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The Association of Physical and Biological Factors of Outdoor Air with Exercise-Induced Bronchoconstriction in Children

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Dedicated to my family.

ABSTRACT

Background: Outdoor free-running exercise test is commonly used for diagnosing asthma in children, but the real-life influence of outdoor environmental factors on test outcomes remains unclear. While temperature and humidity are known to associate with exercise-induced bronchoconstriction (EIB) in adults, the generalization of the results to children is uncertain. Although exposure to pollens or air pollutants worsens asthma symptoms, it is not known how much they affect the outcome of free-running exercise test outdoors.

Aim: The aim of our study was to identify which outdoor air physical factors are associated with a reduction in lung function after exercise and whether the study results are different in younger (lung function measured with forced oscillation technique (FOT)) and older (lung function measured with spirometry) children. We also aimed to determine which factor of outdoor air is the most powerful in affecting the severity and incidence of EIB. We also aimed to examine whether there is an increased incidence of EIB in pollen season and whether elevated pollution concentrations are associated with the increased incidence or more severe EIB.

Methods: We analyzed data from 868 reliable exercise challenge tests with FOT measurements conducted in preschool children between January 2012 and April 2015, as well as 321 exercise tests with spirometry in children aged 6-16 years between January 2012 and December 2017 at Tampere University Hospital. Children were tested because of suspicion of asthma or a need to assess the efficacy of treatment in persistent asthma. We examined the relationship between post-exercise lung function measured with FOT or spirometry with outdoor air factors temperature, relative humidity, and absolute humidity through regression analysis. We also compared the occurrence of EIB defined as a decrease in forced expiratory volume in one second (FEV_1) of ≥ 15% in spirometry or as a ≥40% increase in respiratory resistance measured with FOT at 5 Hz (R_5) at different levels of temperature, relative humidity, and absolute humidity. For children who underwent FOT tests (n=799) between January 2012 and December 2014, we also had data on pollen concentrations of outdoor air. We examined the relationship between EIB occurrence and pollen concentrations. Furthermore, we analyzed pollutant concentrations ($PM_{2.5}$, NO_2, and

O_3) of outdoor air at the time of the exercise tests and examined their association with the occurrence and severity of EIB, adjusting analysis for air humidity and pollen counts. The data on air humidity, temperature, pollen, and pollution levels were obtained from public registers.

Results: In regression analysis, the relative change in R_5 after exercise was related to temperature (regression coefficient (β)=-0.22, p=0.020) and absolute humidity (β=-0.89, p=0.002), but not to relative humidity. There were also more EIB reactions if absolute air humidity was <5 g/m³ in comparison to ≥10 g/m³ (p=0.008). Only absolute humidity was independently associated with change in airway resistance (p=0.009) in multivariable regression analysis in younger children with FOT measurements. In older children with spirometry, absolute humidity ≥5g/m³, but not relative humidity or temperature, was associated with the occurrence of EIB (p=0.035). In multivariable logistic regression, absolute humidity ≥5g/m³ was negatively associated with EIB (OR 0.51, p=0.026). Absolute humidity (Pearson correlation coefficient (r)= -0.12, p=0.028) and temperature (r= -0.13, p= 0.023) correlated with decrease in FEV_1. In multivariable linear regression, only absolute humidity was associated with FEV_1 decrease (β= -0.04, p=0.033). Increase in R_5 after exercise or the frequency of EIB were not related to alder or birch pollen concentrations over 10 grains/m³ (p>0.1). Increase in R_5 after exercise did not correlate with O_3, NO_2 or $PM_{2.5}$ concentrations (p values 0.065-0.884). In multivariable logistic regression, we compared the effects of $PM_{2.5}$ ≥ 10 μg/m³, absolute humidity ≥ 10 g/m³ and alder or birch pollen concentration ≥ 10 grains/m³. In this analysis, absolute humidity ≥ 10 g/m³ was associated with decreased incidence (OR 0.31, p value 0.004), and $PM_{2.5}$ ≥10 μg/m³ was associated with increased incidence (OR 1.69, p value 0.036) of EIB.

Conclusions: We showed in a large real-life clinical sample that high absolute humidity is associated with lower incidence of EIB in children, and the results were similar if lung function was measured with FOT or spirometry. The commonly interpreted association between cold air and EIB seems to be entirely explained by the low absolute humidity of cold air and temperature is not independently explaining EIB. A negative test result at high absolute humidity should be interpreted with caution. In addition, outdoor air $PM_{2.5}$ levels may influence EIB in children, but pollen concentration was not associated with the probability of EIB.

TIIVISTELMÄ

Tausta: Ulkojuoksurasituskoetta käytetään yleisesti astman diagnosointiin lapsilla, mutta ulkoilman tekijöiden vaikutusta kokeen tuloksiin ei täysin tunneta. Vaikka tiedetään, että lämpötila ja kosteus ovat yhteydessä aikuisilla rasituksen laukaisemaan keuhkoputkien supistumiseen, tutkimusten yleistettävyys lapsiin on epävarmaa. Lisäksi ulkoilman siitepölylle altistumisen vaikutusta ulkojuoksurasituskokeen tuloksiin ei tunneta riittävästi. Pitkäaikainen altistuminen ilmansaasteille on yhdistetty astmaan lapsilla, mutta hetkellisten ilmansaastepitoisuuksien vaikutusta ulkojuoksurasituskokeeseen lapsilla ei ole selvitetty.

Tavoite: Tutkimuksen tarkoituksena oli selvittää, mitkä ulkoilman tekijät liittyvät keuhkoputkien supistumiseen rasituksen jälkeen ja ovatko tulokset erilaisia nuoremmilla (keuhkofunktio mitattuna oskillometrisellä menetelmällä) ja vanhemmilla (keuhkofunktio mitattuna spirometrialla) lapsilla. Lisäksi tarkoituksena oli selvittää, mikä ulkoilman ominaisuus vaikuttaa eniten rasituksen laukaiseman keuhkoputkien supistumisen ilmaantumiseen tai vakavuuteen. Tarkoituksena oli myös tutkia, esiintyykö rasituksen laukaisema keuhkoputkien supistuminen useammin siitepölykaudella ja kohonneiden ilmansaastepitoisuuksien aikana.

Menetelmät: Analysoimme kaikki luotettavat rasituskokeiden tulokset, joissa keuhkofunktio oli mitattu oskillometrisellä menetelmällä ja jotka oli tehty lapsilla tammikuun 2012 ja huhtikuun 2015 välisenä aikana, sekä spirometrillä tehdyt testit 6–16-vuotiaille lapsille tammikuun 2012 ja joulukuun 2017 välisenä aikana Tampereen yliopistollisessa sairaalassa. Lapsille oli tehty rasituskoe astmaepäilyn tai diagnosoidun astman seurannan vuoksi. Tutkimme rasituksen jälkeisen keuhkofunktion muutoksen yhteyttä ulkoilman tekijöiden (lämpötila, suhteellinen kosteus ja absoluuttinen kosteus) suhteen regressioanalyysin avulla. Vertailimme myös rasituksen aiheuttaman keuhkoputkien supistumisen (sekuntikapasiteetin (FEV_1) lasku ≥ 15 % spirometriassa tai ≥40 % nousu oskillometriamittauksessa hengitysteiden resistanssissa taajuudella 5 Hz (R_5)) ilmaantumista eri lämpötilan, suhteellisen kosteuden ja absoluuttisen kosteuden tasoilla. Lapsilla, jotka suorittivat oskillometriamittauksen tammikuun 2012 ja joulukuun 2014 välillä, meillä oli myös tiedot rasituksenaikaisista siitepölypitoisuuksista ja tutkimme niiden yhteyttä rasituksen laukaiseman

keuhkoputkien supistumisen ilmaantumiseen. Lisäksi keräsimme tiedot ilmansaasteiden ($PM_{2.5}$, NO_2 ja O_3) pitoisuuksista rasituskokeiden aikana ja arvioimme niiden yhteyttä rasituksen laukaiseman keuhkoputkien supistumisen ilmaantumiseen ja vakavuuteen. Ilman kosteus-, lämpötila-, siitepöly- ja ilmansaastepitoisuustiedot saatiin julkisista rekistereistä.

Tulokset: Tutkimukseen otettiin mukaan yhteensä 868 lasta, joilla oli luotettavat oskillometriamittaukset. Regressioanalyysissä R_5:n suhteellinen muutos rasituksen jälkeen oli negatiivisesti yhteydessä lämpötilaan (regressiokerroin (β) = -0,22, p = 0,020) ja absoluuttiseen kosteuteen (β = -0,89, p = 0,002), mutta ei suhteelliseen kosteuteen. Rasituksen laukaiseman keuhkoputkien supistumisen ilmaantuminen oli suurempaa pienessä (<5 g/m³) kuin suuressa (≥10 g/m³) ilman absoluuttisessa kosteudessa (p = 0,008). Ainoastaan absoluuttinen kosteus oli itsenäisesti yhteydessä R_5 muutokseen (p = 0,009) monimuuttujamallissa. Ulkojuoksurasituskokeen spirometrialla suoritti luotettavasti 321 tutkittavaa ja nämä tulokset otettiin mukaan analyyseihin. Absoluuttinen kosteus ≥5 g/m³, mutta ei suhteellinen kosteus tai lämpötila, oli yhteydessä rasituksen laukaiseman keuhkoputkien supistumisen ilmaantumiseen (p = 0,035). Logistisessa regressiossa monimuuttujamallissa absoluuttisen kosteuden ≥5 g/m³ ja rasituksen laukaiseman keuhkoputkien supistumisen välillä oli negatiivinen yhteys (OR 0,51, p = 0,026) myös spirometrian suorittaneilla lapsilla. Absoluuttinen kosteus (Pearsonin korrelaatiokerroin (r) = -0,12, p = 0,028) ja lämpötila (r = -0,13, p = 0,023) korreloivat negatiivisesti FEV_1:n laskun kanssa. Monimuuttujamallissa lineaarisessa regressiossa ainoastaan absoluuttinen kosteus oli yhteydessä FEV_1:n laskuun (β = -0,04, p = 0,033). Tutkittaessa siitepölypitoisuuksien yhteyttä rasituksen laukaisemaan keuhkoputkien supistumiseen, lepän tai koivun siitepölyn pitoisuuksien ollessa ≥10 kpl/m³, oskillometrisellä menetelmällä mitattuna ei ollut eroa rasituksen laukaisemaan keuhkoputkien supistumisen ilmaantumisella tai R_5:n nousulla (p>0.1). R_5 rasituksen jälkeen ei ollut yhteydessä O_3-, NO_2- tai $PM_{2.5}$-pitoisuuksiin (p = 0,065–0,884) tarkasteltaessa yksittäisiä muuttujia. Monimuuttujamallissa absoluuttinen kosteus ≥10 g/m³ oli yhteydessä rasituksen laukaiseman keuhkoputkien supistumisen vähentymiseen (OR 0,31, p-arvo 0,004) ja $PM_{2.5}$ ≥10 µg/m³ oli yhteydessä rasituksen laukaiseman keuhkoputkien supistumisen lisääntymiseen (OR 1,69, p-arvo 0,036), kun selittävinä muuttujina olivat $PM_{2.5}$ ≥ 10 µg/m³, absoluuttinen kosteus ≥ 10 g/m³ ja lepän tai koivun siitepölypitoisuus ≥10 kpl/m³.

Johtopäätökset: Tosielämän aineistossamme ulkoilman korkea absoluuttinen kosteus liittyi alhaisempaan rasituksen laukaiseman keuhkoputkien supistumisen ilmaantumiseen lapsilla sekä oskillometrisellä menetelmällä, että spirometrialla mitattuna. Yleisesti mielletty yhteys kylmän ilman ja rasituksen laukaiseman keuhkoputkien supistumisen välillä näyttää selittyvän kylmän ilman matalammalla absoluuttisella kosteudella, eikä lämpötila ole itsenäinen selittävä tekijä. Negatiivinen testitulos korkeassa absoluuttisessa kosteudessa tulisi tulkita varoen. Lisäksi $PM_{2.5}$-pitoisuudella saattaa olla vaikutus rasituksen laukaiseman keuhkoputkien supistumisen lisääntymiseen lapsilla jo suhteellisen matalilla pitoisuuksilla. Ulkoilman siitepölypitoisuuden ja rasituksen laukaiseman keuhkoputkien supistumisen välillä ei ollut yhteyttä.

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ABBREVIATIONS

AH = Absolute humidity

CI = 95% Confidence interval

r = Pearson correlation coefficient

r_s = Spearman correlation coefficient

EIB = Exercise-induced bronchoconstriction

FEV_1 = Forced expiratory volume in one second

FeNO= Fractional exhaled nitric oxide

FOT = Forced oscillation technique

FVC = Forced vital capacity

RH = Relative humidity

IOS = Impulse oscillometry

ISO-BMI= Child body mass index corresponding to adult values

IgE= Immunoglobulin E

IL= Interleukin

IQR = Interquartile range

NO_2= Nitrogen Dioxide

O_3= Ozone

$PM_{2.5}$= Particulate matter with a diameter of 2,5 μm or less

R_5 = Respiratory system resistance at 5 Hz

β = Regression coefficient

SD = Standard deviation

VC = Vital capacity

X_5 = Respiratory system reactance at 5 Hz

LIST OF ORIGINAL PUBLICATIONS

This dissertation is based on the following four original publications:

I Tikkakoski AP, Tikkakoski A, Kivistö JE, Huhtala H, Sipilä K, Karjalainen J, Kähönen M, Lehtimäki L. Association of air humidity with incidence of exercise-induced bronchoconstriction in children. *Pediatric Pulmonology*. 2019;54(11):1830–1836

II Tikkakoski AP, Karjalainen J, Sipilä K, Kivistö JE, Kähönen M, Lehtimäki L, Tikkakoski A. Outdoor pollen concentration is not associated with exercise-induced bronchoconstriction in children. *Pediatric Pulmonology*. 2022;57(3):695–701

III Tikkakoski AP, Tikkakoski A, Sipilä K, Kivistö JE, Huhtala H, Kähönen M, Karjalainen J, Lehtimäki L. Exercise-induced bronchoconstriction is associated with air humidity and particulate matter concentration in preschool children. *Pediatric Pulmonology*. 2023 *Apr*;58(4):996-1003.

IV Tikkakoski AP, Reini M, Sipilä K, Kivistö JE, Karjalainen J, Kähönen M, Tikkakoski A, Lehtimäki L. Association of temperature and absolute humidity with incidence of exercise-induced bronchoconstriction in children. Submitted.

AUTHOR´S CONTRIBUTION

The author participated in the planning and conceptualization of the study methodology and design with supervisor and the other members of the research group. The author was responsible for drafting the original manuscripts and finalizing the articles. Data collection, funding acquisition, data processing, data analysis, and reviewing and editing of the articles were conducted by the author with the supervisor and the other authors.

1 INTRODUCTION

Asthma is a heterogenous respiratory disease characterized by airway inflammation and bronchial hyperreactivity, causing symptoms such as cough, mucus secretion, shortness of breath and wheezing. Burden of asthma is increasing globally, especially among children (1). Inflammatory mechanisms in asthma are different, of which the most common among children is allergic eosinophilic asthma, often triggered by airborne allergens (2). Genetic factors are important in the pathogenesis of asthma, but some environmental factors are recognized also (3,4). It is estimated that 13% of asthma incidence in children globally and 33% in Europe may be due to traffic related air pollution or other outdoor pollution (5,6).

One of the common features in asthma is exercise-induced bronchoconstriction (EIB), occurring approximately in half of asthmatic children (7). EIB is triggered by physical activity and characterized by narrowing of the airways during or after exercise, but the mechanism how exercise triggers airway obstruction is not fully understood. It is hypothesized that elevated ventilation in exercise leads to drying of the fluid layer above bronchial epithelium leading to cytokine secretion and smooth muscle contraction (8), but in addition to drying, cooling of the airways is also thought to trigger EIB (9).

Exercise testing with free-running outdoors is a valuable and frequently used tool in diagnosing asthma in children especially before school age (10) although the test environment outdoors can´t be standardized due to changing weather, pollen seasons and variable pollutant levels. The result of the exercise test significantly affects the treatment and follow-up of the patient. However, it is not well known how much the properties of the outdoor air affect the exercise test results in children and how this should be taken into account in clinical practice. The precise roles of temperature, absolute humidity and relative humidity of outdoor air in triggering EIB are not known (11–13). Also outdoor air pollen and pollutant concentrations are connected to airway inflammation but their association with EIB detected in outdoor exercise test is not known (14–17). The aim of this study was to assess the association of EIB with the ambient outdoor air properties in preschool children and school children. Our hypotheses were that the temperature and humidity are associated with the severity and incidence of EIB, pollen concentrations are associated with EIB in atopic subjects and outdoor pollutant concentrations are associated with EIB.

2 REVIEW OF THE LITERATURE

2.1 Exercise-induced bronchoconstriction (EIB) in asthma

Childhood asthma is a heterogenous respiratory disease characterized by airway inflammation and bronchial hyperreactivity, leading to symptoms like wheezing, coughing, and shortness of breath. Its prevalence is increasing globally especially in industrialized countries and in developing countries in parallel to industrialization and urbanization. (1) These environmental changes have led to better hygiene and depleted human microbiome, which, according to so-called hygiene hypothesis and epithelial barrier hypothesis, lead to dysregulation of immune responses and impaired epithelial barrier function triggering auto-immune diseases and allergies and thereby also increasing the prevalence of asthma and atopy (18–20).

Numerous factors -such as allergens, respiratory infections, physical exercise, and environmental factors can exacerbate asthma. Airway hyperreactivity refers to an increased inclination to develop bronchoconstriction in response to those irritants and it can be tested with direct and indirect bronchoprovocation tests. Direct tests (metacholine and histamine challenge) provocate bronchoconstriction directly by stimulating contraction of the bronchial smooth muscle. Indirect tests (e.g., eucapnic voluntary hyperventilation test and mannitol or exercise challenge) affect the bronchoconstriction indirectly by promoting hyperosmolality in the bronchial epithelium, which stimulates bronchial smooth muscle contraction via inflammatory mediators. Direct tests are more sensitive, but indirect tests are more specific to detect asthma (10). Nevertheless, forced expiratory flow in one second (FEV_1) decrease over 15 % in-exercise test does not usually occur in other pulmonary diseases than asthma, thus the specificity is high (21).

One form of hyperreactivity is EIB, when physical exercise triggers airway obstruction (10). During exercise, ventilation of large amounts of air can induce EIB in an estimated 40-60 % of individuals with atopy or asthma (11,22) and approximately half of asthmatic children experience EIB (7). In asthma, there is evidence suggesting that EIB results from inflammation. Asthmatics with EIB have increased levels of cysteinyl leukotrienes and eosinophils in their airways compared to asthmatics without

EIB (23). The level of fractional exhaled nitric oxide (FeNO) predicts the occurrence of EIB (24). Intraepithelial mast cell density is showed to be higher in individuals with asthma who experience EIB (25).

2.2 Diagnosis of EIB

Exercise challenge is the most widely used indirect bronchoprovocation test to detect EIB. The recommended method to perform exercise challenge is running in the treadmill to obtain sufficient ventilation rate, but it is not usually suitable for small children. Free-running outdoors is the most commonly used method to perform exercise-challenge in them (26). Bicycle-ergometer with stepwise increasing exercise level is not recommended for testing bronchial hyperreactivity because of low sensitivity (27).

Exercise is started without warm-up and exercise should be near maximal after 1-3 minutes. Exercise is continued for 4-6 minutes, and total exercise time should be 6-8 minutes (28). The intensity of bronchoconstriction is related to volume of ventilation reached during the exercise and ventilation should be at least 60% of maximal voluntary ventilation. Direct measurement of ventilation is not possible during free-running, and maximal heart rate is measured instead. Exercise is considered sufficient when heart rate is over 85% of the calculated maximal value in the last 4-6 minutes (10,29). Lung function is measured before exercise and 1-2, 5, 10, 15 and 30 minutes after exercise. EIB typically occurs 4-10 minutes after exercise, but it occurs faster in younger children (30,31). After measurements, salbutamol is given to subjects (adults and children over 10 years 400 µg and in Finland children under 10 years 300 µg). Spirometry is measured again after 10-15 minutes after salbutamol inhalation. In children, the lung function measurement used is chosen based on the subjects´ co-operation. Usually, in children 3-6 years old, forced oscillation technique (FOT) is used while spirometry is used in children over 6 years old.

2.2.1 Forced oscillation technique (FOT)

FOT is a technique to measure lung function noninvasively and rapidly during tidal breathing requiring only passive cooperation of the patient (32). It is a standardised method to diagnose asthma in children of age 3-7 years and it is widely used in this age-group (31,33–35). The method measures the mechanical properties of the respiratory system passively and forced expiration is not needed. During measurements, the oscillating pressure (frequencies 5-40 Hz mainly used in clinical practice) is directed into the respiratory system through mouth and its effects on respiratory flow are measured during the normal tidal breathing. As a result, impedance of respiratory system (Z_{rs}) is determined, which represents all the forces resisting proceeding of the pressure wave and thus the mechanical properties of the respiratory system (36–38). Z_{rs} consists of two components: respiratory system resistance (R_{rs}), which describes respiratory system resistance to flow, and reactance (X_{rs}), which reflects out of phase relationship between pressure and flow of the oscillating respiratory system. X_{rs} consists of inertance and capacitance, the latter dominating at low frequencies and reflecting elastic forces opposing flow (and volume) changes. Z_{rs} is not used in clinical practice, but its component R_{rs} is sensitive for airway narrowing and increases in clinical conditions such as bronchoconstriction and mucous plugging. X_{rs} decreases when respiratory system becomes stiffer, lung volume decreases and also in peripheral bronchoconstriction in the small airways (37,38). In bronchoconstriction, frequency dependence in both R_{rs} and X_{rs} increases predominantly due to the heterogeneous narrowing in the airways (38). Impulse oscillometry (IOS) is one of the commercial applications of FOT, in which pressure waves are short impulses consisting of frequencies 5-35 Hz (37).

In IOS, most commonly R_{rs} measurements are reported at 5 Hz (R_5) and 20 Hz (R_{20}). Previous research indicates that R_5 is the most reliable and reproducible parameter for detecting airway obstruction (31,36,39). It is important to observe the frequency dependence of resistance and reactance when assessing impulse oscillometry results (36). Peripheral bronchial obstruction presents as increase in R_5 and a smaller increase in R_{20}, but does not solely reflect small airway disease (32,37,38,40,41). Negative change of X_{rs} also correlates with heterogenous obstruction in the airways. Compared to R_5, respiratory reactance at 5 Hz (X_5) is not as reproducible in children and is more sensitive to lung volume changes (32,36,42).

An increase in R_5 by over 40 % after exercise is interpreted as a positive finding regarding EIB, and if the increase after exercise in R_5 is 35-39 % the finding is indicative for EIB (36,39,42,43). The criterion for significant bronchodilatation response is over 40 % decrease in R_5 compared to baseline (36,39,43)

2.2.2 Spirometry

Spirometry is the most used lung function test in older children (usually child´s co-operation is sufficient after six years of age) and adults. It is based on measuring the flow and volume during maximal exhalation and inhalation. The most important parameters measured during forced exhalation are forced vital capacity (FVC, the total volume of the forced exhalation) and FEV_1, the volume exhaled during the first second. Vital capacity (VC) is measured during a slower not forced exhalation and inhalation. FEV_1 is the most crucial parameter measured in an exercise and bronchodilation test. (44)

A decline in FEV_1 of at least 10% after exercise compared to baseline is considered abnormal. This threshold is based on the variability in healthy population (45,46). In the international recommendations, at least 10% decline is considered a threshold for EIB. However, for the diagnosis of asthma, a more specific threshold of a decline of at least 15% is recommended. (10) The criterion for a significant bronchodilatation effect is considered an increase in $FEV_1 \geq 12\%$ and 200 ml compared to baseline (47).

