associated with reduced student absences due to illness at elementary schools [13].

It is well known that only a part of wheezers will become asthmatic and that early transient wheezing constitutes another entity related to infections [12]. A significant association between CO₂ concentration and reported asthma diagnosis was found in phase II but not in phase I. Whether there is indeed a significant association between asthma and CO₂ concentration and other IAQ characteristics in DCC needs further longitudinal studies of children that attend DCC.

The presented results show that CO₂ concentration was related to the number of children in each room. In the lack of biomass combustion, the rate at which CO₂ is generated in a room is related to the number of occupants, their body mass and their level of physical activity. Other factors were also associated with the CO₂ concentration, namely the practices of opening windows and the outdoor wind velocity. Opening a

window is assumed to be a common ventilation practice in Mediterranean climates, such as Portugal, but windows were closed in most part of the classrooms during the visits. The sampled DCCs are located in urban areas, where opening a window might not be a good strategy when located nearby roads with heavy motor traffic, as air pollutants can readily penetrate indoors [16, 14]. In such cases, the pretreatment of incoming air could be needed, and the availability of tilting windows seems to facilitate the practice of opening windows.

The association between CO₂ and wheezing could result from insufficient air renewal and the number of children per room, which may both contribute to a more prone environment for respiratory virus infections. Respiratory infections were not taken into account in spite of the fact that they are important wheezing triggers and a very common problem in DCCs. This effect could be more relevant for the younger children, explaining the stronger association found among children less than 3 years old.

Higher levels of CO₂ could also reflect a higher concentration of indoor air pollutants due to poor ventilation rates. Previous studies in DCC found high concentrations of air pollutants [11, 21] and a relation between some air pollutants and ventilation [24]. Nevertheless, high CO₂ concentrations may occur without higher levels of other indoor air pollutants.

Despite the flaws on the assumptions for the study design preventing establishing a conclusive causal association between CO₂ and wheezing, these results suggest that poor ventilation in DCC may be related to wheezing in children. Even though natural actions could not be sufficient to provide ventilation and reduce CO₂ to acceptable levels [14], the increment of natural ventilation through effective open window practices, namely the use of tilting windows and adjusting the number of children in each classroom should be taken in consideration.

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