2.3 Theoretical background of EIB – thermal and osmotic hypotheses

The precise mechanism of EIB is not completely understood. However, there is strong evidence that inflammatory mediators, such as leukotrienes and prostaglandin D2, are released in airways during high ventilation in exercise, and that the mediators originate from mast cells and eosinophils in the respiratory tract (23,48). Airway remodeling is also believed to increase bronchial hyperreactivity in response to exercise and environmental exposures. In this process, the increase in extracellular matrix in bronchial submucosa due to fibroblast activation and hypertrophy of bronchial smooth muscle cells, both induced by bronchial epithelial cells and inflammatory cells, play crucial roles (49,50). Epithelial cells are an important barrier between environment and lungs and factors influencing them impact on bronchial responses.

During periods of increased ventilation in exercise, a large amount of air quickly adjusts to the humid conditions of the lower airways. This causes both water and heat to transfer to the incoming air, leading to osmotic stress and a cooling effect on the airway mucosa due to the water vaporization (8). Cold air is believed to increase the sensitivity of the exercise test, but the capacity of cold air to hold water is lower and the isolated impact of low temperature in comparison to low humidity of the inspired air remains a subject of debate. Based on previous studies, cold conditions aren't essential for bronchoconstriction and EIB can still arise when the inhaled air is warmer than body temperature (51–53). There is also evidence that facial cooling with either warm or cool air inhalation can induce bronchoconstriction through vagal mechanism also in non-asthmatic subjects (54,55). There are two main theories about the primary stimulus in triggering EIB: The osmotic hypothesis suggests it is water loss, while the thermal hypothesis suggests it is airway cooling (Figure 1).

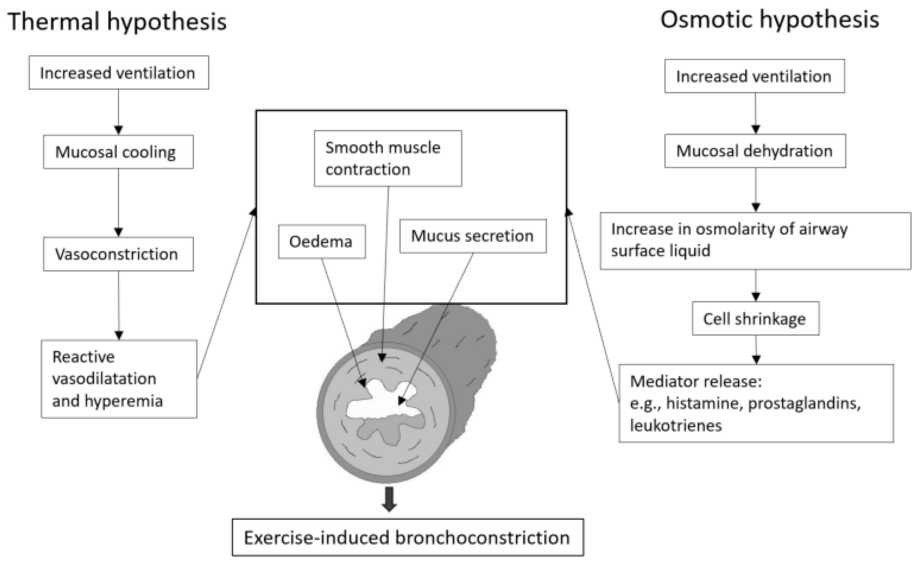

Figure 1. Thermal and osmotic hypothesis in the pathophysiology of exercise-induced bronchoconstriction (EIB). Modified from (56).

According to the thermal hypothesis, the vaporization of water during increased ventilation in exercise cools the surface of the respiratory tract, which is then followed by a rapid rewarming due to vasodilatation when the exercise ends. This results in hyperemia in the bronchial vasculature and swelling of the bronchial wall, and the following bronchoconstriction is a result of these vascular events (9,51). However, there is evidence suggesting that only cooling is not a sufficient trigger for an EIB, and the humidity of the inspired air plays an important role (51,52).

It has been suggested that instead of the heat loss in the airways, the loss of water might be the crucial factor in triggering EIB. This theory is called the osmotic hypothesis. According to it, the elevated ventilation during exercise leads to the vaporization of water from the surface of the airways, causing the liquid layer on the respiratory epithelium to become hypertonic. This results to water transferring from the epithelial cells to the extracellular space, which causes cell shrinkage, which then triggers the release of inflammatory mediators such as histamine and leukotrienes from mast cells, basophils and eosinophils (23,48). This leads to bronchoconstriction through smooth muscle contraction and mucus secretion (8).

2.4 Association of outdoor air temperature and humidity with EIB

Both humidity and temperature of inhaled air are believed to influence the airway's drying and cooling, thereby influencing the occurrence of EIB. However, the precise significance of humidity versus temperature in real-life setting remains unclear. International guidelines suggest that exercise tests should be conducted with inhaled air absolute humidity less than 10 g/m^3, a temperature under 25°C, and a relative humidity of less than 50% (10,57).

In controlled laboratory settings, there is evidence that EIB correlates with either temperature or humidity (52,58–61). Studies in real-life conditions involving adults indicate that humidity and temperature correlate with prevalence of EIB (11,12). However, the pathophysiology and phenotypes in pediatric asthma differ from adults, meaning that findings from adult studies may not directly apply to children (62). Even though outdoor exercise testing is used in pediatric asthma diagnostics, the evidence in real-life setting is sparse in this age group and the conclusions of available research are not completely consistent, primarily because not all studies measured absolute humidity, relative humidity, and temperature simultaneously.

2.4.1 Relation of absolute humidity, relative humidity, and temperature

Absolute humidity, relative humidity, and temperature are related to each other (Figure 2). Absolute humidity measures the water vapor mass per unit volume of air, whereas relative humidity is the proportion of the current absolute humidity to the maximum absolute humidity at that temperature. The warmer the air, the greater its capacity to contain water vapor. If only one of these outdoor air variables is assessed and shows association with EIB, it is difficult to distinguish whether there is a direct cause-and-effect relationship between that specific variable and EIB or if the association is caused by this variable correlating with another that is actually causing EIB – e.g., low absolute humidity rather than low temperature.

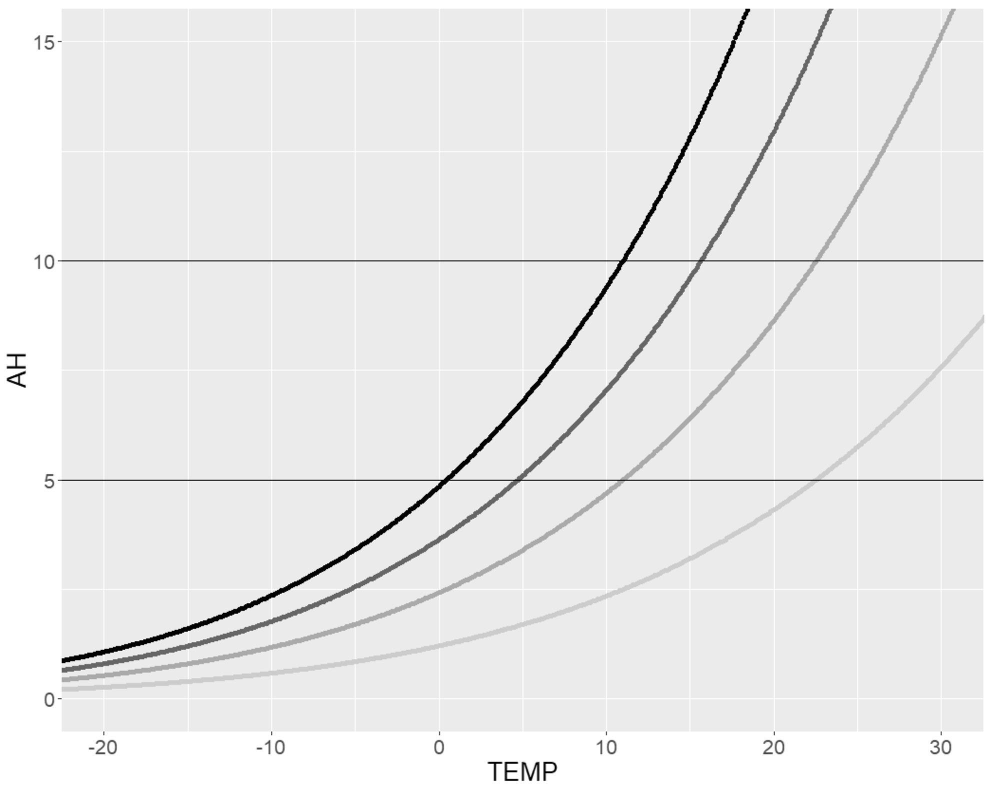

Figure 2. Relation of absolute humidity (AH), relative humidity, and temperature (TEMP).

Note: This figure demonstrates how temperature, relative humidity and absolute humidity are interconnected. Absolute humidity can be hard to assess without calculator or look-up tables due to its exponential relationship with temperature and relative humidity. Horizonal lines are absolute humidity levels 5 and 10 μg/m^3. The faintest gray ascending line represents 25% relative humidity. The other lines indicate relative humidity levels of 50%, 75%, and 100%. AH = absolute humidity (g/m^3), TEMP = temperature (°C).

2.4.2 Association of temperature with EIB

Previous studies have investigated the association of varying temperature levels with EIB in laboratory settings. In one experiment, FOT and spirometry results were compared after exercise tests conducted in cold (-1°C) and room temperature (22°C) (63). During both challenges, subjects inhaled dry, medical bottled air with absolute humidity of less than 3 g/m^3. Although spirometry did not detect any difference in the peak reduction of FEV_1 between the two temperatures, there was a notable increase in peak respiratory system resistance at room temperature only. Another study compared regular conditions at 20°C with cold environment at -18.0°C with both conditions having relative humidity of 39-40% among subjects experiencing EIB (59). The difference in EIB between the conditions was statistically significant, FEV_1 post-exercise reduction being 31% in the cold environment and 24% under regular conditions. These studies demonstrated that temperature alone does not influence EIB when absolute humidity is already low. However, when only relative humidity remains constant between cold and warm climates, the absolute humidity is also low in cold climates, leading to a more pronounced decline in lung function in cold conditions.

Studies examining outdoor free-running test in ambient, uncontrolled environment have found correlations between lung function reduction and temperature. In a Finnish studies (31,64), researchers found that cooler outdoor temperatures correlated with lung function reduction measured with FOT or spirometry in children. Two Korean studies in young adults (11,12) discovered that the outdoor air temperature was lower in tests were subjects experienced EIB. Additionally, temperature correlated with the maximal decrease in FEV_1. Since absolute humidity has not been reported, it cannot be determined whether EIB is actually related to temperature or to lower absolute humidity in colder air.

2.4.3 Association of humidity with EIB

Several studies have been conducted in laboratory environments comparing different humidity levels on EIB incidence and lung function reduction. In 1977, in one study, where the temperature was kept consistent, a comparison between dry and humid conditions revealed a mean FEV_1 reduction of 36.8% in dry settings, compared to a 10% reduction in the humid environment (58). In a study published in 1984 (52), ten subjects with asthma were studied under four different experimental conditions: two were conducted at a temperature of 9-10°C with absolute humidity of 9-10 g/m^3, one at 35°C with absolute humidity of 9-10 g/m^3, and one at 35°C with absolute humidity of 29 g/m^3. The main finding was that there was no difference in the probability of EIB when the humidity was 9-10 g/m^3, despite of changes in temperature, but the probability of EIBs was significantly lower when the absolute humidity was 29 g/m^3. In a study that involved eight asthmatic men and eight healthy controls, the researchers explored the effects of five separate temperature and relative humidity settings in a climate-controlled chamber. The findings revealed that asthmatic participants experienced an average FEV_1 drop of 20-21% under the conditions, where absolute humidity was low (under 7 g/m^3) and the decrease was less than 10% in conditions where absolute humidity was higher (above 7 g/m^3)(61). Another study showed that the reduction in FEV_1 was in humid conditions only half of the FEV_1 reduction detected in dry conditions (12% vs 24%) (60). Taken together, in controlled conditions absolute humidity rather than temperature or relative humidity seems to be the key factor associated with EIB.

In studies conducted outdoors under uncontrolled conditions, there have been conflicting results regarding the effects of humidity when using relative humidity as the variable under investigation. Two studies in Korea found relative humidity to be lower in tests where the subjects experienced EIB, and relative humidity correlated with maximal percent fall in FEV_1(11,12). Conversely, a study conducted in tropical environment in Malaysia in schoolchildren, discovered no link between EIB and ambient air relative humidity, which fluctuated between 41 and 90% (13). The study didn't report or consider absolute humidity levels in their analysis. Based on these studies, we do not know if absolute humidity is the key factor determining risk for EIB also in outdoor exercise as it seems to be in controlled laboratory conditions.

2.5 Outdoor pollens, asthma and EIB

Atopy is known as a strong risk factor for asthma and immunoglobulin E (IgE)-mediated sensitization for outdoor air allergens is common (1). The concentration of allergens in outdoor air varies largely depending on climate and season. In Europe, the primary pollination period spans over approximately 6 months, from spring to autumn (65). Concentration of pollen from birch and its relatives, such as alder, is high in spring time in Northern and Central Europe, leading to the majority of pollen-induced symptoms (66). Highest grass pollen concentrations are usually reached in the middle of summer (65). Climate warming due to climate change contributes to earlier onset of the pollen season and also extends it, which may increase the significance of pollens in asthma morbidity in the future (67,68).

Allergens, for example from birch, entry the lung tissue via bronchial epithelium leading to the activation of dendritic cells (69). Activated dendritic cells promote the differentiation of Th2 cells, which in turn secrete cytokines such as interleukin (IL)-4, IL-5, and IL-13 (2). IL-4 promotes allergen activated B cells to secrete IgE, IL-5 recruits eosinophils and IL-13 enhances IgE production and stimulates mucus secretion in epithelium. Allergens also bind to allergen specific IgE on the surface of mast cells. Activated mast cells then release mediators, of which the most important mediators causing bronchial symptoms are histamine, prostaglandin D2 and leukotrienes. Immediate hypersensitivity reaction is due to these events. Histamine, leukotrienes, and prostaglandin D2 enhance vascular permeability and mucus secretion and they also trigger bronchoconstriction (70). Mast cells and Th2 cells secrete IL-5 which recruits eosinophils to the site of allergen exposure. Eosinophils are the most important cells in the late-phase hypersensitivity reaction, and eosinophils play a significant role in promoting type-2 inflammation in asthma. (71) Allergens can also induce long-term changes through epithelial damage via apoptosis, promoting the proliferation and activation of fibroblasts, smooth muscle cells and goblet cells, leading to airway remodeling (70,72).

2.5.1 Association of pollen concentrations with asthma morbidity

In asthma, various inflammatory mechanisms are recognized, of which eosinophilic allergic asthma is the most common in children and airborne allergens often exacerbate it (2). Many previous studies have shown that outdoor air pollen concentrations are related to the severity of nasal, conjunctival and respiratory symptoms in pollen sensitized individuals (73–76). There is also evidence that increased airway inflammation, measured by FeNO or the number of inflammatory cells, is associated with pollen exposure (15,77,78). Several studies have detected an increase in emergency room visits and asthma morbidity when pollen concentrations are high (79–83). Allergen exposure and airway inflammation correlate also with poorer quality of life (84).

2.5.2 Association of pollen concentrations with EIB and lung function

Although there is evidence that pollen exposure increases airway inflammation, allergy-related symptoms and asthma morbidity, there is no conclusive evidence of its effect on lung function or the probability of EIB. In one Finnish study, it was observed that allergic subjects with asthma experienced a greater decline in FEV_1 after exercise test in spring pollen season than in winter season (85). In a Swedish study, researchers found that grass pollen concentrations correlated with decline in lung function measured by spirometry at rest in pollen sensitized individuals, but the same was not observed with birch pollen concentrations (86). In a recent meta-analysis, it was found that pollen concentrations correlated with allergic symptoms, but there was no evidence of a decrease in lung function in short-term exposure to pollen (14). Based on previous studies, there is no conclusive evidence whether elevated pollen levels during exercise test affect the occurrence or intensity of EIB in allergic and non-allergic children.

2.6 Air pollution´s impact on EIB

Several pollutants are recognized to be associated with childhood asthma morbidity. Particulate matter (PM) arises from vehicular emissions, industrial processes, combustion of solid fuels and natural sources, such as pollen and dust. Particles with a diameter of 2,5 μm or less ($PM_{2.5}$) are remarkably harmful as they can penetrate deep into the alveolar structures in the lungs, leading to inflammation. Ozone (O_3) is formed through a photochemical reaction when nitrogen oxides and volatile organic compounds interact under ultraviolet radiation. Ground-level O_3 is a potent respiratory irritant. Nitrogen dioxide (NO_2) is emitted mainly from traffic and industrial combustion processes (4). It has been linked to reduced lung growth in children, inflammation and airway hyperreactivity (87).

High concentrations of O_3, $PM_{2.5}$, and NO_2 can induce inflammation in the airways measured by the number of inflammatory cells or FeNO (88–93), and gaseous pollutants (O_3 and NO_2) can lead to airway hyperreactivity (88,94). Additionally, pollutants are associated with oxidative stress (95,96). Therefore, exposure to these pollutants has been linked to asthma exacerbations and the onset of asthma, but the mechanisms are not completely understood. High concentrations of air pollutants may have direct inflammatory and irritant effects on the respiratory epithelium through epithelial barrier dysfunction (97,98). However, such concentrations are not typically found in Europe and North America, so lower concentrations likely have different effects.

It is thought that oxidative stress, airway remodeling, inflammatory and immunological pathways and increase in sensitization to aeroallergens may be in the background of onset of asthma and exacerbations of pre-existing asthma (99). Modifications in genes that regulate these mechanisms might predispose individuals to asthmatic responses to pollutants. Such genes include those that code for antioxidant enzymes, such as the glutathione S-transferase. Those genes can also influence the inflammatory responses (100). Increased production of cytokines and chemokines, and also the expression of adhesion molecules, are factors that increase inflammation in pollution exposure (101,102). It is suggested that regulatory T cells act as suppressors in immune responses linked to asthma pathogenesis, and increased exposure to air pollution correlates with reduced regulatory T cell activity and a rise in asthma morbidity. Air pollution may mediate epigenetic alterations in regulatory T cells, potentially exacerbating asthma through an immunological pathway (103). Exposure to particulate matter can increase lymphocyte polarization into Th17 phenotype (104).

Moreover, exposure to house dust mite antigen and diesel exhaust particles induced a combined Th2/Th17 response in mice (105).

2.6.1 The role of air pollution in childhood asthma

Physiological and behavioral features of children make them more vulnerable to harmful effects of pollutants. The structures in children´s lungs are still developing, making them more susceptible to structural and functional changes in pollutant exposure. Compared to adults, children have higher minute ventilation per unit of body weight. That results to greater inhalation of pollutants for their size, enhancing potential lung and systemic effects. Furthermore, due to higher levels of physical activity, children have episodic increases in their ventilation rates, leading to increased amounts of inhaled pollutants. Air pollutants can adversely affect the developing immune system in children, and early exposure to pollutants indicates a longer cumulative lifetime exposure, increasing risk to develop chronic respiratory diseases later in life (106).

Global estimates suggest that traffic-related air pollution or other outdoor pollutants may account for 13% of asthma cases in children worldwide and 33% in Europe (1,5,6). In a study of ten European cities, traffic related air pollution was found to be associated with 14% of new cases of asthma and 15% of exacerbations of asthma in children (107). Prolonged exposure to pollutants, either during prenatal period or in childhood, correlates with decreased lung function as measured by spirometry or FOT (108–110). It can potentially lead to permanent impacts on pulmonary function, and it is presented that early-life exposure to traffic related air pollution is linked to lung function decrease during adolescence (111,111) Studies also indicate that air pollution exposure during pregnancy might decrease the child's lung growth, and exposure in childhood could influence the onset of asthma in later years (16,87,112,113). Some pollutants might facilitate the onset of asthma by inducing airway remodeling and inflammation, as evidenced by their association with increased basal membrane thickness and eosinophilic inflammation in the airways (114).

2.6.2 Short-term air pollution exposure, lung function and EIB

In pediatric asthmatic populations, acute exposure to air pollutants may induce oxidative stress pathways in airways, subsequently correlating with decreased pulmonary function and augmented asthma exacerbations (17). A study conducted in

Finland showed that, amongst school-aged children presenting with chronic respiratory manifestations, pollutant exposures did not significantly correlate to post-exercise lung function reduction measured by spirometry. However, a significant correlation between elevated air pollution levels and decreased spirometry measures at baseline was detected (115). Conversely, another recent investigation conducted in New York City found that an augmented exposure to NO_2 was connected to greater lung function reduction post-exercise in children, and it was thought that airway inflammation may mediate the association between NO_2 and EIB (116). Previous studies do not provide conclusive evidence on whether increased pollutant concentrations in ambient outdoor air during exercise tests influence the incidence or severity of EIB in children.

3 AIMS OF THE STUDY

The overall aim of the study was to assess how different physical and biologic factors of outdoor air affect the probability and severity of EIB among children with suspected or diagnosed asthma being tested with a free-running exercise test outdoors, and if these effects are strong enough to be considered in clinical decisions. The specific aims were:

1. To identify which physical factors – temperature, relative humidity, and absolute humidity – of ambient air are associated with reduction in lung function after exercise in outdoor exercise test. We also aimed to determine which of the factors is the most important in relation to the severity or incidence of EIB and whether there is a difference between younger (lung function measured with FOT) and older (lung function measured with spirometry) children in this regard (original publications I and IV).

2. To examine whether there is an increased incidence of EIB if pollen concentrations are elevated and if this differs between atopic and non-atopic individuals (original publication II).

3. To evaluate whether elevated pollution concentrations are associated with the incidence or severity of EIB (original publication III).

4 MATERIALS AND METHODS

4.1 Study design and subjects

The study was a retrospective chart review. We collected data on all the free-running outdoor exercise tests with FOT, which in our department was measured with device using IOS technique (IOS group, Figure 3) conducted in children under 16 years between January 2012 and April 2015 and free-running exercise tests with spirometry (Spiro group, Figure 3) between January 2012 and December 2017 at Tampere University Hospital. All the children were referred to the exercise test due to suspicion of asthma or a need to assess the efficacy of treatment in persistent asthma. The exercise test and lung function tests were performed as part of normal clinical routine. The lung function measurement method was selected based on children´s age and co-operation. In younger subjects, who were not able to perform forced blows needed in spirometry, lung function was measured with IOS. If several exercise tests had been conducted to a single child, we included only the first one.

We collected data on asthma medications, asthma diagnosed by doctor before or after exercise tests and possible risk factors affecting asthma risk from patient records (117). Those included IgE-mediated sensitization (positive findings in skin prick test or allergen specific IgE levels for animals, house dust mite, food, alder, birch, mugwort, or grass), doctor diagnosis of atopic dermatitis, tobacco exposure during prenatal period or childhood, atopy and asthma in family, domestic animals, respiratory and other symptoms, number of episodes and hospitalizations due to wheezing, other diseases, and medications. None of the subjects were excluded due to the other diseases or medications. We calculated the height adjusted for age as z scores and child body mass index corresponding to adult values (ISO-BMI) (118).

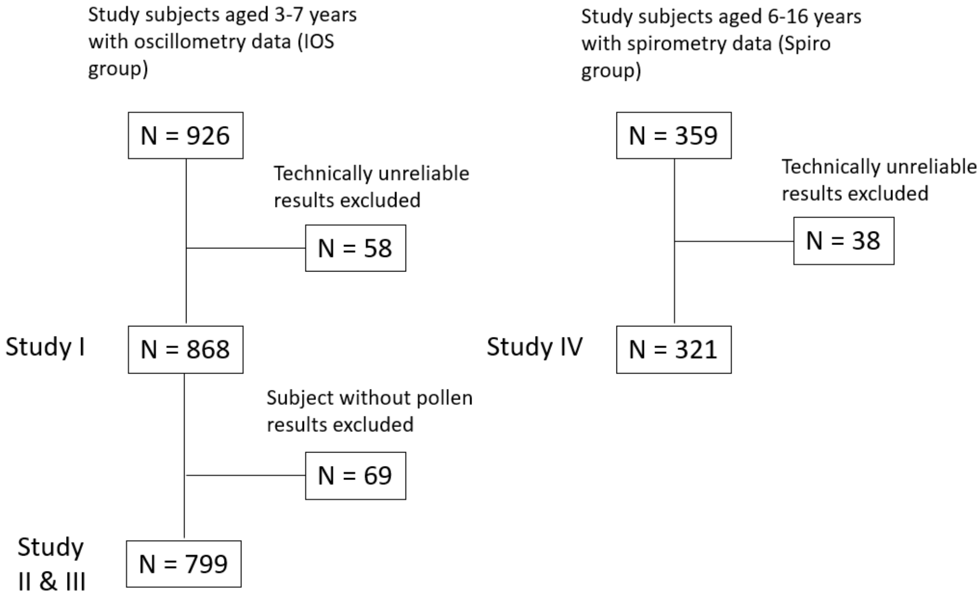

Figure 3. Study subjects divided into groups based on the lung function measurement method used. Subjects included in the-publications are marked on the left side of the graphs.

Note: IOS=impulse oscillometry.

4.2 Air properties

4.2.1 Physical properties of outdoor air

Data on relative humidity, temperature, and pressure of air was collected from the register of Meteorological institute of Finland (license: CC BY 4.0, https://creativecommons.org/by/4.0/). The data originated from the Härmälä weather station, which represents humidity and temperature information for the Tampere urban area. We calculated absolute humidity based on relative humidity, temperature, and pressure. Data was available with 10-minute intervals, and it was matched with the time of each exercise test. Seasons were defined as follows: winter from December to February, spring from March to May, summer from June to August and autumn from September to November.

4.2.2 Pollen concentrations of the outdoor air

Pollen concentrations (expressed as pollen grains per cubic meter of air (grains/m^3)) were collected from the register of Biodiversity Unit of the University of Turku, Finland. Our data originated from the Hirst-Burkard pollen trap, that was located on the roof of the building next to the outdoor exercise test site (119). We collected data on pollen from birch (Betula), grass (Poaceae), alder (Alnus), mugwort (Artemisia vulgaris), and hazel (Corylus avellana) that are the most important pollen allergens in Finland (120).

4.2.3 Pollutant concentrations of the outdoor air

Pollutant concentrations (expressed as micrograms per cubic meter of air (µg/m^3)) were collected from the register of Tampere environmental protection unit. We observed the momentary outdoor air concentrations of NO_2, O_3, and $PM_{2.5}$. Measurements originated from a sampler located approximately 1 km away from the exercise test site in the same city region with similar infrastructure with the testing site. We used 1-hour measures covering the exercise test hour.

4.3 Lung function measurements

4.3.1 Impulse oscillometry (IOS) measurements

IOS measurements (Jaeger Gmbh, Würzburg, Germany) were performed according to international recommendations (43). On the exercise challenge day, children were informed to avoid tea, coffee, cocoa, cola drinks, hard exercise, and large meal before the study. They were also informed that there should be more than two weeks since the last respiratory infection. Subjects were instructed by their treating physicians on whether to use or to pause asthma controller medication. Trained physicians – who were blinded to the outdoor air properties - reviewed the technical properties of the IOS measurements to ensure that they fill the international quality requirements (43). The IOS measurements were considered technically reliable if they were repeatable and the coherence values were within the acceptable limits. Lower coherence values were accepted if there were symptoms suggesting obstruction and results were reproducible. Technical problems during IOS measurements (e.g., opening of the lips, postural problems, movement and holding of breath) were noted and those results were classified as unreliable. We used sex and height -dependent reference values for IOS (39). The criterion for EIB was an increase in R5 of ≥ 40% in IOS after exercise compared to baseline (31,42,43). The criterion for a significant bronchodilatation effect was a decrease in R5 ≥ 40% compared to baseline (39,43).

4.3.2 Spirometry measurements

Spirometry (Vyntus™ pneumo, Vyaire medical inc., Illinois, USA) was measured according to current international standards and the technical properties were evaluated retrospectively by trained physicians who were blinded to the outdoor air properties (121). Technical problems during spirometry measurements were noted by trained nurses and the tests classified as unreliable. We used sex and height-dependent Finnish reference values for spirometry (122). The criterion for EIB was a decrease in FEV_1 of ≥ 15% after exercise compared to baseline (10,47). The criterion for a significant bronchodilatation effect was an increase in FEV_1 of ≥ 12% and 200 ml compared to baseline (47).

4.4 Exercise protocol

Tests were conducted on a pedestrian and bicycle path at Tampere university hospital area between two hospital buildings. In the hospital area, there are some low trees and bushes, and in the immediate surroundings of the hospital area, there is forest consisting of coniferous and deciduous trees. There were no tall grasses in the immediate surroundings of the test site. There is motor vehicle traffic near the test area as in a typical urban environment. Exercise tests involved running between two points outdoors. In younger subjects with co-operation difficulties, exercise involved kicking and fetching a soccer ball. Experienced nurses recorded the possible symptoms during exercise (e.g., wheezing, cough, and dyspnea). Nurses monitored the heart rate (measured with FT4, Polar Ltd, Kempele, Finland) and exercise intensity during the tests: it was considered sufficient, if the heart rate was over 85% of the calculated maximal value (205-age/2) for at least six minutes or if symptoms of EIB appeared (10). We could not fully examine this retrospectively from all subjects due to insufficient entries and problems with heart rate monitors. All results were examined individually and if nurse’s notes reported a sufficient exercise intensity or symptoms of EIB the subjects were included in the results. IOS or spirometry was measured before exercise and 1-2, 5, 10, and 15 minutes after exercise. After measurements, the children were given salbutamol (Ventoline evohaler via Babyhaler® or Volumatic®): 300 µg for children under 10 years and 400 µg for children 10 years or older. Lung function measurements were repeated 15-20 minutes after salbutamol inhalation.

4.5 Statistics

Statistical analysis was performed using R-program version 4.0.2 (R Foundation, Vienna, Austria) and IBM SPSS statistics version 28.0 (IBM Corp, NY, USA). We used logarithmic transformations in linear regression if the distributions of the variables were skewed (relative humidity, maximal decrease in FEV_1, $PM_{2.5}$, NO_2). Due to extremely skewed nature of the pollen data, logarithmic transformation was not suitable, and data was categorized using threshold values for moderate and high pollen level of 10 grains/m^3 and 80 grains/m^3, respectively (73).

Dependent variables in our analysis were EIB or lung function change (R_5 in IOS group and FEV_1 in Spiro group). EIB was defined as increase of at least 40% in R_5 in IOS group. Correspondingly in spirometry, decrease of at least 15% in FEV_1 was defined as EIB in Spiro group. The EIB definitions are according to the Finnish and international guidelines (10,36,47). We selected the 15% cut-off for EIB in FEV_1 because of higher specificity for asthma (10).

For crosstabs and logistic regression analysis, continuous variables were categorized. We used following threshold in IOS group for pollutants: 25 and 40 µg/m^3 for NO_2, 60 and 100 µg/m^3 for O_3 and 10 and 25 µg/m^3 for $PM_{2.5}$ based on World Health Organization´s 2005 and 2021 guidelines (123,124). In statistical analysis of temperature and humidity data we used the following thresholds: < 5 g/m^3, 5-10 g/m^3 and ≥10 g/m^3 for absolute humidity, <50 %, 50-75%, and ≥75% for relative humidity, and <0°C, 0-10°C, and ≥10°C for temperature (52). In Spiro group, the same thresholds were not suitable due to lower number of subjects affecting the power of statistical analyses, and two highest groups were combined. Thus, the temperature and humidity data were categorized using the following thresholds: <5 and ≥5 g/m^3 for absolute humidity, <5 and ≥5°C for temperature, and <75% and ≥75% for relative humidity.

Chi-square, t test, Mann Whitney U-test and Pearson or Spearman correlation analyses were used in single parameter comparisons between different groups. Result of Pearson and Spearman correlation were expressed as correlation coefficients (r and r_s, respectively). Logistic regression was used in analyzing associations of outdoor air factors with the frequency of EIB. Linear regression was used in analyzing associations of outdoor air factors with change in R_5 and maximal decrease in FEV_1 and results were expressed as regression coefficient (β) and 95% confidence interval (CI). P values under 0.05 were considered statistically significant.

4.6 Ethical aspects

The study subjects were not contacted, and their medical treatment was not affected as the study was a retrospective chart review. The study was approved by the Ethics Committee of Tampere University Hospital (R15022). It was funded by non-profit research foundations and no commercial funding was received. None of the researchers had any conflicts of interest regarding the study.

5 RESULTS

5.1 Demographic characteristics of the study subjects

5.1.1 Subjects with IOS measurements

In designated study period, 926 subjects completed the exercise test with FOT measurements, which in our study were implemented by IOS-device (IOS-group, Figure 3). Most of the subjects were 3-7 years old, accounting for 97% of the subjects (with a range from 3 to 14 years, mean 5.4 years (SD 1.4)). Among the subjects, 73 (8.4%) were born with a gestational age of under 37 weeks, with four of them (0.5%) born before the 28^{th} week of gestation. Birth weight was low (<2500g) in 53 subjects (6.1%). Following the exercise challenge, EIB occurred in 13.9% of the study subjects. After assessing the IOS results, 58 tests were considered unreliable and excluded, leaving 868 technically reliable IOS results (94%) for further analysis (Figure 3). Table 1 provides the clinical and demographic characteristics of the participants included in the study divided into groups with and without EIB. Children who experienced EIB were significantly older, taller, had more often IgE-mediated sensitization, and R_5 was lower before exercise. At the time of the exercise test, 79 subjects (9.1 %) were using asthma controller medication. In patient records, 584 subjects (67%) had a diagnosis of asthma before or after the exercise test.

Table 1. Subject characteristics in the IOS group with technically reliable IOS divided in groups with and without exercise-induced bronchoconstriction (EIB) (n=868). Modified from the original publication I.

	EIB -	EIB +	
	n=747	n=121	
	Mean (SD) or %		p value
Age (years)	5.4 (1.4)	5.7 (1.4)	0.013
Height (cm)	113 (10)	115 (10)	0.005
ISO BMI (kg/m^2)	23 (5)	22 (4)	0.419
Gender male (%)	64%	65%	0.884
IgE-mediated sensitization (%)	50%	67%	0.001
Atopic dermatitis (%)	40%	47%	0.131
Any asthma medication (%)	54%	55%	0.890
Asthma in either parent (%)	34%	39%	0.335
Parental smoking (%)	28%	34%	0.259
Heart rate % from calculated maximum	97 (6)	97 (4)	0.314
R5 % of reference values	95 (17)	90 (15)	0.001
Significant bronchodilatation response (%)	4%	2%	0.282

Note: IgE mediated sensitization is a positive finding in skin prick test or allergen specific IgE. EIB=Exercise-induced bronchoconstriction (≥40% increase in impulse oscillometry resistance at 5Hz). Atopic dermatitis was diagnosed by physician and collected from the patient records, IgE=Immunoglobulin E, IOS=impulse oscillometry, R5=impulse oscillometry resistance at 5 Hz. ISO BMI=Body mass index adjusted to adult values. Chi-squared test or t-test was used where appropriate in single parameter comparisons between the groups.

5.1.2 Subjects with spirometry measurements

We reviewed the exercise tests with spirometry in 359 children (Spiro group, Figure 3), age ranging from 6.4 to 15.9 years (mean 10.7 years), in the designated study period. Only exercise tests that were considered technically reliable (n=321, 89%) were included in further analysis (Figure 3). Table 2 provides details about the demographic and clinical characteristics of the subjects included in the analysis divided into groups with and without EIB. IgE-mediated sensitization, atopic dermatitis and obstruction before exercise were more prevalent among subjects who experienced EIB. FEV_1 at baseline as Z-score were significantly lower in subjects with EIB. There was no difference in age, height, weight, gender, parental smoking, asthma status of parents, FVC at baseline as Z-score and controller medication pause between the groups. Asthma was diagnosed before or after exercise test in 57% of the subjects.

Table 2. Subject characteristics in Spiro group with reliable spirometry (n=321) divided into groups with and without exercise-induced bronchoconstriction (EIB). Table is modified from original publication IV.

	EIB (-)	EIB (+)	
	n=246	n=75	
	Mean (SD), Median [IQR] or %		p value
Age (years)	10.8 (2.6)	11.0 (2.5)	0.566
Height (cm)	146 (16)	147 (15)	0.654
Weight (kg)	39.0 [30.0–53.0]	40.0 [31.0–54.0]	0.294
Gender male (%)	58%	57%	0.952
IgE-mediated sensitization (%)	68%	82%	0.029
Atopic dermatitis (%)	42%	59%	0.009
Parental smoking (%)	39%	29%	0.315
Asthma in either parent (%)	40%	32%	0.342
Controller medications pause ≥ 28 days (%)	75%	67%	0.134
FEV_1 at baseline as Z-score	-0.5 (1.2)	-0.9 (1.3)	0.027
FVC at baseline as Z-score	-0.03 (1.1)	0.2 (1.2)	0.524
Obstruction before exercise (%)	25%	44%	0.001

Note: Criterion for exercise-induced bronchoconstriction was ≥15% decrease in forced expiratory volume in one second (FEV_1) after exercise compared to baseline. IgE-mediated sensitization = any positive finding in either skin prick test or serum allergen specific IgE. Atopic dermatitis was diagnosed by physician and collected from the patient records. Controller medication pause = period without inhaled corticosteroids, leukotriene receptor antagonists or long-acting beta-2-agonists before exercise test. Obstruction before exercise is spirometry FEV_1/FVC Z-score under -1.65 before exercise. Abbreviations: EIB = Exercise-induced bronchoconstriction, IgE = Immunoglobulin E, FEV_1 = forced expiratory volume in one second, FVC = Forced vital capacity. Chi-squared test, t-test or Mann-Whitney U test was used where appropriate in single parameter comparisons between the groups.

5.2 The association of outdoor physical factors with EIB in univariate analyses

In IOS data, the average absolute humidity of outdoor air during the tests was 5.8 g/m^3, ranging from 0.6-16.2 g/m^3 with a standard deviation (SD) of 3.1 g/m^3. Mean temperature was 5.0°C with SD 9.2°C and range -23.3°C-28.0°C. The distribution of relative humidity was markedly skewed, and median was 84% with interquartile range (IQR) of 69%-92% and range from 20%-100%. The distribution of absolute humidity in different seasons during years 2012-2015 is presented in Figure 4.

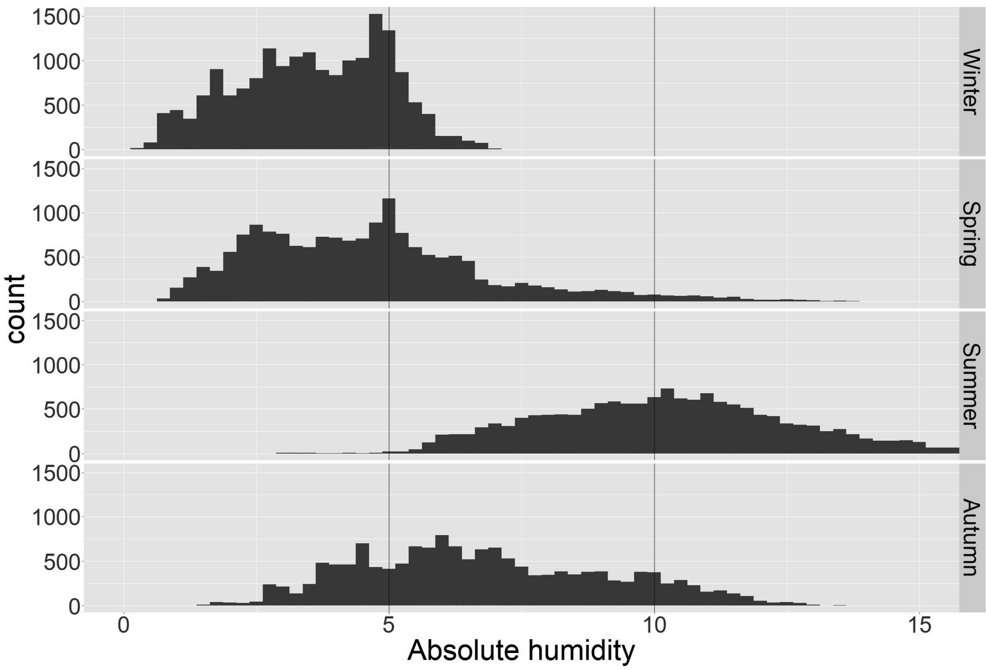

Figure 4. Distribution of absolute humidity in Finland during years 2012-2015 at different seasons. Figure is modified from supplemental material of original publication I.

Note: Absolute humidity is presented as g/m^3. Data is recorded in 10-minute intervals on Monday to Friday during working hours (8-16). Winter is from December to February, spiring is from March to May, summer is from June to August and autumn is from September to November.

5.2.1 The association of absolute humidity with EIB

In IOS group, mean absolute humidity of outdoor air during exercise test was significantly lower in subjects who experienced EIB (Table 3). Change in R_5 following the outdoor free running test demonstrated a significant correlation with absolute humidity (β=-0.89, CI [-1.45 – -0.34], p= 0.002). The prevalence of EIB was higher in tests conducted at lower absolute humidity levels, occurring in 17.6% of cases when absolute humidity was less than 5 g/m^3, 12.1% when absolute humidity ranged from 5 to 10 g/m^3, and only 5.9% when absolute humidity exceeded 10 g/m^3. A statistically significant difference in EIB occurrence was observed between absolute humidity levels below 5 and exceeding 10 g/m^3 (p = 0.008, Figure 5).

Table 3. Outdoor air physical factors in IOS group with technically reliable impulse oscillometry (IOS) (n=868) divided into those with and without exercise-induced bronchoconstriction (EIB).

	EIB -	EIB +	
	n=747	n=121	
	Mean (SD) or %		p value
Temperature (°C)	5.4 (9.2)	2.9 (8.8)	0.005
Temperature ≥5°C (%)	50%	38%	0.024
Relative humidity (%)	78 (18)	79 (18)	0.710
Relative humidity ≥75% (%)	69%	70%	0.787
Absolute humidity (g/m^3)	6.0 (3.2)	5.1 (2.7)	0.002
Absolute humidity ≥5 g/m^3 (%)	53%	38%	0.003

Note: EIB=Exercise-induced bronchoconstriction (≥40% increase in R5). IOS = impulse oscillometry. Chi-square test, Mann Whitney U-test or t-test were used where appropriate in single parameter comparison between groups.

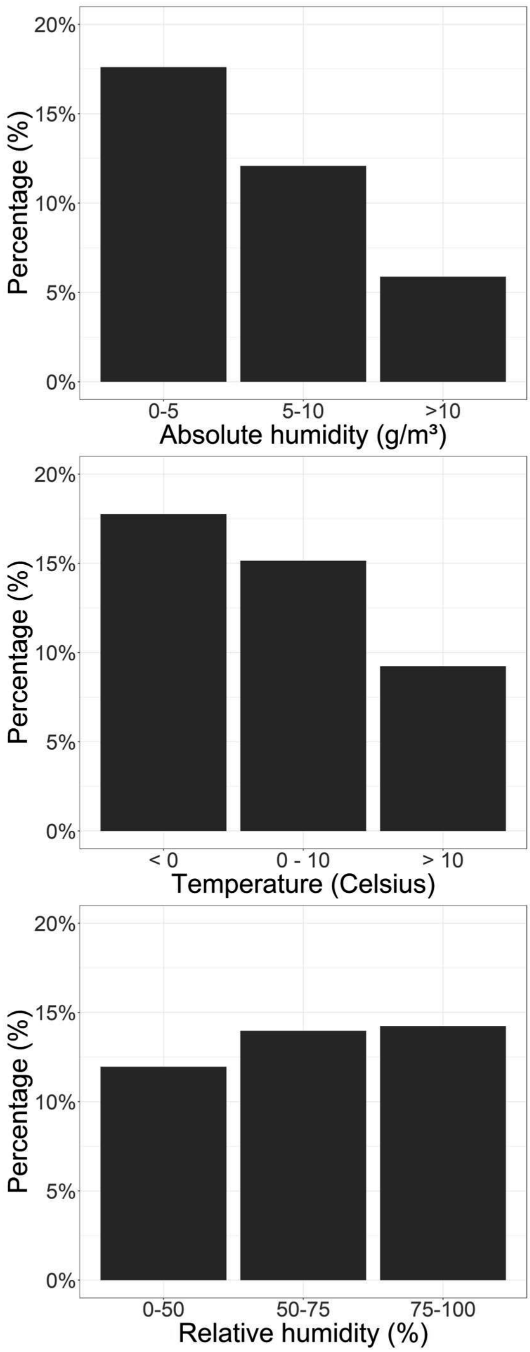

Figure 5. Proportion of reliable exercise tests with exercise-induced bronchoconstriction (EIB) at different absolute humidity, temperature and relative humidity levels (n=868). Figure is modified from original publication I.

Table 4 presents the data on physical properties of outdoor air during the exercise tests in EIB positive and negative subjects in Spiro group. Mean absolute humidity did not differ significantly between the groups. However, in the EIB positive group, 38% had absolute humidity ≥5 g/m^3, and in the EIB negative group, 51% had absolute humidity ≥5 g/m^3 and this difference was statistically significant. Subjects who underwent an exercise test in environment with low absolute humidity (levels ≤5 g/m^3) exhibited a significantly higher incidence of EIB of 28 %, compared to those tested in higher absolute humidity when incidence was 18 % (p = 0.035). In univariable logistic regression analysis absolute humidity levels of 5 g/m^3 or higher were associated with a decreased likelihood of experiencing EIB (OR 0.57, CI [0.33 ─ 0.96], p=0.036). Maximal decrease in FEV_1 correlated negatively with absolute humidity (r= -0.123, p=0.028).

We performed the linear univariate analysis also in subgroups with (pause < 28 days) and without (never medication or pause ≥28 days) controller medication (inhaled corticosteroids, leukotriene receptor antagonists or long-acting beta-2-agonists). In children without controller medication (n=234), the results were similar and absolute humidity (β=-0.146, CI [-0.269 ─ -0.018), p=0.026) was associated with FEV_1 decrease. In children with controller medication (n=85), absolute humidity was not associated with FEV_1 decrease (p>0.1).

Table 4. Subject characteristics and outdoor air physical properties in subjects with technically reliable spirometry (n=321) in Spiro group divided into those with and without exercise-induced bronchoconstriction (EIB). Table is from original publication IV.

	EIB -	EIB +	
	n=246	n=75	
	Mean (SD), Median [IQR] or %		p value
Absolute humidity (g/m^3)	5.6 (3.0)	5.0 (2.5)	0.102
Absolute humidity ≥5 g/m^3 (%)	51%	37%	0.035
Temperature (°C)	4.6 (9.9)	3.3 (9.0)	0.295
Temperature ≥5°C (%)	46%	35%	0.107
Relative humidity (%)	83 [64–92]	83 [65–92]	0.294
Relative humidity ≥75% (%)	65%	64%	0.827

Note: Criterion for exercise-induced bronchoconstriction was ≥15% decrease in forced expiratory volume in one second (FEV_1) after exercise compared to baseline. IgE-mediated sensitization = any positive finding in either skin prick test or serum allergen specific IgE. Atopic dermatitis was diagnosed by physician and collected from the patient records. Abbreviations: EIB = Exercise-induced bronchoconstriction (≥15% decrease in FEV_1 after exercise compared to baseline), FEV_1 = forced expiratory volume in one second, IgE = Immunoglobulin E. Chi-squared test, t-test or Mann-Whitney U test was used where appropriate in single parameter comparisons between the groups.

5.2.2 The association of relative humidity with EIB

In IOS group, relative humidity was not significantly related to R_5 change. Mean relative humidity was not statistically different between groups with and without EIB (Table 3). Additionally, there were no differences in frequencies of EIB at different levels of relative humidity: EIB occurred in 12.0 %, 14.0 % and 14.2 % of cases at relative humidity levels of < 50, 50–75 and ≥ 75 % (Figure 4).

Also in Spiro group, Table 4 shows that there was no statistically significant difference in mean relative humidity between subjects who did and did not have EIB (p>0.1). Furthermore, the proportions of tests with EIB were not significantly different in different levels of relative humidity (p>0.1). Also, univariate logistic regression analysis showed that relative humidity ≥75% (OR 0.94, CI [0.55─1.62], p>0.1) was not associated with EIB. Maximal decrease in FEV_1 did not correlate with relative humidity (r_s=-0.046, p>0.1).

5.2.3 The association of temperature with EIB

In IOS group, EIB was more frequent at lower temperatures and occurred in 17.8%, 15.1% and 9.2 % of cases at temperature levels of < 0, 0–10 and ≥ 10 ˚C, and there was a statistically significant difference between groups < 0 and ≥ 10 ˚C (p = 0.025) (Figure 4). Table 3 also shows that the mean temperature during the test was significantly lower in subjects who experienced EIB. R_5 change after outdoor free running test was related to temperature in linear regression (β = -0.22, CI [-0.41 – -0.04], p = 0.020).

In Spiro group, as detailed in Table 4, there wasn't a statistically significant difference in the mean temperature of outdoor air during the test between subjects who experienced EIB and those who didn't. The incidence of EIB also didn't differ across varying temperature levels. From the univariate logistic regression analysis, a temperature of ≥5°C (OR 0.64, CI [0.38 ─ 1.10], p>0.1) was not associated with the occurrence of EIB. A negative correlation was observed between the maximal decrease in FEV_1 and temperature (r= -0.127, p= 0.023).

We performed the linear univariate analysis also in subgroups with and without (subjects with never medication or pause at least 28 days) controller medication. In children without controller medication (n=234), the results were similar and temperature (β=-0.157, CI [-0.280 ─ -0.029), p=0.017) associated with FEV_1 decrease.

In children with controller medication (n=85), temperature was not associated with FEV_1 decrease (p>0.1).

5.3 Association of EIB and outdoor air physical factors in multivariable analyses

5.3.1 Results in IOS group

In the IOS group, a linear regression analysis with absolute humidity, relative humidity, and temperature as independent variables identified absolute humidity as the only statistically significant predictor of the change in R_5 (β = -2.14, CI [-3.91 – -0.37], p = 0.018). When the model was adjusted for age, gender, and height, the association between absolute humidity and the change in R_5 persisted as statistically significant (β = -2.26, CI [-4.02 – -0.50], p = 0.012). Even after integrating IgE-mediated sensitization and season as independent variables, this relationship remained significant (β = -2.67, CI [-4.67 – -0.66], p = 0.009, Table 5). Further, when other factors such as gestational age, birth weight, the interaction term of age and height, or a comparison of height to reference values were included, the association consistently remained statistically significant (β = -2.66 – -2.74, p = 0.009 – 0.013). In a subgroup analysis in subjects with asthma diagnosis (n=584), linear regression analysis of absolute humidity, relative humidity, temperature, age, height and gender, the results remained similar and absolute humidity was the only significant factor associated with R_5 change (β=-2.7, CI [-5.2 – -0.4], p=0.021). In non-asthmatics (n=284) the association did not remain statistically significant (p > 0.1).

Using a logistic regression model with independent variables absolute humidity, temperature and relative humidity levels, age, gender, IgE-mediated sensitization and season, a significant effect on EIB incidence was observed only in absolute humidity ≥ 10 g/m^3 and having IgE-mediated sensitization (p=0.036 and p<0.001, Table 6).

Table 5. Relative change of respiratory system resistance at 5Hz (R_5) explained with absolute humidity, relative humidity, and temperature using linear regression in IOS group. Results adjusted with age, gender, season, Immunoglobulin E (IgE)-mediated sensitization, and height (n = 868). Table is modified from original publication I.

	β	95% CI	p-value
Absolute humidity (g/m³)	-2.67	-4.67 — -0.66	0.009
Temperature (°C)	0.54	-0.11 — 1.18	0.103
Relative humidity (%)	0.13	-0.66 — 0.33	0.179
Age (years)	0.26	-2.20 — 2.72	0.837
Gender (male as reference)	-0.89	-4.47 — 2.69	0.626
Height (cm)	0.25	-0.10 — 0.60	0.164
IgE-mediated sensitization	7.13	3.66 — 10.6	<0.001
Season (winter as reference)			
Spring	0.94	-5.50 — 7.37	0.775
Summer	2.83	-6.20 — 11.86	0.539
Autumn	2.73	-2.62 — 8.09	0.316

Note: IgE mediated sensitization is a positive finding in skin prick test or allergen specific IgE. Winter is from December to February, spring is from March to May, summer is from June to August and autumn is from September to November. Abbreviations: IgE = Immunoglobulin E, CI = Confidence interval, IOS = impulse oscillometry, R_5 = Respiratory system resistance at 5 Hz, β = regression coefficient.

Table 6. Incidence of exercise-induced bronchoconstriction (EIB) explained with different absolute humidity, relative humidity and temperature levels using logistic regression in IOS group. Results adjusted with age, gender, height, Immunoglobulin E (IgE)-mediated sensitization and season (n = 868). Table is from supplemental material of original publication I.

	OR	95% CI			p-value
AH (g * m³)					
< 5	1.00				
5–10	0.63	0.35	–	1.16	0.137
≥ 10	0.28	0.09	–	0.92	0.036
Temperature (˚C)					
< 10	1.00				
0–10	1.12	0.64	–	1.93	0.696
≥ 10	1.15	0.46	–	2.85	0.762
RH (%)					
< 50	1.00				
50-75	1.39	0.60	–	3.23	0.439
≥ 75	1.24	0.50	–	3.06	0.643
Age (y)	0.98	0.75	–	1.30	0.912
Gender (male)	0.98	0.65	–	1.49	0.938
Height (cm)	1.03	0.99	–	1.07	0.195
Any IgE test positive (yes)	2.03	1.34	–	3.08	<0.001
Season					
Winter	1.00				
Spring	0.83	0.41	–	1.69	0.605
Summer	0.72	0.24	–	2.14	0.550
Autumn	0.88	0.49	–	1.58	0.667

Note: IgE mediated sensitization is a positive finding in skin prick test or allergen specific IgE. Winter is from December to February, spring is from March to May, summer is from June to August and autumn is from September to November. Abbreviations: AH = Absolute humidity, RH = Relative humidity, IgE = Immunoglobulin E, OR = Odds ratio, CI = Confidence interval.

5.3.2 Results in Spiro group

In Spiro group, we used stepwise approach in multivariable analyses. To study which of the variables were the independent predictors of EIB, we included absolute humidity ≥5 g/m³, temperature ≥5°C, atopic dermatitis, IgE-mediated sensitization, and obstruction before exercise in the model using stepwise logistic regression. In this model, absolute humidity ≥5 g/m³ was associated with a lower probability of EIB (OR 0.51, p=0.026, Table 7). Obstruction before exercise (OR 2.11, p=0.015) and IgE-mediated sensitization (OR 2.24, p=0.025) were associated with higher probability of EIB.

We used multivariable stepwise linear regression to further test if absolute humidity and temperature were independently associated with maximal decrease in FEV_1. Absolute humidity, temperature, obstruction before exercise, IgE-mediated sensitization, and atopic dermatitis were included in the model. Only absolute humidity (β -0.044, p=0.033, table 8) was associated with maximal decrease in FEV_1.

Table 7. Results of a multivariable stepwise logistic regression analysis in Spiro group (n=321) on the incidence of exercise-induced bronchoconstriction (EIB) explained with absolute humidity ≥5 g/m^3, temperature ≥5°C, obstruction before exercise, Immunoglobulin E (IgE)-mediated sensitization, and atopic dermatitis. Table is from original publication IV.

	OR	95% CI	p value
Absolute humidity ≥5 g/m^3	0.51	0.28, 0.92	0.026
Obstruction before exercise	2.11	1.16, 3.86	0.015
IgE-mediated sensitization	2.24	1.11, 4.51	0.025

Note: Variables not statistically significant in the model were temperature ≥5°C and atopic dermatitis. IgE-mediated sensitization = any positive finding in either skin prick test or serum allergen specific IgE. Atopic dermatitis was diagnosed by physician and collected from the patient records. Obstruction before exercise was spirometry FEV_1/FVC Z-score under -1.65 before exercise. Abbreviations: EIB = Exercise-induced bronchoconstriction, IgE = Immuglobulin E, OR = Odds ratio, CI = Confidence interval, FEV_1 = Forced expiratory volume in one second, FVC = Forced vital capacity.

Table 8. Decrease in forced expiratory volume in one second (FEV_1) explained with absolute humidity, temperature, obstruction before exercise, Immunoglobulin E (IgE)-mediated sensitization, and atopic dermatitis using multivariable stepwise linear regression in Spiro group. Subjects with technically reliable spirometries included (n=321). Table is from original publication IV.

	β	95% CI	p value
Absolute humidity (g/m^3)	-0.044	-0.085, -0.004	0.033

Note: Variables not statistically significant in the model were temperature, obstruction before exercise, IgE-mediated sensitization, and atopic dermatitis. IgE-mediated sensitization = any positive finding in either skin prick test or serum allergen specific IgE. Atopic dermatitis was diagnosed by physician and collected from the patient records. Obstruction before exercise was spirometry FEV_1/FVC Z-score under -1.65 before exercise. Due to marked skewness of decrease in FEV_1 it was log transformed in linear regression analysis. Abbreviations: IgE = Immuglobulin E, β = Regression coefficient, CI = Confidence interval, FEV_1 = Forced expiratory volume in one second.

5.4 The association of pollen concentrations with EIB

Data about outdoor air pollen concentrations was available for children who underwent IOS tests (n=799) between January 2012 and December 2014 (Figure 3). Birch pollen concentration was measurable (>0 grains/m^3) in 127 cases (16%), and similarly, alder pollen concentration was measurable in 58 cases (7%). Birch pollen concentration was ≥10 grains/m^3 in 94 cases (12%), and similarly, alder pollen concentration in 34 cases (4%). Either birch or alder pollen count was ≥10 grains/m^3 in 127 cases (16%).

Table 9 presents subject characteristics divided to either birch or alder pollen count being over or both being under 10 grains/m^3 at the time of the test. Subjects who underwent testing during periods of low pollen counts were, on average, older and taller than those tested when either of the pollen counts was high. Individuals tested during periods of low pollen counts were also more frequently sensitized to birch or alder pollen.

Frequency of EIB did not differ statistically significantly between groups tested when either birch or alder pollen count was moderate or both were low (13% versus 14%, p>0.1, Table 9). Also, there were no statistically significant differences in baseline R_5 (96 % versus 95 %, p>0.1) or mean change in R_5 (15 % versus 17 %, p>0.1) between these groups (Table 9). If the subjects were categorized based on whether either alder or birch pollen was over or both were under 80 grains/m^3 (720 vs 79 tests), or when either pollen was measurable (pollen grain count ≥ 1 grains/m^3, 627 vs 127 tests), no significant differences were found between the groups in terms of either the frequency of EIB or the change in R_5 (p>0.1 in all scenarios).

Table 9. Subject characteristics and exercise test results in IOS group (n=799) divided according to either alder or birch pollen count over or both being under 10 grains/m³ at the time of exercise test. Data is mean (standard deviation (SD)) or percentage. Table is modified from original publication II.

	both pollen counts < 10 grains/m³	either pollen count ≥ 10 grains/m³	
	n=672	n=127	p-value
Age (years)	5.5 (1.4)	5.0 (1.2)	0.001
Height (cm)	113.3 (9.6)	110.4 (9.0)	0.002
Gender Male (%)	65%	61%	0.457
Alder or Birch IgE test positive (%)	38%	22%	0.001
R_5 of reference values (% of reference)	94.5 (17.4)	95.7 (15.9)	0.450
R_5 change after exercise (% of baseline)	17.4 (26.3)	15.5 (24.8)	0.449
Obstruction before exercise (%)	3%	2%	0.792
Exercise induced bronchoconstriction (%)	14%	13%	0.968

Note: Alder or birch IgE positive is a positive finding in skin prick test or allergen specific IgE for alder or birch. IgE = Immunoglobulin E, R_5 = Respiratory system resistance at 5 Hz measured using impulse oscillometry. Chi-squared test or t-test was used where appropriate in single parameter comparisons between the groups.

In the subgroup of alder or birch pollen sensitized subjects (n=286), there were no statistically significant differences in frequency of EIB (16.7 % versus 21.4 %, p>0.1) or mean change in R_5 (18.9 % versus 25.7 %, p>0.1) between subjects tested at lower (< 10 grains/m³) and higher (≥ 10 grains/m³) pollen concentrations. Additionally, we made a subgroup analysis of alder and birch pollen separately. When comparing tests with alder pollen concentration under or over 10 grains/m³, there were no statistically significant differences in baseline R_5 (95 % versus 96 %, p>0.1), frequency of EIB (13.6 % versus 20.6 %, p>0.1) or mean change in R_5 (17.1 % versus 16.1 %, p>0.1). Similarly, when comparing tests with birch pollen concentration under or over 10 grains/m³, there were no differences in baseline R_5 (95 % versus 96 %, p>0.1), frequency of EIB (14.3 % versus 10.6 %, p>0.1) or mean change in R_5 (17.4 % versus 14.8 %, p>0.1).

In logistic multivariable regression analysis of EIB using alder or birch pollen over 10 grains/m³, absolute humidity ≥ 10 g/m³, IgE-mediated birch or alder sensitization, and interaction factor of alder or birch sensitization and alder and birch pollen, showed that only absolute humidity ≥ 10 g/m³ was associated with EIB (OR 0.32, p= 0.006, Table 10). In multivariable linear regression analysis, using alder or birch pollen count over 10 grains/m³, absolute humidity and IgE-mediated sensitization for alder or birch pollen, the R_5 change after outdoor free running test was significantly related only to absolute humidity (β= -0.877, p= 0.003).

In multivariable linear analysis using any IgE-mediated sensitization, alder or birch pollen ≥ 10 grains/m^3 and absolute humidity as a covariate, both any IgE-mediated sensitization (p-value < 0.001) and absolute humidity (p=0.003) were significantly correlated with change in R_5 after outdoor free running test. Similarly, the multivariable logistic regression analysis including any IgE-mediated sensitization, absolute humidity ≥ 10 g/m^3 and alder or birch pollen ≥ 10 grains/m^3 as covariates, only any IgE-mediated sensitization (p<0.001) and absolute humidity ≥ 10 g/m^3 (p=0.006) were associated with EIB incidence. We wanted to investigate whether the factors mentioned above were related to baseline lung function: any IgE-mediated sensitization, absolute humidity ≥ 10 g/m^3 or alder or birch pollen ≥ 10 grains/m^3 were not related to R_5 as a percentage of reference values (p>0.1 in all scenarios). When adding asthma controller medication pause over 14 days, use of antihistamines or nasal corticosteroid as covariates, these did not significantly associate to EIB incidence or R_5 change (p > 0.1 for all) and again only absolute humidity ≥ 10 g/m^3 correlated significantly with EIB incidence and change in R_5. In a subgroup of alder or birch sensitized subjects (n=286), linear regression analysis using absolute humidity ≥ 10 g/m^3 and alder or birch pollen ≥ 10 grains/m^3, neither of the variables were associated with R_5 change.

Table 10. Incidence of exercise-induced bronchoconstriction (EIB) explained with absolute humidity ≥over 10 g/m^3, alder or birch pollen count over 10 grains/m^3 and alder or birch pollen IgE-mediated sensitization using logistic regression (n = 799). Table is from original publication II.

	OR	95% CI			p-value
Alder or birch pollen ≥ 10 grains/m^3	0.90	0.43	–	1.73	0.754
Absolute humidity ≥ 10 g/m^3	0.32	0.13	–	0.67	0.006
Alder or birch IgE-mediated sensitization	1.46	0.94	–	2.27	0.093
Alder or birch pollen ≥ 10 grains/m^3 * Alder or birch IgE-mediated sensitization	1.78	0.51	–	5.83	0.346

Note: Alder or birch IgE positive is a positive finding in skin prick test or allergen specific IgE for alder or birch. IgE = Immunoglobulin E, EIB = Exercise-induced bronchoconstriction, OR = Odds ratio, CI = Confidence interval.

5.5 The association of pollutant concentrations with EIB

The average O_3 of outdoor air during the tests was 48 μg/m^3, ranging from 2-106 μg/m^3. The distributions of $PM_{2.5}$ and NO_2 were markedly skewed. $PM_{2.5}$ median was 6 μg/m^3 with IQR of 4-8 μg/m^3 and range from 0-38 μg/m^3. NO_2 median was 12 μg/m³ with IQR of 8-15 μg/m³ and range from 1-81 μg/m³. Concentrations of $PM_{2.5}$ and NO_2 were higher in cold season and concentration of O_3 was higher in summer months. Percentage of days when $PM_{2.5}$ exceeded 10 μg/m³ is presented in Figure 6.

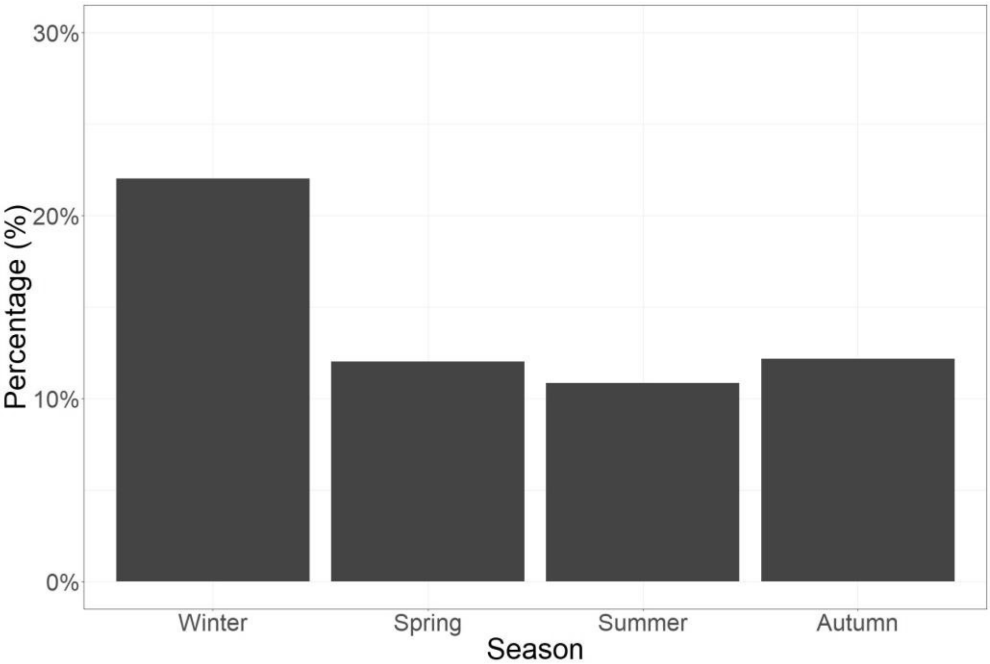

Figure 6. In different seasons, the percentage of days when the particulate matter with a diameter < 2,5 μm concentration exceeded 10 μg/m³. Winter is from December to February, spiring is from March to May, summer is from June to August and autumn is from September to November.

5.5.1 Relation between pollutant levels and airway obstruction after exercise

The concentrations of $PM_{2.5}$, NO_2, and O_3 displayed no significant correlation with the change in R_5 in the univariate linear regression analyses (β = 0.018, CI [-0.05 –0.09], p = 0.603 for $PM_{2.5}$; β = 0.005, CI [-0.06 –0.07], p = 0.884 for NO_2; β = 0.064, CI [-0.00 –0.13], p = 0.065 for O_3). The O_3 concentration was selected for subsequent multivariate analysis due to its strongest, but not statistically significant, correlation with the change in R_5. Consequently, we performed a multivariate linear regression analysis of the change in R_5, using O_3, absolute humidity, alder or birch pollen counts ≥ 10 grains/m³, and age as predictor variables. In this analysis, only absolute humidity demonstrated a significant association with the change in R_5 (β= -0.84, CI [-1.43 –-0.25], p = 0.005), whereas O_3 did not show a significant association (β= 0.08, CI [-0.02 –0.18], p = 0.115).

5.5.2 Relation between pollutant levels and probability of EIB

EIB was observed in 13.1% of cases when the $PM_{2.5}$ concentration remained below 10 μg/m^3 and in 18.9% of cases when the $PM_{2.5}$ concentration exceeded 10 μg/m^3. The $PM_{2.5}$ concentration excessed 10 μg/m^3 in 22.9% and 16.2% of cases with and without EIB, respectively. However, this difference did not reach statistical significance in either scenario (p = 0.096). Statistically significant differences were not observed between the EIB-positive and EIB-negative groups regarding the percentage of cases in which other pollutant concentrations exceeded the predefined thresholds (Table 11).

Table 11. Proportions of subjects with pollutant levels above established thresholds at the time of the exercise test in subjects with technically reliable impulse oscillometry (IOS) (n=868) divided into those with and without exercise-induced bronchoconstriction (EIB). Table is from original publication **III**.

	EIB (-)	EIB (+)	
	n=747	n=121	p-value
$NO_2 \geq 25$ μg/ m^3 (%)	12%	17%	0.198
$NO_2 \geq 40$ μg/ m^3 (%)	3%	3%	1.000
$O_3 \geq 60$ μg/m^3 (%)	28%	31%	0.701
$O_3 \geq 100$ μg/m^3 (%)	0.4%	0%	1.000
$PM_{2.5} \geq 10$ μg/m^3 (%)	16%	23%	0.096
$PM_{2.5} \geq 25$ μg/m^3 (%)	0.3%	0%	1.000

Note: Abbreviations: EIB = Exercise-induced bronchoconstriction, IOS = impulse oscillometry, NO_2 = Nitrogen dioxide, O_3 = Ozone, $PM_{2.5}$ = Particulate matter < 2.5 μm. The Chi-squared test or Fisher´s exact test was used.

As the largest difference between the EIB-positive and EIB-negative groups was noted in the proportion of tests having $PM_{2.5} \geq 10$ μg/m^3 (Table 11), we selected it for subsequent logistic multivariable regression analysis of the EIB incidence (n=799). In this analysis, $PM_{2.5} \geq 10$ μg/m^3, absolute humidity ≥ 10 g/m^3, alder or birch pollen counts ≥ 10 grains/m^3, and age were used as independent variables. Our findings showed a significant connection between the occurrence of EIB and both absolute humidity ≥ 10 g/m^3 (OR 0.31, CI [0.13–0.65], p = 0.004, Table 12) and $PM_{2.5}$ ≥ 10 μg/m^3 (OR 1.69, CI [1.02–2.75], p = 0.036).

Table 12. Results of a multivariate regression analysis on the incidence of exercise-induced bronchoconstriction (EIB) explained with particulate matter < 2.5 µm ($PM_{2.5}$) ≥ 10 µg/ m^3, absolute humidity ≥ 10 g/m^3, alder or birch pollen count ≥ 10 grains/m^3 and age (n = 799). Table is from original publication III.

	OR	95% CI			p-value
Absolute humidity ≥ 10 g/m^3	0.31	0.13	–	0.65	0.004
$PM_{2.5}$ ≥ 10 µg/ m^3	1.69	1.02	–	2.75	0.036
Alder or birch pollen ≥10 grains/m^3	1.09	0.60	–	1.89	0.755
Age	1.18	1.02	–	1.35	0.017

Note: Abbreviations: EIB = Exercise-induced bronchoconstriction, $PM_{2.5}$ = Particulate matter < 2.5µm, OR = Odds ratio, CI = Confidence interval. Age is included as a covariate and its results are reported only for reference.

In the subgroup analysis focusing on allergic subjects only (n= 425), the results were similar and both absolute humidity ≥ 10 g/m^3 (OR 0.41, CI [0.16–0.89], p = 0.036) and $PM_{2.5}$ ≥ 10 μg/m^3 (OR 1.83, CI [1.00–3.28], p = 0.045) were statistically significant predictors of EIB.

6 DISCUSSION

6.1 Absolute humidity and temperature in relation to EIB

In univariable analyses, both lower temperature and lower absolute humidity of outdoor air were associated with more frequent EIB and more severe obstruction in outdoor exercise tests, but in multivariable analyses only absolute humidity was independently associated with the outcomes. The correlation of absolute humidity with the frequency of EIB was meaningful and clinically significant. Clinicians should take these findings into account – especially when assessing negative exercise test results in environments with high outdoor air absolute humidity.

To our knowledge, this is the first study to simultaneously evaluate the influence of temperature, relative humidity, and absolute humidity on the likelihood and severity of EIB post free-running exercise test with a large clinical sample of preschool and school-aged children. As temperature, relative humidity, and absolute humidity are related to each other, univariable analyses may show any of these factors to correlate with EIB. However, since all inhaled air is warmed up to body temperature in airways and saturated with water vaporizing from epithelial lining fluid, theoretically the rate of water loss from airways is determined only by absolute humidity of inspired air and minute ventilation—not by relative humidity or temperature. Our findings that only absolute humidity has a direct association with EIB, support the established theories on airway water loss and hyperosmolarity triggering EIB.

Our real-life results are in line with previous laboratory-based studies that examined EIB in relation to different humidity levels. Specifically, three separate studies evaluated the average decline in FEV_1 post-exercise under varying humidity and temperature settings (58–60). These studies consistently reported a more pronounced reduction in FEV_1 in dry conditions. Interestingly, even these “dry” settings usually had an absolute humidity above 5 g/m^3. One study (52) showed that the frequency of EIB in different temperature levels didn´t change when absolute humidity was kept constant but at very high absolute humidity fewer EIB reactions were recorded. This showed that the influence of absolute humidity is more significant on EIB frequency than the influence of temperature preferring the osmotic hypothesis over the thermal hypothesis for the pathogenesis of EIB. It's noteworthy that the absolute humidity levels examined in the laboratory studies were relatively high compared to what we observed outdoors in Finland. Moreover, while the earlier studies focused on a few specific humidity and temperature settings, our findings present a linear relationship between temperature, absolute humidity, and the decline in FEV_1 and increase in R_5 over a large range of temperature and humidity. This linear association could not be observed in prior laboratory studies.

Some of the previous studies have focused on examining the effect of temperature on EIB. If relative humidity was kept constant (59), there were more decrease in lung function after exercise in cold environment – when the absolute humidity was also lower. On the other hand, when the absolute humidity was kept constant at a low level (63), temperature did not have effect on post-exercise lung function reduction in spirometry. Furthermore, couple of studies have indicated that the incidence of EIB can fluctuate across different seasons and incidence of EIB is notably lower in the summer (125,126), which could be due to the higher absolute humidity during the summer (Figure 4). This is in line with our findings. It also supports the theory that temperature is not directly related to EIB, but rather that lower absolute humidity in cold air is the important factor in the onset of EIB. There is also evidence that facial cooling while inhaling either warm or cool air can induce bronchoconstriction through vagal mechanism also in non-asthmatic subjects (54,55). This may also be one underlying mechanism in the background of EIB, which we were not able to take into account in our study setting.

In previous studies about outdoor free-running tests in uncontrolled conditions, absolute humidity has not been taken into account. In some studies, lower temperature was associated with a decline in lung function after exercise (11,12,31), but based on our findings and previous laboratory studies, these findings may be due to lower absolute humidity in cold air. When temperature was constantly near 30°C (13), changes in relative humidity did not affect the EIB incidence. Absolute humidity in such conditions is anyway high (> 10 g/m^3), which is also in line with our finding that high absolute humidity is associated with lower EIB incidence. Relative humidity and temperature did not independently explain EIB in our study. Instead, absolute humidity is the driving factor. Based on international guidelines, exercise testing is recommended to be conducted with inhaled air having absolute humidity of <10 g/m^3 or temperature of <25°C, and a relative humidity of <50% (10,57). Based on our study findings, this occurs in Nordic climate in most seasons, but in the summer, absolute humidity is often > 10 g/m^3 (Figure 4).

In the subgroup analyses of our study, the association of outdoor air physical factors with post-exercise lung function reduction was found in children with asthma diagnosis in IOS group and in those who were not using controller asthma medication at the time of the exercise test in Spiro group. In Spiro group in the subgroup of children without controller medication, absolute humidity and temperature correlated with FEV_1 decrease similarly as in the whole study population, but this was not found in children without a 28 day controller medication pause (n=85). In IOS group, we found that absolute humidity associated with R_5 change in the subgroup of children with asthma diagnosis before or after exercise test (n=584), but not in non-asthmatic children (n=284). Negative results in the smaller subgroups (e.g. non-asthmatics and children without adequate controller medication pause) may be due to lack of statistical power or they may reflect the importance of inflammation in the onset of EIB.

Earlier research reports higher prevalence of EIB across different patient populations (11,125) in comparison to our population. The lower EIB incidence in our study is likely due to Finland's high suspicion rate for asthma and that the national guideline recommends objective lung function measures for asthma diagnosis in children aged three and above (47). While prior studies on outdoor air physical factors and EIB are conducted mainly in adults, it is important to verify results in pediatric populations due to differences in characteristics in childhood asthma compared to adults (62,127,128). Despite the challenges of isolating individual mechanisms in uncontrolled outdoor conditions where humidity and temperature are closely linked, our study in preschool and schoolchildren confirmed that absolute humidity plays an important role in the incidence of EIB and post-exercise lung function decline.

6.2 Pollen concentration and EIB

To our knowledge, our study was the first to explore the link between outdoor pollen levels and lung function in preschool children using FOT. We found that outdoor pollen levels had no significant impact on EIB incidence in preschool children during a free running test. Additionally, there was no difference in results between allergic and non-allergic children.

Atopy is characterized as a risk factor for asthma, and allergic asthma represents one of the asthma phenotypes (2). There is a lot of evidence about connection between high pollen concentrations and allergic symptoms such as rhinitis and conjunctivitis, as well as bronchial symptoms (75,76). Additionally, there is evidence about association of increased allergen concentrations with higher number of emergency department visits for asthma and allergies (81–83). Inflammation markers associated with asthma have been observed to increase with allergen exposure, explaining increased symptom rate and asthma morbidity (15,78). Findings of our study do not question this previously observed association between high allergen concentrations and asthma-related inflammation and morbidity in allergic individuals. However, our study results do indicate that in a large dataset of children under school age, the frequency or severity of EIB during the pollen season did not stand out.

It is thought that elevated pollen levels during exercise test increase the risk of EIB. A small Finnish study (85) managed to show this phenomenon by investigating the decline in lung function measured by spirometry in young adults with asthma during the spring pollen season and in winter. Half of the participants were sensitized to pollen, while the other half were not. The results indicated that lung function declined more after exercise in individuals allergic to pollen during the pollen season. Non-allergic individuals experienced a greater decline in lung function during the winter, possible due to lower absolute humidity, but absolute humidity was not measured or reported in the study.

However, studies have not been able to establish a connection between the decline in lung function and all types of pollen. In a Swedish study, the amount of grass pollen, but not birch pollen, correlated with decrease in lung function during the pollen season, although the impact on exercise test results was not investigated (86). One former study showed that grass pollen counts increase as urbanity decreases in Helsinki metropolitan area, and occasionally grass pollen concentrations can reach such high levels in most urban areas that they can trigger allergic symptoms (129). In our research, the occurrence of elevated grass pollen counts was so infrequent that a statistical analysis could not be conducted due to low statistical power. Our data was collected using Hirst-Burkard pollen traps positioned on the roof of the building adjacent to the hospital. Grass pollen grains, being relatively heavy particles, tend to create high concentrations only in localized areas because they do not spread widely. However, there is no significant presence of intensive grass growth near the location where the outdoor exercise test was conducted, which explains, together with the location of our pollen collector, low grass pollen concentrations in our data.

Some children were diagnosed with asthma based on signs and symptoms, like recurrent obstructions and wheezing, at a very young age in our study population. Among these children, asthma typically has a favourable prognosis, with spontaneous recovery being common. This could potentially introduce a confounding factor in our study. Some of these children may later develop allergies and allergic asthma, but due to the retrospective design of our study, we cannot analyse them as a separate subgroup and follow-up is not possible.

Some patients experience bronchial symptoms during allergen exposure in the pollen season but not in other seasons. If the symptoms are severe, the patients are typically treated during the pollen season and diagnostic tests, such as exercise test, are scheduled outside of the allergy season after a pause in medication. Our data showed that children tested when the pollen concentrations were high, were significantly less likely to be sensitized to pollen. Consequently, there may have been a selection bias in our study such that the most pollen-sensitive subjects were more likely to be tested outside pollen season. This may have affected our results on the relation between pollen concentrations and EIB. Our study may also lack the statistical power to investigate the effects of very high pollen concentrations that patients may encounter in their daily lives but not necessarily during the time of the exercise test. There is also a possibility for wrong negative result in children with pollen sensitization and moderate pollen counts due to a small sample and lack of statistical power. Due to the retrospective study design, we were not able to take other allergen exposures (e.g., domestic animals, house dust mite) into account and they can be confounding factors.

6.3 Pollution concentration and EIB

In our data with a large number of subjects, we identified a positive correlation between elevated momentary concentrations of $PM_{2.5}$ and an increased occurrence of EIB in preschool children in a multivariable analysis. Notably, the average levels of pollutants examined in our study remained relatively modest, and we did not observe any significant associations between the presence or severity of EIB and levels of NO_2 or O_3.

To our knowledge, there haven't been any prior studies that have used real-life clinical data to investigate whether outdoor pollution levels during exercise tests relate to the occurrence of EIB in preschool children, using FOT to measure lung function. In a former study from Finland, researchers didn´t find any association with different pollutant exposures and lung function decline after exercise in children with chronic respiratory symptoms. However, air pollution was connected to lower baseline spirometry results (115). In a more recent study, heightened exposure to NO_2 was associated with lower post-exercise lung function evaluated with spirometry in children living in New York City (116). In our study, $PM_{2.5}$ was the only air pollutant associated with the incidence of EIB. In previous studies, higher $PM_{2.5}$ levels have been associated with lower lung function, airway inflammation, airway oxidative stress and asthma exacerbations (17,95,96,130,131). It is suggested that PM exposure enhances Th17 polarization in lymphocytes increasing neutrophilic inflammation (104,132). Particulate matter, diesel exhaust and ozone have also been shown to damage epithelial barrier in airways in animal studies (97,98,133). Increased inflammation, epithelial barrier damage and variable airway hyperreactivity are the mechanisms that could explain the association of higher $PM_{2.5}$ concentrations with EIB occurrence observed in our study.

In epidemiological research where pollutants naturally exist as a gaseous mixture in ambient air, their effects are difficult to study. Attempts have been made to distinguish the individual effects of each pollutant. This often masks the overall health impact of the gas mixture. Furthermore, controlled exposure studies in humans are limited by a small sample size and challenges in examining highly vulnerable subgroups, such as children. In our study, we observed a statistically significant association between EIB and $PM_{2.5}$ concentrations, but not with NO_2 or O_3 levels. However, prior studies have reported reduced lung function in adults linked to substantially higher NO_2 and O_3 concentrations compared to what we measured in our study (134,135). Therefore, it's possible that the NO_2 and O_3 levels during the tests in our data were so low that any connection with EIB would be unlikely.

In our study, we observed a minor increase in the incidence of EIB when $PM_{2.5}$ concentrations exceeded 10 $\mu g/m^3$ during the exercise test. Our measurements were taken hourly, although there are no established recommendations for hourly $PM_{2.5}$ concentrations. The European Union's annual threshold value for $PM_{2.5}$ is higher than the health-based limit in the recently (2021) updated recommendation by the World Health Organization (25 $\mu g/m^3$ versus 5 $\mu g/m^3$). During our study, the hourly $PM_{2.5}$ concentration exceeded the European Union's guideline value only twice. Thereby our findings suggest that even relatively low levels of ambient $PM_{2.5}$ may be linked to EIB occurrence in preschool children. The significance of air pollution, including pollutants beyond $PM_{2.5}$, is likely more significant in areas with higher air pollution levels and further studies are needed to examine the association between EIB and pollution in areas with different ambient air pollution concentrations.

6.4 Absolute humidity as the most powerful predictor of EIB

We discovered that reduced air humidity was linked to a higher likelihood and severity of EIB, while pollen concentrations did not show a significant association with EIB in univariable or multivariable analysis. Notably, in this study conducted in a small city in Finland, among various air properties, absolute humidity appeared as the strongest predictor of EIB. In addition to low absolute humidity, $PM_{2.5}$, even at relatively low concentrations, may be an independent predictor of EIB. However, in multivariable analysis absolute humidity was a more powerful predictor of EIB than $PM_{2.5}$ level.

Given that all inhaled air is warmed to body temperature within the airways and becomes saturated with water vapor from the epithelial lining fluid, the rate of water loss from the airways depends on both the minute ventilation and the absolute humidity of the inhaled air. In epithelium, dehydration is linked to cooling through evaporation. Initially, the warming of cold air results in a reduction in bronchial epithelial temperature, and as the air becomes warmer, the relative humidity of the air decreases. Consequently, moisture is evaporated from the surface epithelium due to humification of dry air, resulting in additional temperature loss. If only the warming of cold air is studied, thermal hypothesis cannot be excluded. However, it has been shown that breathing dry warm air causes more lung function decrease than breathing dry cold air (63). Our study results demonstrated that absolute humidity was the most important factor associated with the occurrence of EIB. The finding is in line with theories about airway water loss and the osmotic theory of EIB.

6.5 Strengths and weaknesses of the study

Our study's primary strength lies in its large clinical sample, consisting of individuals with suspected asthma or need to assess the efficacy of asthma medication, the same population for which exercise tests are commonly conducted in real-world clinical practice. This enhances the applicability of our findings to clinical settings with comparable climatic conditions. Furthermore, our comprehensive dataset on outdoor air properties was provided by official sources (the Meteorological Institute of Finland, Biodiversity Unit of the University of Turku, and city of Tampere) minimizing any substantial error or gaps on data.

In our study, there are also some weaknesses. We could have drawn better conclusions about causality with a prospective study and repeated exercise tests in same individuals in different conditions. However, such research would have been expensive and laborious to apply on the scale of our sample size. Due to the retrospective study design, we did not have all the information available for the subjects, and some clinical data were missing, such as symptom severity during the time of the exercise test. Our study population consisted of a heterogeneous group of children, in which some were asthmatics, and some were non-asthmatics with respiratory symptoms. Asthma is a heterogenous disease with varying types and intensities of inflammation, and we did not have reliable data on the type, activity, and severity of asthma at the time of the exercise test due to the retrospective study design. These factors may modify our results, because underlying active inflammation is an important factor in the pathophysiologic mechanism of EIB. Some of the subjects were on controller medication at the time of the exercise test, which also may modify the results. These factors may reduce the generalizability of the results.

Humidity, temperature, and airborne particles interact with each other, and examining them as individual phenomena is difficult. We have collected large clinical data and used statistical methods to try to account for confounding factors as effectively as possible. However, eliminating all confounding factors is impossible in real-life data. Furthermore, we were unable to investigate the impacts of markedly elevated concentrations of pollutants due to the relatively clean air in our study site. Notably, significant associations between pollutant levels and EIB were not detected through simple group comparisons or univariate regression analyses; instead, they emerged only in multivariate analyses. To validate these findings, additional replication studies are warranted in locations with higher levels of air pollution.

The international recommendation suggests measuring lung function up to 30 minutes after exercise challenge (10). In our study, the last lung function measurement was conducted 15 minutes post-exercise, based on clinical practice at Tampere University Hospital. This is because previous research has shown that EIB in children typically occurs within 15 minutes of exercise (30,136).

We did not have access to data regarding pollution and pollen concentrations in the participants' residential areas prior to the exercise test, which was therefore not considered in our analysis. Since both pollen and pollution can trigger asthmatic inflammation, future studies may benefit from including pre-exercise test exposures into their analyses. Climatic data was recorded at 10-minute intervals, while pollution data was collected hourly near the study site. However, pollen data was measured in daily concentrations, which could introduce some inaccuracies when assessing the exact timing of pollen exposure during the exercise tests.

7 CONCLUSION

Corresponding previously defined aims of the study, the conclusions were as follows:

1. Absolute humidity is the key factor out of the physical properties of outdoor air in determining the likelihood and severity of EIB. Relative humidity is not connected to EIB and temperature is associated with EIB only by its correlation with absolute humidity. The results were the same in younger children, whose lung function was measured with FOT, and older children with lung function measurements by spirometry. Absolute humidity should be reported during exercise tests so that clinicians can make a reliable assessment of the predictive value of a negative test. Reporting only temperature and relative humidity is not sufficient because without complex calculations or a look-up table, assessing absolute humidity reliably is difficult.

2. The levels of pollen did not have a notable impact on the outcomes of outdoor exercise tests in children with suspected or diagnosed asthma. Findings were similar in atopic and non-atopic children. Our findings indicate that, in the case of mildly to moderately allergic children, it may not be necessary to account for fluctuations in pollen counts when interpreting the results of clinical exercise tests or determining their timing. Moreover, our research underscores that absolute humidity holds greater significance in the EIB compared to pollen count. In future studies, it is recommended to include absolute humidity as a variable of interest when investigating the influence of pollen or other outdoor factors.

3. Short-term exposure to $PM_{2.5}$ may raise the occurrence of EIB in preschool children, even when the concentration falls below recent EU, US, and WHO short-term thresholds. However, the observed effect was so marginal that considering $PM_{2.5}$ concentration when interpreting the outcomes of outdoor exercise tests in areas with low pollution levels does not seem necessary. In our analysis, we did not find any associations between EIB incidence and O_3 or NO_2 levels. In multivariable analysis with pollutants and other outdoor air factors, the most significant outdoor air factor associating EIB in our study was again absolute humidity.

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LIST OF ORIGINAL PUBLICATIONS

I Tikkakoski AP, Tikkakoski A, Kivistö JE, Huhtala H, Sipilä K, Karjalainen J, Kähönen M, Lehtimäki L. Association of air humidity with incidence of exercise-induced bronchoconstriction in children. *Pediatric Pulmonology*. 2019;54(11):1830–1836

II Tikkakoski AP, Karjalainen J, Sipilä K, Kivistö JE, Kähönen M, Lehtimäki L, Tikkakoski A. Outdoor pollen concentration is not associated with exercise-induced bronchoconstriction in children. *Pediatric Pulmonology*. 2022;57(3):695–701

III Tikkakoski AP, Tikkakoski A, Sipilä K, Kivistö JE, Huhtala H, Kähönen M, Karjalainen J, Lehtimäki L. Exercise-induced bronchoconstriction is associated with air humidity and particulate matter concentration in preschool children. *Pediatr Pulmonol.* 2023 *Apr*;58(4):996-1003.

IV Tikkakoski AP, Reini M, Sipilä K, Kivistö JE, Karjalainen J, Kähönen M, Tikkakoski A, Lehtimäki L. Association of temperature and absolute humidity with incidence of exercise-induced bronchoconstriction in children. Submitted.

PUBLICATION
I

Association of air humidity with incidence of exercise-induced bronchoconstriction in children

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ORIGINAL ARTICLE: DIAGNOSTIC TESTING

PEDIATRIC PULMONOLOGY WILEY

Association of air humidity with incidence of exercise-induced bronchoconstriction in children

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Abstract

Background: The effects of humidity and temperature on results of free running test in children are not known.

Objective: Assess the relation of outdoor air temperature, relative humidity (RH), and absolute humidity (AH) to airway obstruction in children after free running exercise test.

Methods: We analyzed all exercise challenge tests with impulse oscillometry in children between January 2012 and April 2015 in the Tampere University Hospital. The associations of AH, RH, and temperature of outdoor air with change in airway resistance were studied using regression analysis and by comparing the frequency of exercise-induced bronchoconstriction (increase ≥40% in resistance at 5 Hz) at different levels of temperature and humidity.

Results: Overall, 868 children with reliable results were included (mean age: 5.4 years; range: 3.0-14.1). In regression analysis, the relative change in resistance at 5 Hz after exercise was related to temperature (regression coefficient = −0.223, $P = .020$) and AH (regression coefficient = −0.893, $P = .002$), but not to RH. If absolute air humidity was <5 g/m^3, exercise-induced bronchoconstriction (EIB) occurred in 17.6% of study subjects and at AH levels ≥10 g/m^3, it occurred in 5.9% of study subjects ($P = .008$). In multiple regression analysis comparing the effects of temperature and humidity and adjusting for covariates, only AH was independently associated with change in airway resistance ($P = .009$).

Conclusion: High AH of air is associated with lower incidence of EIB after outdoor exercise test in children. A negative test result at AH ≥10 g/m^3 should be interpreted with caution.

KEYWORDS
asthma, exercise test, exercise-induced asthma, pulmonary function tests

1 | INTRODUCTION

Asthma is one of the most prevalent chronic diseases in children worldwide.[1] Exercise-induced bronchoconstriction (EIB) is a common

Abbreviations: AH, absolute humidity of air; CI, confidence interval; EIB, exercise-induced bronchoconstriction; IgE, immunoglobulin E; IOS, impulse oscillometry; RH, relative humidity; R5, impulse oscillometry resistance at 5 Hz.

Anna P. Tikkakoski and Antti Tikkakoski contributed equally to this study.

feature in asthma, but its mechanisms are not fully understood.[2] There are two major hypotheses explaining the link between exercise and bronchial smooth muscle contraction, the thermal and the osmotic hypotheses. Exercise can cause cooling of the airways due to elevated ventilation, which is followed by rewarming at rest. Thermal hypothesis suggests that these effects initiate reactive hyperemia and vasoconstriction of lung microvasculature, which cause bronchial obstruction.[3,4] The osmotic hypothesis is based on the assumption that during exercise, more water is vaporized from the liquid layer above airway epithelium of the bronchial tree because of elevated ventilation.[5,6] This would cause the airway surface fluid to become hypertonic which, in turn, would activate leukocytes to release inflammatory mediators like leukotrienes, histamine, and tryptase.[7] These would finally cause smooth muscle cell contraction and elevate mucus secretion, leading to obstruction.[6]

Impulse oscillometry (IOS) is a noninvasive and rapid technique to measure lung function requiring only passive cooperation from the patient.[8] IOS is therefore widely used and is a reliable method in diagnosing asthma in children from age 3 to 7 years.[9-12] IOS can be used to detect responsiveness to inhaled beta-2-agonists or bronchial hyperresponsiveness in connection with exercise.[11,13] IOS can be used to measure the airway resistance at different frequencies; most commonly, the numeric evaluation is done at 5 and 20 Hz. A peripheral bronchial obstruction presents as rise in resistance at 5 Hz (R5) and a smaller rise in resistance at 20 Hz.[8,14,15] Change in reactance at 5 Hz (X5) can also be used to evaluate obstruction, but it is not as repeatable in children and is hypothesized to be more in connection with the elastic properties of the lung.[8,16] In addition, other values, such as the reactance and impedance, can be used.[13,17] Previous studies have shown that R5 is the most reliable and reproducible parameter in diagnosing obstruction.[11,13,18-21]

In adults, there are data showing that the probability of EIB is affected by temperature, humidity, and season.[22-26] However, there are only two studies assessing the relation between probability of EIB and outdoor conditions during free running test. One study in children from Ghana using peak expiratory flow rate measurements showed more EIB reactions during dry season than wet season,[27] and another study from Finland showed partial correlation between the change in R5 measured with IOS and outdoor temperature.[11]

Absolute humidity (AH) is the mass of water vapor divided by the mass of dry air in a volume of air at a given temperature. Relative humidity (RH) is the ratio of the current AH and the maximal AH at current temperature. The previous studies assessing the relation between physical properties of inhaled air and probability of EIB have not consistently taken into account temperature and both AH and RH.[11,22-25,27-29] These three factors are closely related (Figure SE1), and their relative importance in provoking EIB is not known. Theoretically, low AH should be the most important factor driving water vaporization from airway epithelial fluid. The aim of this study was to assess if physical factors of outdoor air affect the probability of EIB during outdoor free running exercise testing in children and which factor is the most important.

2 | MATERIALS AND METHODS

2.1 | Study design and subjects

The study was a retrospective chart review. We analyzed all IOSs with exercise challenge test in children between January 2012 and April 2015 in Tampere University Hospital, Finland, who had been tested because of suspicion of asthma or need to assess efficacy of treatment in subjects with persistent asthma. If several exercise tests had been conducted in one child, we included only the first. We collected the known risk factors of asthma from patient records. Those were the results of allergy testing (skin prick test or allergen-specific immunoglobulin E [IgE] levels), atopy and atopic dermatitis, gestational age, birth weight and height, tobacco exposure during pregnancy and childhood, atopy and asthma in parents and siblings, pets at home, respiratory symptoms, number of episodes and hospitalizations of wheezing, other diseases, medications, and whether asthma was diagnosed or not.[30] Major respiratory infection within 4 weeks was considered contraindication for exercise challenge. We additionally calculated the height adjusted to age as *z*-scores.[31]

The city of Tampere lies on 61°30′ northern latitude and has a borderline humid continental climate/subarctic climate (daily mean temperature of −6.9°C in February and +16.9°C in July). The AH of air is mostly under 5 g/m^3 in winter and near 10 g/m^3 in summer (Figure SE2). The physical properties of air (RH, temperature, and pressure) at the time of each exercise test were collected from the register of Meteorological Institute of Finland (license: CC BY 4.0, https://creativecommons.org/licenses/by/4.0/). The meteorological institute had recorded values at 10-minute intervals, so time periods were well matched. AH was calculated using RH, temperature, and pressure. The study was approved by the Ethics Committee of Tampere University Hospital (R15022).

2.2 | IOS measurements and exercise challenge

IOS (Jaeger Gmbh, Würzburg, Germany) was measured according to international recommendations.[19] Trained nurses performed the measurements and commented on the possible technical problems during the measurements, such as opening of the lips, postural problems, physical movement, or holding of breath. To ensure the measurements meet the international quality criteria,[14,16] their technical properties were reviewed retrospectively by trained physicians blinded to the humidity and temperature. The physicians defined the results technically acceptable if they were repeatable and coherence values were within acceptable limits. The exercise challenge was conducted as free running outdoors. Possible symptoms (wheezing, cough, and dyspnea) were recorded. The exercise level was considered sufficient if heart rate (measured with FT4, Polar Ltd, Kempele, Finland) was >85% from calculated maximal value (205−age/2) and the duration of exercise was over 6 minutes.[32] IOS was measured before exercise and 1 to 2, 5, 10, and 15 minutes after exercise. Then children were given 300 µg of salbutamol

(Ventoline evohaler via Babyhaler) and IOS was measured again 15 to 20 minutes after inhalation. IOS reference values were chosen according to the age group of the subject.[13,33] R5 and X5 values were retrieved for analyses from each time point for each subject. The criterion for EIB was increase in IOS R5 ≥40% after exercise compared to baseline.[11,19,21] The criterion for the significant bronchodilation effect was a decrease in R5 ≥ 40% compared to baseline.[11,13,18-20,34]

2.3 | Statistics

Statistical analysis was performed by using R-program version 3.5.1 (R foundation, Vienna, Austria). Seasons were defined as follows: from December to February as winter, from March to May as spring, from June to August as summer, and from September to November as autumn. The χ^2 test, Mann-Whitney *U* test, or *t* test were used, where appropriate, in single parameter comparison between EIB-positive and -negative groups. Relations between physical factors of outdoor air and EIB were studied in two ways. First, continuous variables were used in univariable regression analysis (AH, RH, and temperature against relative change in R5). Multiple linear regression analysis was used to adjust for covariates (age, sex, height, any positive IgE test, and season) and to compare the effects of temperature, RH, and AH on the change in R5. The results were reported as coefficients and confidence intervals (CI). Second, to account for possible linearity problems, the key variables were categorized, and the probability of EIB was calculated at different levels of AH (<5, 5-10, and ≥10 g/m^3), RH (<50, 50-75, and ≥75%) and temperature (<0, 0-10, and ≥10°C). Further, logistic regression was used to adjust for same covariates and to compare the effects of temperature, AH and RH. *P* values under .05 were considered statistically significant.

3 | RESULTS

3.1 | Demographic characteristics of study subjects

Altogether, 926 patients had gone through the exercise test at study period and data from these were collected. After a review of the IOS results, technically unreliable IOS results were excluded (n = 58). IOS was technically reliable in 94% (n = 868), and these subjects were chosen for the main analyses. There were no significant differences in the environmental conditions between accepted and non-accepted IOS tests (AH: 5.3 g/m^3 vs 5.9 g/m^3, *P* = .142; RH: 76% vs 78%, *P* = .375; temperature 4.0°C vs 5.0°C, *P* = .392). Ninety-seven percent of study subjects were 3 to 7 years old (range: 3.0-14.1 years and six subjects were 10 years old or older). In 78 subjects (8.4%), the gestational age was under 37 weeks, of which four subjects (0.4%) were born before 28 weeks. Birth weight was under 2500 g in 56 subjects (6.0%). In the study population, 13.9% had EIB after exercise challenge. Table 1 represents the clinical and demographic characteristics of the study population.

TABLE 1 Subject characteristics in the whole study population (n = 926). Values are mean (SD) or percentages

	Mean, %	SD
Age, y	5.4	1.4
Birth weight, g	3451	631
Gestational age, wk	39.0	2.4
Sex (male; %)	65	
IOS technically reliable (%)	94	

Abbreviation: IOS, impulse oscillometry.

Table 2 presents subject characteristics and IOS values in subjects with and without EIB. Children with EIB were older and taller and had more often IgE-mediated sensitization. There were no significant differences in sex, birth weight, gestational age, use of asthma medication, child body mass index adjusted to adult values, frequencies of parental smoking, and asthma or allergy in parents or siblings. There was no difference in the proportion of subjects who had asthma controller medication paused for at least 14 days before the test. However, the proportion of subjects with pause at least 28 days was significantly lower in children with EIB. Symptoms and wheezing in auscultation during or after exercise were more frequent in children with EIB. There was no difference in the baseline level of X5 between subjects with and without EIB.

3.2 | Association between physical properties of air and EIB

In our study, the mean outdoor temperature during exercise tests was 5.3°C (standard deviation [SD]: 9.2), RH was 78% (SD: 18.2), and AH was 5.8 g/m^3 (SD: 3.1). R5 change after outdoor free running test was related to AH (regression coefficient = −0.89, CI = −1.45 to −0.34, *P* = .002; Figure SE3A) and temperature (regression coefficient = −0.22, CI = −0.41 to −0.04, *P* = .020; Figure SE3B). RH was not significantly related to R5 change (Figure SE3C).

When AH, RH, and temperature were put to the same linear regression analysis, only AH was a statistically significant predictor of change in R5 (regression coefficient = −2.14, CI = −3.91 to −0.37, *P* = .018; Table 3). When the model was further adjusted with age, sex, and height, the relation between AH and change in R5 remained statistically significant (regression coefficient = −2.26, CI = −4.02 to −0.50, *P* = .012; Table SE1). This relation was statistically significant (regression coefficient = −2.67, CI = −4.67 to −0.66, *P* = .009; Table 4) even after adding the presence of any positive IgE test result and season as an independent variable to the model. In addition, if gestational age and birth weight, interaction term of age and height, or height compared to reference values were added, the association remained statistically significant (regression coefficient = −2.68 to −2.74, *P* = .009-.013; Table SE3-E5).

EIB was more frequent at lower levels of AH and occurred in 17.6%, 12.1%, and 5.9% of exercise tests at AH levels of <5, 5 to 10, and ≥10g/m^3, respectively. There was a statistically significant difference in EIB incidence between AH levels of <5 and ≥10 g/m^3

TABLE 2 Subject characteristics and exercise test results in subjects with technically reliable IOS (n = 868) divided into those with or without EIB

	EIB (−)		EIB (+)		
	n = 747	SD	n = 121	SD	*P*
Age, y	5.4	1.4	5.7	1.4	**.013**
Iso BMI (kg/m^2)	23	5	22	4	.419
Height (cm)	113	10	115	10	**.005**
Height of reference values in *z*-score	−0.18	1.09	−0.10	1.00	.424
Sex, male (%)	64		65		.884
Any IgE positive allergy (%)	50		67		**.001**
Atopic dermatitis (%)	40		48		.131
Any asthma medication (%)	54		55		.890
Controller medication pause over 14 d (%)	91		92		.977
Controller medication pause over 28 d (%)	64		49		**.048**
Asthma in either parent (%)	34		39		.335
Parental smoking (%)	28		34		.259
Asthma in siblings (%)	15		14		.806
Heart rate from the calculated maximum (%)	97	6	97	4	.314
R5 baseline (kPa/l^{-1}/s^{-1})	0.84	0.18	0.76	0.16	**<.001**
R5 of reference values (%)	95	17	90	15	**.001**
X5 baseline (kPa/l^{-1}/s^{-1})	−0.31	0.11	−0.30	0.12	.404
R5 change after exercise (%)	9	13	67	31	**<.001**
Marked symptoms in exercise (%)	4		54		**<.001**
Wheezing in auscultation (%)	6		53		**<.001**
Marked bronchodilator response (%)	4		2		.282
Temperature (°C)	5.4	9.2	2.9	8.8	**.005**
Relative humidity (%)	78	18	79	18	.710
Absolute humidity (g/m^3)	6.0	3.2	5.1	2.7	**.002**

Note: Data are either percentages or mean (SD). IOS = impulse oscillometry, ISO BMI = Body mass index adjusted to adult values. Any IgE positive allergy = any positive allergy test in either prick or RAST. Controller medication pause = period without either inhaled corticosteroids or long acting beta-2-agonists before exercise test in the whole study population. R5 = resistance at 5 Hz measured using impulse oscillometry. X5 = reactance at 5 Hz measured using impulse oscillometry. The χ^2 test, Mann-Whitney *U* test, or *t* test were used where appropriate in single parameter comparison between groups.
Bold value indicates *P* < 0.05.

(*P* = .008; Figure 1). Similarly, EIB was more frequent at lower temperatures and occurred in 17.8%, 15.1%, and 9.2% of cases at temperature levels of <0°C, 0°C to 10°C, and ≥10°C. There was a statistically significant difference in EIB incidence between <0°C and ≥10°C temperature levels (*P* = .025; Figure SE4). However, there were no differences in frequencies of EIB at different levels of RH: EIB occurred in 12.0%, 14.0%, and 14.2% of cases at RH levels of <50%, 50% to 75%, and ≥75% (Figure SE5). In logistic regression model with covariates and AH, temperature and RH levels, there was statistically significant difference in EIB incidence only between AH groups <5 and ≥10 g/m^3 (odds ratio = 0.28, CI = 0.09-0.92, *P* = .036; Table SE2).

TABLE 3 Relative change of resistance at 5 Hz explained with absolute humidity, temperature and relative humidity using linear regression. Subjects with technically reliable lung function studies included (n = 868)

Intercept = 18.70	Coefficients	95% Confidence interval	*P*
Absolute humidity (g/m^3)	−2.14	−3.91, −0.37	**.018**
Temperature (°C)	0.46	−0.17, 1.09	.149
Relative humidity (%)	0.11	−0.06, 0.29	.189

Bold value indicates *P* < 0.05.

4 | DISCUSSION

Our results show that AH of the outdoor air is an important physical factor affecting the probability of EIB in children undergoing free running test. Higher AH was associated with lower incidence of EIB, whereas RH, temperature, and season were not significant independent predictors of EIB.

There are not only patient-related factors but also environmental factors that affect the appearance of EIB. International recommendations suggest that exercise testing should be performed with the

TABLE 4 Relative change of resistance at 5 Hz explained with absolute humidity, relative humidity, and temperature using linear regression. Results adjusted with age, sex, season, any IgE test positive, and height (n = 868)

Intercept = −14.6	Coefficient	95% confidence interval	*P*
Absolute humidity (g/m^3)	−2.67	−4.67, −0.66	**.009**
Temperature (°C)	0.54	−0.11, 1.18	.103
Relative humidity (%)	0.13	−0.66, 0.33	.179
Age (y)	0.26	−2.20, 2.72	.837
Sex (male)	−0.89	−4.47, 2.69	.626
Height (cm)	0.25	−0.10, 0.60	.164
Any IgE test positive (yes)	7.13	3.66, 10.61	**<.001**
Season (winter as reference)			
Spring	0.94	−5.50, 7.37	.775
Summer	2.83	−6.20, 11.86	.539
Autumn	2.73	−2.62, 8.09	.316

Abbreviation: IgE, immunoglobulin E.
Bold value indicates *P*<0.05.

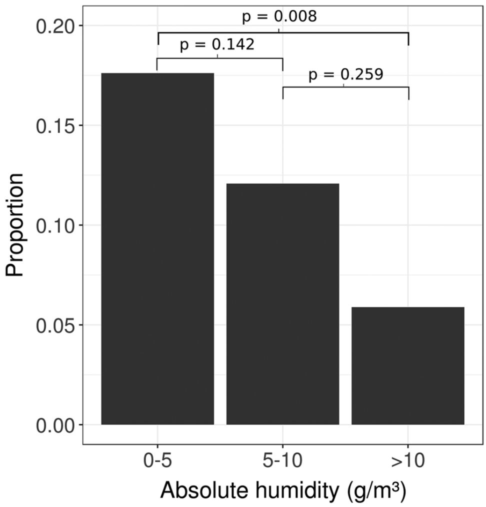

FIGURE 1 Proportion of free running tests with exercise-induced bronchoconstriction reactions at different absolute humidity levels (only reliable impulse oscillometry studies included, n = 868)

inhaled air AH of <10 g/m^3.[3,32] This is based on one relatively small experimental study with 10 asthmatic male subjects published in 1984.[35] In the study, four exercise tests were conducted for each subject: two were conducted randomly in AH of 29 g/m^3 or 9 to 10 g/m^3 at a temperature of 35°C, and two at a temperature of 9°C to 10°C and AH 9 to 10 g/m^3. The result was that temperature was not as significant a factor as AH in affecting the probability of EIB. The authors also noticed that high AH inhibited the development of EIB, suggesting that the osmotic hypothesis is more valid than the thermal hypothesis regarding pathogenesis of EIB. However, although important in advancing our understanding on the basic mechanisms of EIB and relative effects of AH and temperature, this was a quite small study conducted in laboratory conditions using only two fixed levels of AH and temperature.

Although the temperature was not an important factor provoking EIB in the seminal laboratory study, later on, several clinical studies have shown that temperature correlates with the incidence of EIB in free running exercise test outdoors.[11,24,25] Two of the studies also measured RH[24,25] and found that it also correlates with the probability of EIB, but none of them measured and reported AH. Some of the studies have also reported that the probability of EIB varies between seasons.[22,23,26]

To our knowledge, this is the first study in a large clinical sample simultaneously assessing the effects of season, temperature, RH, and AH on the probability of EIB after free running exercise test. Since temperature, RH, and AH are closely interrelated, any of these can be found to correlate with EIB in univariate analysis. However, as RH is defined as the ratio of the partial pressure of water vapor to the equilibrium vapor pressure of water at a given temperature, RH is actually determined by AH and temperature. Since all inhaled air is warmed up to body temperature in airways and saturated with water vaporizing from epithelial lining fluid, the rate of water loss from airways is determined by minute ventilation and AH of inhaled air, not by RH or temperature. The results of this study showed that only AH was independently associated with occurrence of EIB, which fits well the theoretical background of water loss from airways and the osmotic theory of EIB.

Previous studies focusing on physical factors of outdoor air and EIB have been conducted in older children and adults. Childhood asthma and its pathology and phenotypes are different from adult asthma, and, therefore, the study results in adult patients should be verified in children.[36-38] Atopic sensitization defined as positive IgE test to any usual pollen or animal allergen was related with a more marked increase in resistance after exercise test.

IOS measurement of resistance is thought to represent the dissipative mechanical properties of the respiratory system.[8,16] Higher values especially in lower frequencies are related to small airway disease.[39] Reactance is thought to represent the elastic properties of the lung. R5 was statistically significantly lower in subject with EIB. However, the difference was probably clinically insignificant, because the mean difference was only 5%. In addition, there was no difference in baseline X5 values between the groups.

Some previous studies have reported higher prevalence of EIB in different patient populations.[23,25] The lower incidence of EIB in our study probably reflects high suspicion rate for asthma in Finland and that according to the national guideline all cases of asthma in children at least 3-years old should be diagnosed based on objective lung function measures.[40]

Our study has some limitations. Due to the retrospective study design, we could not collect all the clinical data in every patient. The study was also finalized in the year 2015 and most recent results are not included. With longitudinal study design, we could have made better assumptions about causality between AH and EIB. However, a longitudinal study of this magnitude would be laborious and expensive to perform. Though spirometry is gold standard for older children and adults, IOS has been shown to be a reliable tool in asthma diagnostics in small children.[9,11,12,34] Further, asthma can be inactive during certain seasons, but due to retrospective study design we were not able to measure the activity of asthma and symptom frequencies in individual patients at the time of exercise test. The mechanism of EIB is complicated with environmental and host-relating factors affecting it. Removal of all confounding factors would be difficult in practice.

In summary, we have shown in a large clinical sample that AH of outdoor air rather than temperature or RH is an important physical factor that is markedly and independently associated with the probability of EIB in children undergoing free running test. Our study is the first to show the association between AH and EIB in children. Higher AH was associated with lower incidence of EIB. We conclude that AH of outdoor air should be recorded in connection with free running test to guide clinicians with decision making. RH should be left out of the reports because it often creates only confusion and often is not relevant to the result. Our results suggest that a negative free running test at AH of >10 g/m^3 should be interpreted with caution. However, more studies are needed to verify the results.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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PUBLICATION
II

Outdoor pollen concentration is not associated with exercise-induced bronchoconstriction in children

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Outdoor pollen concentration is not associated with exercise-induced bronchoconstriction in children

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Abstract

Background: Free running exercise test outdoors is an important method to diagnose asthma in children. However, the extent of how much exposure to pollens of outdoor air affects the results of the test is not known.

Methods: We analyzed all reliable exercise challenge tests with impulse oscillometry in children ($n = 799$) between January 2012 and December 2014 in Tampere University Hospital. Pollen concentrations at the time of the test were collected from the register of Biodiversity Unit of the University of Turku. We compared the frequency of exercise-induced bronchoconstriction and pollen concentrations.

Results: The analyses were restricted to birch and alder pollen as high counts of grass and mugwort pollen were so infrequent. The relative change in resistance at 5 Hz after exercise or the frequency of exercise-induced bronchoconstriction were not related to alder or birch pollen concentrations over 10 grains/m^3 ($p = 0.125$–0.398). In logistic regression analysis comparing the effects of alder or birch pollen concentrations, immunoglobulin E (IgE)-mediated alder or birch allergy and absolute humidity over 10 g/m^3 only absolute humidity was independently associated with change in airway resistance (odds ratio [OR]: 0.32, confidence interval [CI]: 0.13–0.67, p: 0.006).

Conclusions: In our large clinical sample, outdoor air pollen concentration was not associated with the probability of exercise-induced bronchoconstriction in free running test in children while low absolute humidity was the best predictor of airway obstruction.

KEYWORDS

asthma, exercise test, exercise-induced asthma, pollen, pulmonary function tests

Abbreviations: AH, absolute humidity of air; CI, confidence interval; EIB, exercise-induced bronchoconstriction; FEV1, forced expiratory volume during 1 s; grains per cubic meter, grains/m^3; grams per cubic meter, g/m^3; IgE, immunoglobulin E; IOS, impulse oscillometry; NO, nitric oxide; R5, impulse oscillometry resistance at 5 Hz; RH, relative humidity of air; SD, standard deviation.

1 | INTRODUCTION

Asthma is one of the most common chronic diseases among children and adults worldwide.[1] Asthma is characterized by reversible or variable bronchial obstruction associated with airway inflammation.[2] Exercise-induced bronchoconstriction (EIB) is a common finding in asthma and it can be provoked with exercise challenge testing.[3] Impulse oscillometry (IOS) is a fast and noninvasive lung function test requiring only minimal co-operation from the patient. IOS is therefore a commonly used and reliable lung function test in diagnosing asthma in preschool children.[4,5] It can be used to detect hyperresponsiveness in exercise or reversibility with inhaled beta-2-agonists.[6,7] Airway resistance at 5 Hz (R5) is the most reliable and repeatable parameter in diagnosing obstruction in children under school age.[4]

There are different inflammatory mechanisms in asthma, of which the most common among children is allergic eosinophilic asthma often triggered by airborne allergens.[8] The allergen content of air varies a lot with climate. The key pollination period in Europe is about 6 months from spring to autumn.[9] Pollen from birch and other related trees, for example, alder, are the major tree pollen type in Northern and Central Europe and causes the majority of pollen-related symptoms.[10]

Many previous studies have shown that there is an association between outdoor pollen counts and severity of nasal, conjunctival, and bronchial symptoms in pollen sensitized patients.[11–14] Level of allergen exposure is also related to decreased quality of life.[15] There is evidence that pollen exposure is related to increased airway inflammation in asthmatic patients measured with exhaled nitric oxide (NO) or amounts of inflammatory cells.[16–18] In addition, increased pollen counts in outdoor air correlate with emergency room visits and asthma morbidity in several previous studies.[19–23]

There are contradictory results on how short-term exposure to pollen affects lung function in asthmatics and if outdoor exercise test is affected by pollen count at the time of the test. To our knowledge, there is only one previous study in adults suggesting that pollen season affects the probability of EIB in outdoor exercise test in allergic subjects with asthma.[24] However, there are no previous studies in preschool children on the correlation between lung function measured with IOS and outdoor pollen count during exercise test. The aim of this study was to assess if pollen concentration of air affects the probability of EIB in outdoor exercise test with IOS in preschool children.

2 | MATERIALS AND METHODS

2.1 | Study design and subjects

This study is a retrospective chart review, where all IOSs with exercise challenge test in children between January 2012 and December 2014 at Tampere university hospital, Finland, were analyzed. Children had been tested because of suspicion of asthma or need to assess the efficacy of treatment in subjects with persistent asthma. We included only the first exercise test to the study if several tests had been performed in one child. Additionally, the recognized risk factors of asthma were collected from patient records, which were the results of allergy testing (skin prick test or allergen-specific immunoglobulin E [IgE]-levels), atopy and atopic dermatitis, gestational age, birth weight and height, tobacco exposure during pregnancy and childhood, atopy and asthma in parents and siblings, pets at home, respiratory symptoms, number of episodes and hospitalizations of wheezing, other diseases, medications and whether asthma was diagnosed or not.[25] Contraindication for exercise challenge was significant respiratory infection within 4 weeks. The height adjusted to age as *z*-scores was also calculated.[26] Study was approved by the Ethics Committee of Tampere University Hospital (R15022).

2.2 | Air properties and pollen concentration

The most important pollen particles in Finland are from birch (Betula), grass (in Finland the most common are timothy, meadow fescue, and common meadow-grass) (Poaceae), and alder (Alnus). The allergens of alder and birch resemble each other structurally and immunochemically and cross-reactivity is common. In addition, we collected pollen data of mugwort (Artemisia vulgaris) and hazel (Corylus avellana). Pollen counts expressed as pollen grains per cubic meter (grains/m^3) of air at the time of each exercise test were collected from the register of Biodiversity Unit of the University of Turku, Finland. The Hirst–Burkard pollen trap, from where our data originated, was on the roof of the building next to the outdoor exercise test location.[27]

Relative humidity (RH), temperature, and pressure of air at the time of each exercise test were collected from the register of Meteorological Institute of Finland (license: CC BY 4.0, https://creativecommons.org/licenses/by/4.0/). Absolute humidity (AH) of air was calculated using RH, temperature, and pressure. City of Tampere is located 61°30' northern latitude and has a borderline humid continental climate/subarctic climate, daily mean temperature of −6.9°C in February and +16.9°C in July. AH is mostly under 5 grams per cubic meter (g/m^3) in winter and near 10 g/m^3 in summer.[28]

2.3 | IOS measurements and exercise challenge

IOS (Jaeger Gmbh) was measured by trained nurses according to international recommendations.[29] Possible technical problems during the measurements were noted, such as opening of the lips, postural problems, physical movement, or holding of breath. To ensure the measurements meet the international quality criteria, technical properties of the measurements were reviewed retrospectively by trained physicians blinded to the outdoor air properties and pollen concentration.[30,31] The results were defined technically acceptable if they were repeatable and the coherence values were within acceptable limits. The exercise challenge was performed outdoors as free running. Exercise level was considered sufficient if heart rate (measured with FT4, Polar Ltd) was > 85% of calculated maximal value (205-age/2) and the time of exercise was over 6 min.[32] Possible objective findings and symptoms (such as wheezing, cough, and dyspnea) were documented. IOS was measured before exercise and 1–2, 5, 10, and 15 min after exercise. Children were given 300 µg of salbutamol (Ventoline evohaler via Babyhaler®) after these measurements and IOS was repeated 15–20 min after salbutamol inhalation. Reference values for IOS were chosen according to the age of the child.[33] R5 values were retrieved for analyses from each time point for each subject. The criterion for EIB was increase in IOS R5 ≥ 40% after exercise compared with baseline.[6,28,30] Criterion for significant bronchodilation effect was decrease in R5 ≥ 40% compared with baseline.[7,29,34]

2.4 | Statistics

Statistical analysis was made by using R-program version 4.0.2 (R foundation). Alder and birch pollen concentrations were significantly skewed and thus they were categorized by using thresholds of 10 and 80 grains/m^3 (moderate and high pollen count, respectively).[11] As cross-reactivity between alder and birch pollen is frequent and either of these pollen counts was over the threshold limit in only a small number of cases, they were combined in the analyses. Fisher exact test, χ^2, Mann– Whitney *U* test, or *t* test were used in single parameter comparison between different groups. Logistic regression was used to compare the effects of AH, alder, or birch pollen concentration over 10 grains/m^3 and positive alder or birch IgE on the frequency of EIB. Linear regression was used to compare the effect of AH, alder, or birch pollen concentration over 10 grains/m^3 and positive alder or birch IgE on R5. *p*-values under 0.05 were considered statistically significant.

3 | RESULTS

3.1 | Demographic characteristics of the study subjects

In total 854 subjects had completed exercise test with IOS at study period and we collected data from those. After excluding technically unreliable IOS results (*n* = 55) 799 (94%) children were selected for analyses. Ninety-seven percent of the study subjects were 3–7 years old (range between 3.0 and 14.1 years). The gestational age was under 37 weeks in 72 subjects (9.0%) of whom four subjects (0.5%) were born before 28 weeks. Birth weight was low (<2500 g) in 50 subjects (6.3%).

EIB occurred in 13.9% of the study subjects after exercise challenge. Table 1 presents demographic characteristics of the study population (including only those with reliable IOS) and the frequency of significant pollen concentrations at the time of exercise tests. Fifty-three percent of study subjects were atopic or had at least one IgE-mediated sensitization in prick or radioallergosorbent test testing and 35.8% had alder or birch IgE test positive (Table 1). Grass, mugwort, and hazel pollen concentrations were over 10 grains/m^3 only in 3.0%, 1.0%, and 0.6% of tests, and these pollen counts were excluded from analyses due to the lack of statistical power. Alder and birch pollen concentrations were over 10 grains/m^3 in 4.3% and 11.8% of the cases. Either alder or birch was over 10 grains/m^3 in 15.9% of cases.

TABLE 1 Subject characteristics and frequency of significant pollen concentrations at the date of exercise test in the study population with technically reliable IOS (*n* = 799)

	Mean (SD) or %
Age (years)	5.4 (1.4)
Height (cm)	112.9 (9.6)
Males (%)	64.7
R5 change after exercise (%)	17.1 (26.1)
Exercise-induced bronchoconstriction	13.9
Any IgE test positive	53.2
Alder or birch IgE test positive	35.8
Any asthma controller medication	56.4
Inhaled corticosteroids	54.9
Leukotriene receptor antagonists	11.1
Long-acting beta-agonists	7.0
Antihistamine	9.0
Nasal corticosteroid	2.3
Alder or birch pollen ≥ 10 grains/m^3 (%)	15.9
Birch pollen ≥ 10 grains/m^3 (%)	11.8
Alder pollen ≥ 10 grains/m^3 (%)	4.3
Grass pollen ≥ 10 grains/m^3 (%)	3.0
Mugwort pollen ≥ 10 grains/m^3 (%)	1.0
Hazel pollen ≥ 10 grains/m^3 (%)	0.6

Note: Data are either mean (SD) or percentages.

Abbreviations: IOS, impulse oscillometry; IgE, immunoglobulin E; R5, resistance at 5 Hz.

Subject characteristics according to the presence of EIB are presented in Table 2. Children with EIB were on average slightly older and taller than those without EIB. Parental smoking and the presence of asthma were not different between the groups. R5 before the test was lower, R5 rise after the test was higher, and abnormal auscultation findings were more frequent in subjects with EIB. There were no differences in frequencies of birch or alder pollen counts being at least moderate (>10 grains/m^3) or high (>80 grains/m^3) at the time of the exercise test between subjects with or without EIB.

3.2 | Association between pollen concentrations and EIB

Table 3 presents subject characteristics according to either birch or alder pollen count at the time of test over or under 10 grains/m^3.

TABLE 2 Subject characteristics, exercise test results, and frequencies of moderate or high pollen grain counts in subjects with technically reliable IOS (*n* = 799) divided into those with or without EIB

	EIB (−) *n* = 688	EIB (+) *n* = 111	*p* value
Age (years)	5.4 (1.4)	5.7 (1.4)	0.009
Height (cm)	112.5 (9.5)	115.3 (9.6)	0.003
Iso BMI (kg × m^{-2})	22.8 (4.7)	22.4 (4.5)	0.416
Gender male (%)	64.0	69.4	0.317
Parental smoking (%)	28.5	34.2	0.262
Asthma in either parent (%)	34.4	36.9	0.687
Controller medication pause over 14 days (%)	96.5	97.1	0.974
Alder or Birch IgE test positive (%)	34.4	44.1	0.061
R5 of reference values (% of reference)	95.5 (17.3)	89.3 (15.3)	<0.001
R5 change after exercise (% change)	9.1 (12.8)	66.9 (31.6)	<0.001
Wheezing in auscultation (%)	6.5	51.4	<0.001
Alder or birch pollen ≥ 10 grains/m^3 (%)	16.0	15.3	0.968
Birch pollen ≥ 10 grains/m^3 (%)	12.2	9.0	0.417
Birch pollen ≥ 80 grains/m^3 (%)	9.4	5.4	0.227
Alder pollen ≥ 10 grains/m^3 (%)	3.9	6.3	0.368
Alder pollen ≥ 80 grains/m^3 (%)	0.7	2.7	0.156

Note: Values are either mean (SD) or percentages.

Abbreviations: BMI, body mass index; EIB, exercise-induced bronchoconstriction; R5, resistance at 5 Hz.

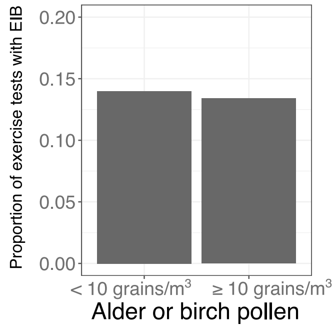

FIGURE 1 Proportion of free running tests with exercise-induced bronchoconstriction reactions at alder or birch pollen counts over 10 grains/m^3 at the date of exercise test (*p*: 0.968)

Subjects who were tested when both pollen counts were low were on average slightly older and taller than those tested when either of the pollen counts were high. Interestingly, those tested when both pollen counts were low were slightly more often sensitized to birch or alder pollen. There was no statistically significant difference in the frequency of EIB between groups tested when either birch or alder pollen count was under or over 10 grains/m^3 (14.0% vs. 13.4%, *p*: 0.968) (Figure 1). Also, there were no differences in baseline R5 (94.5% vs. 95.7%, *p*: 0.450) or mean change in R5 (17.4% vs. 15.5%, *p*: 0.449) (Table 3) between these groups. If the subjects were divided according to either alder or birch pollen being over or under 80 grains/m^3 (720 vs. 79 tests), or when either pollen was measurable (pollen grain count ≥ 1 grains/m^3, 627 vs. 127 tests), there were no significant differences between the groups in either frequency of EIB or change in R5 (*p*: 0.125–0.613).

When we analyzed the subgroup of birch or alder pollen IgE-positive subjects (*n* = 286) there were no differences in the frequency

TABLE 3 Subject characteristics and exercise test results in subjects with technically reliable IOS (*n* = 799) divided according to alder or birch pollen count over or under 10 grains/m^3

	< 10 grains/m^3 *n* = 672	≥ 10 grains/m^3 n = 127	*p* value
Age (years)	5.5 (1.4)	5.0 (1.2)	0.001
Height (cm)	113.3 (9.6)	110.4 (9.0)	0.002
Gender male (%)	65.3	61.4	0.457
Alder or Birch IgE test positive (%)	38.4	22.0	0.001
R5 of reference values (% of reference)	94.5 (17.4)	95.7 (15.9)	0.450
Obstruction before exercise (%)	3.3	2.4	0.792
R5 change after exercise (% change)	17.4 (26.3)	15.5 (24.8)	0.449
Exercise-induced bronchoconstriction (%)	14.0	13.4	0.968
Marked symptoms in exercise (%)	10.9	11.8	0.874
Wheezing in auscultation (%)	12.8	12.6	1.000

Note: Data are mean (SD) or percentage.

Abbreviations: IOS, impulse oscillometry; R5, resistance at 5 Hz.

of EIB (16.7% vs. 21.4%, *p*: 0.711) or mean change in R5 (18.9% vs. 25.7%, *p*: 0.184) between subjects tested at higher and lower pollen concentrations (Table 4). In an additional subgroup analysis, when comparing tests with alder pollen concentration over or under 10 grains/m³, there were no differences in baseline R5 (95% vs. 96%, *p*: 0.702), frequency of EIB (13.6% vs. 20.6%, *p*: 0.368) or mean change in R5 (17.1% vs. 16.1%, *p*: 0.826) between subjects tested at higher and lower alder pollen concentrations. Similarly, when comparing tests with birch pollen concentration over or under 10 grains/m³, there were no differences in baseline R5 (95% vs. 96%, *p*: 0.436), frequency of EIB (14.3% vs. 10.6%, *p*: 0.417) or mean change in R5 (17.4% vs. 14.8%, *p*: 0.370) between subjects tested at higher and lower birch pollen concentrations.

The logistic regression analysis of EIB using alder or birch pollen over 10 grains/m^3, AH ≥ 10 g/m^3 and IgE positive birch or alder allergy showed that only AH ≥ 10 g/m^3 was correlated with EIB (odds ratio [OR]: 0.32, *p*: 0.006) (Table 5). In multivariate linear regression analysis, using alder or birch pollen count over 10 grains/m^3, AH and positive birch or alder IgE-test, the R5 change after outdoor free running test was significantly related only to AH (regression coefficient: −0.8769, *p*: 0.003). When adding the asthma controller medication pause over 14 days, antihistamine medication or nasal corticosteroid as covariate, these did not significantly affect the results (*p* value for all > 0.100) and only AH ≥ 10 g/m^3 correlated significantly with EIB incidence and change in R5 after the test. In multivariate regression analysis of any IgE-mediated sensitization, alder or birch pollen over 10 grains/m^3 and AH ≥ 10 g/m^3 both any IgE-mediated sensitization (*p* < 0.001) and AH ≥ 10 g/m^3 (*p*: 0.003–0.006) were significantly correlated with change in R5 after outdoor free running test. If R5 as a percentage of reference values was modeled using the same cofactors as above, no factor remained statistically significant (*p*: 0.346–0.390).

4 | DISCUSSION

To our knowledge, this is the first study concerning the connection between outdoor air pollen concentration and lung function tested with IOS. This study shows that the ambient outdoor pollen concentration does not significantly affect the incidence of EIB in children under school age undergoing free running test. There was no difference between allergic and nonallergic children.

In our previous study, we showed that AH of the outdoor air was an important physical factor affecting the probability of EIB in children, and higher AH was related to lower incidence of EIB.[28] As well in this study, the effect of AH on the incidence of EIB was higher than the effect of pollen concentration.

IgE-mediated allergy is thought to be a predisposing factor for asthma and high amounts of pollen in the air during exercise test have been thought to predispose for EIB. Several previous studies have shown that the high pollen concentrations are connected to allergic (rhinitis and conjunctivitis) and bronchial symptoms.[13,14] There is also evidence, that when allergen concentrations are high, the emergency department visits due to asthma and allergy increase.[21,23] The inflammation markers of asthma have been shown to increase in allergen exposure in several studies.[17,18] One small Finnish study[24] compared asthmatic men allergic and nonallergic to birch pollen in exercise test in winter season and in the spring pollen season. It was shown that there was more decrease in forced expiratory volume during 1 s (FEV1) after exercise in spring pollen season in allergic patients. Nonallergic patients had more decrease in FEV1 in winter season which was possibly due to different AH levels between seasons, but AH was not measured. However, studies have not managed to show that pollen concentrations have significant correlation with the change of lung function tests in all sorts of pollens.[35–37]

In a Swedish study,[35] grass but not birch pollen count was correlated to decreasing of lung function measured with spirometry during the pollen season. Effects on exercise test were not studied. The correlation was stronger in pollen sensitized children. In our study, high grass pollen counts occurred so rarely that reliable analysis could not be performed. Our data were originated from Hirst–Burkard-collectors located on the roof of the building next to the hospital. Grass pollen grains are heavy molecules and cause high concentrations only locally because they do not spread widely. However, there is no rich grass growth close to the place where the outdoor exercise test was performed, which could have caused local high pollen concentrations. In a Finnish study,[38] grass pollen counts were compared between urban and rural areas in Helsinki metropolitan area. In the study, the grass pollen concentration increased with decreasing urbanity, but pollen concentrations can occasionally increase to such high levels that they can cause allergic symptoms also in most urban areas.

TABLE 4 Subject characteristics and exercise test results in subjects with technically reliable IOS and positive birch or alder IgE test (*n* = 286) divided according to alder or birch pollen over or under 10 grains/m³

	<10 grains/m^3 *n* = 258	≥10 grains/m^3 *n* = 28	*p* value
R5 of reference values (% of reference)	93.8 (17.3)	92.0 (15.9)	0.608
Obstruction before exercise (%)	3.5	3.6	1.000
R5 change after exercise (% change)	18.9 (25.5)	25.7 (27.1)	0.184
Exercise-induced bronchoconstriction (%)	16.7	21.4	0.711

Note: Data are mean (SD) or percentage.

Abbreviations: IgE, immunoglobulin E; IOS, impulse oscillometry; R5, resistance at 5 Hz.

TABLE 5 Incidence of EIB explained with absolute humidity ≥over 10 g/m^3, alder, or birch pollen count over 10 grains/m^3 and alder or birch IgE positive using logistic regression (n = 799)

	OR	95% CI	*p* value
Alder or birch pollen ≥ 10 pcs/m^3	0.90	0.43–1.73	0.754
AH ≥ 10 g/m^3	0.32	0.13–0.67	0.006
Alder or birch IgE positive	1.46	0.94–2.27	0.093
Alder or birch pollen ≥ 10 pcs/m^3 × Alder or birch IgE positive	1.78	0.51–5.83	0.346

Abbreviations: CI, confidence interval; EIB, exercise-induced bronchoconstriction; IgE, immunoglobulin E; OR, odds ratio.

There are some limitations in our study. Because of the retrospective study design, all the clinical data of every subject were not in our reach. However, major confounders were covered. For example, the data about asthma or allergy medication and IgE-mediated sensitization were widely available in the patients. There are also minor confounders which could not be collected due to retrospective study design, such as asthma and allergy symptoms around the time of the exercise test and information about pollen concentrations in the subject´s living area before the exercise test. The climatic data were recorded every 10 min close to the study site, but pollen data were measured as daily concentrations. That may cause inaccuracy in pollen measurements in the exact time of exercise tests. Better assumptions about causality between pollen counts and EIB could be made with a longitudinal study design, but a longitudinal study of this extent would be difficult and expensive to conduct. There are several environmental and host-relating factors affecting EIB and the mechanism of EIB is complicated. Because of that, elimination of all confounding factors is impossible in practice. A priori power calculations were difficult since there was no previous definition for a clinically significant difference in the probability of EIB or the change in R5 to be used in a study like this. If 10% difference in EIB incidence or 10% change in R5 was considered clinically significant between low and moderate/high alder or birch pollen groups, our study had a statistical power higher than 80%. Due to the lack of power, we could not study the effects of some allergens, most notably grass pollen.

In our study population, there are children whose asthma is diagnosed based on symptoms of repeatable obstructions and wheezing in very young age. Among these children, the prognosis of asthma is very good and spontaneous recovery is very common and allergies do not often develop. This may be a confounding factor in our study. Some of these children develop later allergies and allergic asthma, and because of our retrospective study design, we cannot analyze them as a separate subgroup. In clinical practice, there are patients who have bronchial symptoms in allergen exposure in pollen season but not in other seasons. If patients have difficult symptoms, they are treated during the pollen season based on symptoms and the exercise test is often scheduled outside allergy season. Due to elimination of the most severely symptomatic patients, there might be a selection bias in our study population. This study might be underpowered for studying the effects of markedly high pollen concentrations which patients can encounter in daily lives but not necessarily on the time of the exercise test.

In conclusion, in this large clinical real-life cohort, pollen concentrations did not significantly affect the results of outdoor exercise tests in children with suspected or diagnosed asthma. Our results suggest that there is no need to consider variations in pollen counts in mildly or moderately allergic children when interpreting clinical exercise test results or planning timing of the test. Furthermore, our results demonstrate that AH is a more significant factor in EIB than pollen count. In future studies, AH should be considered when studying the effects of pollen or other outdoor factors.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

Anna P. Tikkakoski, Jussi Karjalainen, Juho E. Kivistö, Kalle Sipilä, Mika Kähönen, Lauri Lehtimäki, Antti Tikkakoski: Conceptualization; Mika Kähönen, Lauri Lehtimäki, Kalle Sipilä: Resources; Anna P. Tikkakoski, Jussi Karjalainen, Juho E.Kivistö, Kalle Sipilä, Lauri Lehtimäki, Antti Tikkakoski: Methodology; Anna P. Tikkakoski, Antti Tikkakoski: Data collection; Statistical analysis; writing - original draft; Jussi Karjalainen, Juho E. Kivistö, Kalle Sipilä, Mika Kähönen, Lauri Lehtimäki: writing - review and editing.

DATA AVAILABILITY STATEMENT

Data not available due to legal restrictions.

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PUBLICATION III

Exercise-induced bronchoconstriction is associated with air humidity and particulate matter concentration in preschool children

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Exercise-induced bronchoconstriction is associated with air humidity and particulate matter concentration in preschool children

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Abstract

Background: Long-term exposure to air pollution is connected to asthma morbidity in children. Exercise-induced bronchoconstriction (EIB) is common in asthma, and the free running test outdoors is an important method for diagnosing asthma in children. It is not known whether momentary air pollution exposure affects the results of outdoor exercise tests in children.

Methods: We analyzed all reliable exercise challenge tests with impulse oscillometry in children ($n = 868$) performed between January 2012 and April 2015 at Tampere University Hospital. Pollutant concentrations ($PM_{2.5}$, NO_2, and O_3) at the time of the exercise test were collected from public registers. We compared the pollutant concentrations with the proportion and severity of EIB and adjusted the analyses for air humidity and pollen counts.

Results: Pollution levels were rarely high (median $PM_{2.5}$ 6.0 μg/m^3, NO_2 12.0 μg/m^3, and O_3 47.0 μg/m^3). The relative change in resistance at 5 Hz after exercise did not correlate with O_3, NO_2 or $PM_{2.5}$ concentrations (p values 0.065–0.884). In multivariate logistic regression, we compared the effects of $PM_{2.5}$ over 10 μg/m³, absolute humidity (AH) over 10 g/m³ and alder or birch pollen concentration over 10 grains/m³. High (over 10 g/m^3) AH was associated with decreased incidence (OR 0.31, p value 0.004), and $PM_{2.5}$ over 10 μg/m³ was associated with increased incidence (OR 1.69, p value 0.036) of EIB.

Conclusions: Even low $PM_{2.5}$ levels may have an effect on EIB in children. Of the other properties of air, only AH was associated with the incidence of EIB.

KEYWORDS
air pollution, air quality, asthma, exercise test, exercise-induced bronchoconstriction, impulse oscillometry, particulate matter, pulmonary Function Tests

Abbreviations: AH, absolute humidity of air; CI, confidence interval; EIB, exercise-induced bronchoconstriction; grains/m^3, grains per cubic meter; IgE, immunoglobulin E; IOS, impulse oscillometry; IQR, interquartile range; ISO-BMI, child body mass index corresponding to adult values; NO_2, nitrogen dioxide; O_3, ozone; $PM_{2.5}$, particulate matter diameter under 2.5 μm; R5, impulse oscillometry resistance at 5 Hz; RH, relative humidity; TRAP, traffic-related air pollution; WHO, World Health Organization; μg/m^3, μg per cubic meter.

1 | INTRODUCTION

Asthma is a heterogeneous disease characterized by chronic airway inflammation and bronchial hyperreactivity. It is one of the most common long-term diseases among children, and its burden is increasing globally.[1] In addition to genetic factors,[2] environmental exposure also increases the risk for asthma.[3] The Global Burden of Disease report lists outdoor air pollution as a marked cause of death and disability globally, contributing an estimated more than 3 million premature deaths per year.[4] Children are more vulnerable than adults to the adverse effects of air pollution because their lungs and immune systems are still developing, their breathing rate is higher, they spend more time outdoors and they are also physically more active.[5] It has been estimated that 13% of asthma incidence in children globally and 33% in Europe may be due to traffic-related air pollution (TRAP) or other outdoor pollution.[1,6,7]

The most important air pollutants, nitrogen dioxide (NO_2) and particulate matter with aerodynamic diameter < 2.5 µm ($PM_{2.5}$), are emitted, for example, in motor vehicle exhaust, and $PM_{2.5}$ is also emitted during wood combustion. Ozone (O_3) is formed when nitrogen oxides and volatile organic compounds react with ultraviolet radiation. Several previous studies have addressed the long-term and short-term adverse effects of air pollution in children. Long-term exposure to air pollution during the prenatal period or during childhood is associated with lower lung function as measured by spirometry,[8–10] impulse oscillometry (IOS)[11] or forced oscillation techniques[12] and with persistent wheezing and asthma morbidity.[13–15] In asthmatic children, acute exposure to air pollution may induce oxidative stress in the airways and is connected to lower lung function and to exacerbation of asthma.[16,17]

In 2005, the World Health Organization (WHO) updated threshold limits[18] for various air pollutants based on information about the adverse effects of different pollutant levels, and these air quality guidelines were further updated in September 2021.[19] Nevertheless, safe long-term threshold values are difficult to define, and proper threshold values for momentary exposure are not described for all pollutants. However, recent studies have shown that adverse health effects occur at pollutant levels below the former long-term threshold values and that these adverse effects are dose-dependent.[20,21] The long-term threshold value defined by the WHO in 2005 for NO_2 was 40 $\mu g/m^3$ mean annual concentration, whereas the 1-h threshold for acute exposure was 200 $\mu g/m^3$. The updated long-term thresholds defined in 2021 for NO_2 are markedly lower, 10 $\mu g/m^3$ for mean annual concentration and 25 $\mu g/m^3$ for mean daily (24-h) concentration. Correspondingly, threshold values for $PM_{2.5}$ were previously 10 $\mu g/m^3$ mean annual concentration and 25 $\mu g/m^3$ mean daily concentration, and in the recent guideline they are markedly lower, 5 and 15 $\mu g/m^3$, respectively. The threshold for shorter acute exposure is not described. The 8-h threshold value of 100 $\mu g/m^3$ for O_3 remained the same in the recent guidelines as in 2005, but a mean threshold value of 60 $\mu g/m^3$ for the peak season was also defined.[18,19]

Exercise is thought to increase the effects of air pollution on airways in various ways. Elevated minute ventilation increases the total amount of inspired particles. Shifting from nose-breathing to mouth-breathing at exercise decreases the absorption of pollutants in the upper airway. However, increased ventilation causes turbulence of the inhaled air, increasing the absorption of particles in the upper airway. Exercise-induced bronchoconstriction (EIB) is a frequent finding in asthma and can be triggered by exercise challenge testing.[1] In preschool children, IOS is generally used as a lung function test to detect hyperresponsiveness during exercise or reversibility with inhaled beta-2-agonists. It is the most important diagnostic test when asthma is suspected in this age group.[22,23] IOS is a rapid, noninvasive and reliable lung function test that requires minimal cooperation from the patient and is thus commonly used.[24] To our knowledge, there are no previous studies of the connection between short-term exposure to ambient outdoor air pollution and EIB measured with IOS in children.

Our aim in this study was to assess whether the concentrations of various air pollutants at the time of the free-running exercise test conducted outdoors affect the probability or severity of EIB and whether pollen concentrations and absolute humidity (AH) modify the results.

2 | MATERIALS AND METHODS

2.1 | Study design and subjects

We analyzed the IOS results obtained during all outdoor free-running exercise challenge tests conducted in children between January 2012 and April 2015 at Tampere University Hospital, Finland. Exercise tests were performed due to suspicion of asthma or a need to evaluate the efficacy of treatment in children with persistent asthma. If several exercise tests were conducted in a single child, we included only the results of the first test. We collected information from patient records on possible asthma diagnoses before or after exercise testing and known risk factors for asthma, including the results of allergy testing (skin prick test or allergen-specific immunoglobulin E [IgE] levels), atopy and atopic dermatitis, gestational age, birth weight and height, tobacco exposure during pregnancy and childhood, atopy and asthma in parents and siblings, pets at home, respiratory symptoms, number of episodes of and hospitalizations for wheezing, and other diseases and medications.[25] We also calculated the children's heights adjusted for age as z scores and child body mass index corresponding to adult values (ISO-BMI).[26] The study was a retrospective chart review of subjects studied under normal clinical routine due to suspicion of asthma. The Ethics Committee of Tampere University Hospital approved the study (R15022).

2.2 | Air properties and pollution concentration

In this study, we observed the momentary outdoor air concentrations of NO_2, O_3, and $PM_{2.5}$ at the time of each exercise test. Information on these concentrations was collected from the database maintained by the city of Tampere environmental protection unit; those data are based on measurements performed at a location approximately 1 km from the exercise test site. We used 1-h measures covering the hour in which the exercise test was conducted. We also collected data on the most important pollen particles in Finland, birch (*Betula*) and alder (*Alnus*). Pollen counts at the time of each exercise test were collected from the register of the Biodiversity Unit of the University of Turku, Finland, and were expressed as pollen grains per cubic meter (grains/m^3) of air. The Hirst-Burkard pollen trap from which our data were originated was located on the roof of a building located next to Tampere University Hospital and the exercise test site.[27] Pollen data from Tampere were available only for the period between January 2012 and December 2014.

Relative humidity (RH), temperature and air pressure at the time of each exercise test were collected from the register of the Meteorological Institute of Finland (license: CC BY 4.0, https://creativecommons.org/licenses/by/4.0/). The AH of air was calculated based on RH, temperature and pressure.

The city of Tampere has a borderline humid continental climate/subarctic climate and is located at 61°30′ northern latitude. It experiences daily mean temperatures of −6.9°C in February and +16.9°C in July. The AH of the air is usually under 5 g/m^3 in winter and close to 10 g/m^3 in summer.[28] Approximately 240,000 inhabitants live in Tampere, and the level of air pollution is relatively low. However, during winter, occasional temperature inversions and peaks in emissions due to residential heating may cause a significant increase in air pollution close to the ground.

2.3 | IOS measurements and exercise challenge

IOS (Jaeger Gmbh) was measured according to international recommendations.[29] Technical problems during the measurements, such as opening of the lips, postural problems, physical movement or holding of breath, were noted. To confirm that the measurements met the international quality criteria, the technical properties were reviewed retrospectively by trained physicians who were blinded to the pollution concentrations and properties of the outdoor air.[30] If the measurements were repeatable and the coherence values were within acceptable limits, the results were considered technically acceptable. The majority of the exercise tests involved running between two points outdoors. In younger subjects with cooperation difficulties, some tests also included other high-intensity activities such as kicking and fetching a soccer ball. Experienced nurses monitored the heart rate and the intensity of the exercise. The intensity of the exercise was considered sufficient if maximal heart rate (measured with FT4, Polar Ltd) was >85% of the calculated maximal value (205-age/2) or if clear symptoms of obstruction appeared.[31] The nurses controlled that the heart rate was greater than 85% of the maximal value for at least 6 min and that the exercise was continuous. Possible symptoms during exercise (such as wheezing, cough and dyspnea) were documented. IOS was measured before exercise and 1–2, 5, 10, and 15 min after exercise. As specified in the provocation protocol, all the children were given 300 µg of salbutamol (Ventoline evohaler via Babyhaler®), and IOS was measured 15–20 min after salbutamol inhalation. Height-dependent reference values were used for IOS.[23] R5 values were retrieved for each subject at each time point. An increase in R5 of ≥40% after exercise compared to baseline was the criterion for EIB.[22,29,30] The criterion for a significant bronchodilation effect was a decrease in R5 of ≥40% compared to baseline.[23,29]

2.4 | Statistics

Statistical analysis was performed using R-program version 4.0.2 (R Foundation). The distributions of NO_2 and $PM_{2.5}$ concentrations were markedly skewed; therefore, pollutant concentrations were categorized as under or over specific threshold levels. Commonly defined thresholds for momentary exposure were not suitable for our data because measured concentrations did not exceed the thresholds. Instead, we used the following thresholds: 25 and 40 µg/m^3 for NO_2, 60 and 100 µg/m^3 for O_3 and 10 and 25 µg/m^3 for $PM_{2.5}$. The distributions of pollen concentrations were also markedly skewed; therefore, they were categorized using threshold values for a moderate pollen level of 10 grains/m^3.[27] The allergens produced by alder and birch resemble each other structurally and immunochemically, and cross-reactivity is common. Alder and birch pollen counts were ≥10 grains/m^3 in only a small number of cases; thus, these counts were combined in the analysis (if either the alder or the birch pollen count was ≥10 grains/m^3, the combined count was defined as true). Subjects with missing values were excluded from the analysis. Logarithmic transformation was used for skewed variables (NO_2 and $PM_{2.5}$). The χ^2 test, the *t* test, Pearson's correlation or Fisher's exact test was used in single-parameter comparisons between different groups and correlations. Linear multivariate regression was used to analyze the effects of O_3, pollen concentration, AH and age on the change in R5. Logistic multivariate regression was used to analyze the effects of $PM_{2.5}$, pollen concentration, AH and age on the incidence of EIB. *p* values under 0.05 were considered to indicate statistical significance.

3 | RESULTS

3.1 | Demographic characteristics of the study subjects and mean pollutant levels

Overall, 926 subjects completed the IOS exercise test during the study period. Following a review of the IOS results, tests that were considered unreliable (n = 58) were excluded, and only technically reliable IOS results, n = 868 (94%), were used in the analyses. Pollutant and pollen data had variable numbers of missing values (n = 14–69), and these were excluded from the analyses case-by-case. Table 1 presents the clinical and demographic characteristics of the study population. Ninety-seven percent of the study subjects

TABLE 1 Subject characteristics and frequency of exercise-induced bronchoconstriction in the study population with technically reliable IOS ($n = 868$)

	Mean (SD) or %
Age (years)	5.4 (1.4)
Height (cm)	113.0 (9.6)
Males (%)	64.2
R5 change after exercise (% from baseline)	17.4 (25.9)
Exercise induced bronchoconstriction (%)	13.9
Any IgE test positive (%)	52.2
Significant bronchodilator effect (%)	3.8
Physician diagnosed asthma (%)	73.6

Note: R5 change is reported as percentage change compared to baseline (mean and SD). Criterion for exercise-induced bronchoconstriction was ≥40% increase in R5 after exercise compared to baseline. Criterion for significant bronchodilation effect was ≥40% decrease in R5 compared to baseline. Asthma diagnosed by physician was collected from patient records and is a combination of diagnoses before or after exercise test (missing values not included).

Abbreviations: IgE, immunoglobulin E; IOS, impulse oscillometry; R5, impulse oscillometry resistance at 5 Hz.

were 3–7 years old (range 3.0–14.1). Gestational age at birth was under 37 weeks in 73 subjects (8.4%), of whom four (0.5%) were born before the 28th gestational week. In 53 subjects (6.1%), birth weight was low (<2500 g). After exercise challenge, EIB occurred in 13.9% of the study subjects. Median (interquartile range) pollutant concentrations during the exercise tests were 6.0 $\mu g/m^3$ (4.4–8.3 $\mu g/m^3$) for $PM_{2.5}$, 12.0 $\mu g/m^3$ (8.0–18.0 $\mu g/m^3$) for NO_2 and 47.0 $\mu g/m^3$ (33.0–61.0 $\mu g/m^3$) for O_3.

Table 2 presents subject characteristics and IOS results for subjects with or without EIB. Children with EIB more often had IgE-mediated sensitization, and they were on average older and taller. There were no statistically significant differences between groups in ISO-BMI, gender, parental smoking, asthma or allergy in parents or siblings, birth weight, gestational age or use of asthma or allergy medications. Abnormal auscultation findings and symptoms during or after exercise were more frequent in children with EIB. Children with EIB had lower R5 at baseline. There was no difference between groups in the prevalence of bronchial obstruction before exercise.

3.2 | Relation between pollutant levels and degree of airway obstruction after exercise

There was no correlation between the O_3 concentration and the change in R5 (Pearson's correlation coefficient $r = 0.064$, confidence interval [CI] [−0.00 to 0.13], p value 0.065). The logarithmically transformed $PM_{2.5}$ or NO_2 concentrations also had no correlation with the change in R5 ($r = 0.018$, CI [−0.05 to 0.09], p value 0.603 and $r = 0.005$, CI [−0.06 to 0.07], p value 0.884, respectively). We chose

TABLE 2 Subject characteristics and exercise test results in subjects with technically reliable IOS ($n = 868$) divided into those with and without EIB

	EIB (−) $n = 747$	EIB (+) $n = 121$	
	Mean (SD) or %		p Value
Age (years)	5.4 (1.4)	5.7 (1.4)	0.013
Height (cm)	112.6 (9.6)	115.2 (9.5)	0.005
Iso-BMI ($kg*m^{-2}$)	22.8 (4.8)	22.5 (4.4)	0.419
Gender Male (%)	64.0	65.3	0.861
Parental smoking (%)	28.4	33.9	0.259
Asthma in either parent (%)	33.9	38.8	0.335
Controller medications pause over 14 days (%)	96.8	97.3	0.979
Any IgE test positive (%)	49.8	66.9	0.001
R5 percent of reference value	95.4 (17.1)	89.8 (15.5)	0.001
Obstruction before exercise (%)	22.2	24.8	0.610
R5 change after exercise (% from baseline)	9.4 (12.8)	66.8 (31.3)	<0.001
Wheezing in auscultation (%)	6.3	52.9	<0.001
Physician diagnosed asthma (%)	69.6	95.4	<0.001

Note: Any IgE test positive = any positive allergy test in either prick or RAST. Controller medication pause = period without either inhaled corticosteroids, Leukotriene receptor antagonists or long-acting beta-2-agonists before exercise test. Asthma diagnosed by physician was collected from patient records and is a combination of diagnoses before or after exercise test (missing values not included). The χ^2 test or t-test was used where appropriate in single parameter comparison between groups.

Abbreviations: Any IgE test positive, any positive allergy test in either prick or RAST; Controller medication pause, period without either inhaled corticosteroids; EIB, Exercise-induced bronchoconstriction, IOS, impulse oscillometry, ISO-BMI, Body mass index corresponding to adult values, R5, resistance at 5 Hz measured using impulse oscillometry.

the O^3 concentration for further multivariate analysis because it showed the highest correlation with the change in R5. Thus, a multivariate linear regression analysis of the change in R5 was performed using O_3, AH, alder or birch pollen ≥ 10 grains/m^3 and age as independent variables. Only AH (coefficient −0.84, CI [−1.43 to −0.25], p value 0.005) but not O_3 (coefficient 0.08, CI [−0.02 to 0.18], p value 0.115) was significantly related to the change in R5.

3.3 | Relation between pollutant levels and probability of EIB

EIB occurred in 13.1% of cases when the $PM_{2.5}$ concentration was <10 $\mu g/m^3$ and in 18.9% of cases when the $PM_{2.5}$ concentration was

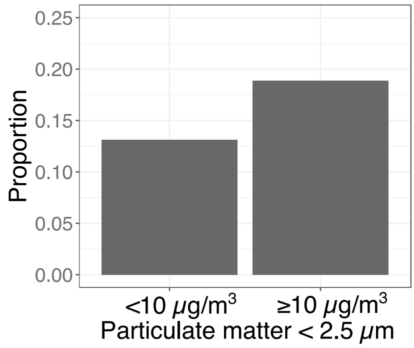

FIGURE 1 Proportion of free running tests with exercise-induced bronchoconstriction reactions at different particulate matter < 2.5 µm levels (only reliable impulse oscillometry studies included, $n = 868$, p Value 0.096, χ^2 test)

TABLE 3 Proportions of subjects with pollutant levels above established thresholds at the time of the exercise test in subjects with technically reliable IOS ($n = 868$) divided into those with and without EIB

	EIB (−) $n = 747$	EIB (+) $n = 121$	p Value
$NO_2 \geq 25$ µg/m^3 (%)	11.9	16.5	0.198
$NO_2 \geq 40$ µg/m^3 (%)	3.3	3.3	1.000
$O_3 \geq 60$ µg/m^3 (%)	28.3	30.5	0.701
$O_3 \geq 100$ µg/m^3 (%)	0.4	0	1.000
$PM_{2.5} \geq 10$ µg/m^3 (%)	16.2	22.9	0.096
$PM_{2.5} \geq 25$ µg/m^3 (%)	0.3	0	1.000

Note: The χ^2 test or Fisher's exact test was used.

Abbreviations: EIB, Exercise-induced bronchoconstriction, IOS, impulse oscillometry, NO2, Nitrogen dioxide, O3, Ozone, PM2.5, Particulate matter < 2.5 µm.

≥10 µg/m^3, but the difference was not statistically significant (p value 0.096, Figure 1). The concentration of $PM_{2.5}$ exceeded 10 µg/m^3 in 22.9% and 16.2% of cases with and without EIB, respectively, but the difference was not statistically significant (p value 0.096). There were no statistically significant differences between the EIB-positive and EIB-negative groups in the percentage of cases in which other pollutant concentrations exceeded the predefined thresholds (Table 3).

As $PM_{2.5} \geq 10$ µg/m^3 showed the largest difference between the EIB-positive and EIB-negative groups, we performed a logistic multivariate regression analysis ($n = 799$) of the incidence of EIB using $PM_{2.5} \geq 10$ µg/m^3, AH ≥ 10 g/m^3, alder or birch pollen ≥ 10 grains/m^3 and age as independent variables. We found that AH ≥ 10 g/m^3 (OR 0.31, CI [0.13–0.65], p value 0.004) and $PM_{2.5} \geq 10$ µg/m^3 (OR 1.69, CI [1.02–2.75], p value 0.036) were significantly related to the incidence of EIB (Table 4). In the subgroup of allergic subjects ($n = 425$), the results were similar; in that subgroup, both AH ≥ 10 g/m^3 (OR 0.41, CI [0.16–0.89], p value 0.036) and $PM_{2.5} \geq 10$ µg/m^3 (OR 1.83, CI [1.00–3.28], p value 0.045) were statistically significant predictors of EIB.

TABLE 4 Results of a multivariate regression analysis on the incidence of EIB explained with $PM_{2.5} \geq 10$ µg/m^3, absolute humidity ≥ 10 g/m^3, alder or birch pollen count ≥ 10 grains/m^3 and age ($n = 799$)

	OR	95% CI			p Value
AH ≥ 10 g/m^3	0.31	0.13	–	0.65	0.004
$PM_{2.5} \geq 10$ µg/m^3	1.69	1.02	–	2.75	0.036
Alder or birch pollen ≥ 0 grains/m^3	1.09	0.60	–	1.89	0.755
Age	1.18	1.02	–	1.35	0.017

Note: Age is included as a covariate and its results are reported only for reference.

Abbreviations: AH, Absolute humidity; CI, Confidence interval; EIB, Exercise-induced bronchoconstriction; OR, Odds ratio; PM2.5, Particulate matter < 2.5 µm.

4 | DISCUSSION

In our large set of real-life data, we found that higher momentary $PM_{2.5}$ concentration was associated with a higher incidence of EIB in the outdoor free-running test in preschool children. In our study, the average pollutant levels were relatively low, and we did not find NO_2 or O_3 levels to be associated with the presence or severity of EIB. In our previous publications, we reported the effects of other physical and biological characteristics of the outdoor air on EIB incidence in preschool children. We found that lower air humidity was associated with a higher probability of EIB and more severe EIB but that pollen concentrations were not related to EIB.[27,28] As higher AH was associated with a lower probability of EIB, negative test results obtained under conditions of high AH should be interpreted with caution. Additionally, in this study conducted under the climatic conditions of a small city in Finland, when pollutant levels were considered, the strongest predictor of EIB among the properties of air was AH. Since all inhaled air is warmed to body temperature in the airways and saturated with water vaporizing from the epithelial lining fluid, the rate of water loss from airways is determined by the minute ventilation and the AH of the inhaled air. The results of this study showed that AH was independently associated with the occurrence of EIB, a finding that is very consistent with the theories that explain water loss from airways and the osmotic theory of EIB. In addition to low AH, $PM_{2.5}$, even at relatively low concentrations, may be an independent predictor of EIB.

To our knowledge, this is the first study to use real-life clinical data to evaluate the relationship between outdoor pollution levels at the time of exercise testing and the incidence of EIB measured using IOS. In one small previous study, exposure to a number of different pollutants did not affect the exercise test results measured with spirometry in schoolchildren with chronic respiratory symptoms, but air pollution was connected to poorer spirometry results at baseline.[32] The exercise tests performed in that study used a bicycle ergometer, possibly decreasing the sensitivity of the test. In addition, RH rather than AH was reported in that study; this may be another confounding factor, since low AH is the most important factor that triggers EIB. Another recent study showed that greater exposure to NO_2 was associated with greater lung-function reduction postexercise measured by spirometry in children in New York City.[33] The association was attenuated when adjustment was made for exhaled nitric oxide, suggesting that inflammation may mediate the association between NO_2 and EIB.

In our study, $PM_{2.5}$ was the only pollutant that was found to be associated with the probability of EIB. One previous study has shown that exposure to elevated indoor $PM_{2.5}$ levels on the previous day is related to higher airway resistance and airway inflammation at rest.[34] Higher levels of $PM_{2.5}$ have also been reported to reduce lung function in schoolchildren[35] and to be associated with emergency department visits due to respiratory causes in children.[16] The mechanisms underlying the effects of $PM_{2.5}$ on the respiratory system are not completely understood. $PM_{2.5}$ may increase airway oxidative stress and inflammation and decrease small airway function.[17,36] Animal and human studies suggest that inflammation due to PM exposure is due to phenotypic differentiation of Th2 and Th17 cells.[37,38] These mechanisms may increase bronchial hyperresponsiveness during exercise.

In a previous study,[16] $PM_{2.5}$, but not other pollutants such as NO_2, O_3 and PM_{10}, was associated with emergency visits due to respiratory causes in children. The concentrations noted in that study were lower than the upper limits recommended by the European Union. Additionally, in our study, only $PM_{2.5}$ concentrations, not NO_2 or O_3 concentrations, were associated with EIB. However, decreased lung function in adults has been reported previously in connection with much higher concentrations of NO_2 and O_3 than measured in our study.[39,40] Thus, the NO_2 and O_3 levels that were present when the tests reported in our data were performed may have been so low that an association with EIB would be unlikely.

Recent evidence from epidemiological studies shows that long-term exposure to air pollution, even at relatively low concentrations, may cause morbidity. It has been estimated that approximately 92% of pediatric asthma cases that may be attributable to NO_2 exposure occurred in areas in which the annual average NO_2 concentration did not exceed the WHO 2005 threshold.[6] Furthermore, long-term exposure to air pollution, even at exposure levels near the current limits, has been reported to be associated with adult-onset asthma.[20] Exposure to air pollution in childhood may have long-term effects on lung function, since exposure to TRAP during early life has been shown to correlate with lung function measured using IOS in adolescence.[9]

Further studies are needed to determine safe threshold values for pollutants so that regulations regarding air pollution concentrations can be made based on scientific evidence. In our study, $PM_{2.5}$ concentrations of $10\,\mu g/m^3$ or more had a small effect on the incidence of EIB after short-term exposure during the exercise test. Our measured values were recorded hourly, but there are no known limits for hourly concentrations of $PM_{2.5}$. The European Union directive-based annual threshold value for $PM_{2.5}$ is higher than the health-based limit recently recommended by WHO ($25\,\mu g/m^3$ vs. $5\,\mu g/m^3$). The hourly $PM_{2.5}$ concentration reported in our data exceeded the European Union's guideline value only twice during the study period. In California, a positive effect of improved air quality on children's lung function has already been detected.[41] Additionally, the United States Environmental Protection Agency's current annual guideline of $12\,\mu g/m^3$ for $PM_{2.5}$ is higher than the recent WHO guideline.

Our study has some limitations. Better assumptions about causality between outdoor air properties and EIB could have been made with longitudinal study design. However, a longitudinal study of this scale would be difficult and costly to perform. Although spirometry is the gold standard test of lung function in older children and adults, IOS has been shown to be a reliable instrument for detecting asthma in preschool children who are not able to undergo spirometry.[22,24] Removing all confounding factors and collinearity between pollution concentrations and other physiological properties of the outdoor air, for example, air humidity, would be difficult in practice. Additionally, we were not able to study the effects of high concentrations of pollutants other than $PM_{2.5}$. We did not find significant associations between pollutant levels and EIB in simple group comparisons or univariate regression analyses, only in multivariate analyses. Replication studies are needed to verify the results. The strengths of the study are a large sample of real-world data, technical validation of the measurements, and reliable data on pollutants and other characteristics of the outdoor air at the time of each exercise test.

In conclusion, momentary exposure to $PM_{2.5}$ may increase the incidence of EIB in preschool children, even when the concentration is below recent EU, US, and WHO short-term thresholds. However, the observed effect was so small that it is probably not necessary to take the $PM_{2.5}$ concentration into account when interpreting the results of free-running outdoor exercise tests at low pollution levels. O_3 and NO_2 levels were not associated with the incidence of EIB in our analysis. The most significant physical characteristic of the outdoor air that affected the incidence of EIB in our study is AH, and this factor should be taken into account when planning exercise test timetables and interpreting exercise test results. Further studies are needed to verify the results presented here and to study the effects of higher pollution concentrations.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Research data are not shared.

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PUBLICATION
IV

Association of temperature and absolute humidity with incidence of exercise-induced bronchoconstriction in children

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