

ENVIRONMENTAL DETERMINANTS

Endotoxin Exposure and Childhood Wheeze and Asthma: A Meta-Analysis of Observational Studies

ANGELICO MENDY, M.D., M.PH.,¹ JANVIER GASANA, M.D., PH.D.,^{1,*} EDGAR RAMOS VIEIRA, PH.D.,² ERICK FORNO, M.D., M.PH.,³ JASMIN PATEL, M.B.B.S.,¹ PRASAD KADAM, M.PH.,¹ AND GILBERT RAMIREZ, DR.P.H.¹

¹Robert Stempel School of Public Health, Florida International University, Miami, FL, USA.

²Department of Physical Therapy, Florida International University, Miami, FL, USA.

³Division of Pediatric Pulmonology, Department of Pediatrics, University of Miami, Miami, FL, USA.

Background. Exposure to endotoxin has been widely investigated as a potential factor for asthma and associated symptoms in children with different results. To clarify a potential relationship, we performed the present meta-analysis to integrate the results of studies examining the association of endotoxin exposure with wheeze and asthma in children. **Methods.** A search for relevant studies and reviews was conducted in MEDLINE, Highwire, CINAHL, and The Cochrane Library databases. Adjusted odds ratio (OR) with corresponding 95% confidence interval (CI) for endotoxin exposure and wheeze or asthma were retrieved and pooled to generate summary effect estimates in STATA 11.1. **Results.** Nineteen studies were included in the meta-analysis. The summary estimates suggested that endotoxin was positively associated with wheeze in infants and toddlers (meta-OR: 1.48, 95% CI: 1.10–1.98), but negatively related to asthma in school-aged children (meta-OR: 0.82, 95% CI: 0.69–0.97 for endotoxin concentration and 0.68, 95% CI: 0.50–0.93 for endotoxin load). **Conclusions.** Based on the studies evaluated, endotoxin is a risk factor for wheeze in younger children, but a protective factor for asthma in older children. Thus, this study supports the “hygiene hypothesis.”

Keywords asthma, children, endotoxin, hygiene hypothesis, meta-analysis, wheeze

INTRODUCTION

Asthma affects approximately 300 million individuals in the world and is the most common chronic illness in children (1). In 2007, the Centers for Disease Control and Prevention evaluated to 13% the percentage of American children who had been diagnosed with the disease in their lifetime. According to the National Center for Health Statistics, the number of drug prescriptions for childhood asthma has increased two and a half times from 1994 to 2004 (2–4).

The differences in asthma prevalence between developing and developed countries (>10% in Europe and North America, 3–7% in Asia, 4–8% in Africa) suggest that environmental factors play an important role in the development of the allergic disease (5–7). To explain these disparities, bacterial components such as endotoxin—a substance of the gram negative bacteria outer membrane—have been widely investigated. It has been reported that endotoxin could protect against asthma, but might increase the risk of asthma symptoms in children (8–10). However, epidemiological studies have yielded conflicting results. While some found a protective effect of endotoxin (11–13), a majority of articles did not report significant results (14–21). Tavernier et al. (22),

for their part, found endotoxin exposure to be higher in asthmatic children in comparison to non-asthmatic ones.

We performed the present meta-analysis to examine the relationship between endotoxin and childhood wheeze and asthma, given the inconsistency of existing individual epidemiological study results. To our knowledge, this is the first meta-analysis on the topic, and it adds in a unique manner to the existing literature by quantitatively synthesizing observational studies on endotoxin and wheeze and asthma in children. This study evaluates the relationship of endotoxin exposure with the prevalence of wheeze and asthma in children, and will help increase the effect size precision of a probable association.

MATERIALS AND METHODS

Data Sources and Searches

To estimate the odds of wheeze and asthma associated with endotoxin exposure, we searched MEDLINE, Highwire, CINAHL, and The Cochrane Library using the keywords “endotoxin asthma* child*” and “endotoxin wheez* child*” up to March 2011. The search strategies in the different databases were as follows:

- In MEDLINE: (“endotoxins” [MeSH] OR “endotoxins” [All] OR “endotoxin” [All]) AND (“asthma” [MeSH] OR “asthma” [All]) AND (“child” [MeSH] OR “child” [All] OR “children” [All]), using the keywords “endotoxin,” “asthma,” and “children”; and (“endotoxins” [MeSH] OR “endotoxins” [All] OR

*Corresponding author: Janvier Gasana, M.D., Ph.D., Robert Stempel School of Public Health, Florida International University, 1200 S.W. 8 St. University Park, HLS II 595, Miami, FL 33199, USA; Tel: +305-348-0371; Fax: +305-348-4901; E-mail: gasanaj@fiu.edu

“endotoxin” [All]) AND (“respiratory sounds” [MeSH] OR “respiratory” [All]) AND (“sounds” [All] OR “respiratory sounds” [All] OR “wheeze” [All]) AND (“child” [MeSH] OR “child” [All] OR “children” [All]), using the keywords “endotoxin,” “wheeze,” and “children.”

- In Highwire: “endotoxin* wheez* child* (all words in title or abstract)” and “endotoxin* asthma* child* (all words in title or abstract).”
- In CINAHL: “endotoxin*” AND “wheez*” AND “child*,” “endotoxin*” AND “asthma*” AND “child*.”
- In The Cochrane Library: “endotoxin* wheez* child* (title, abstract, or keywords)” and “endotoxin* asthma* child* (title, abstract, or keywords)”.

In addition to the databases search, we screened the list of references in the previous reviews and selected papers to identify additional relevant studies.

Study Selection

Studies that met the following criterion were included in the meta-analysis: observational studies published in English that reported or contained data to calculate the odds of childhood wheeze and/or asthma related to endotoxin exposure with corresponding 95% confidence interval (CI). All references were independently screened by two authors (A.M. and J.P.) according to the selection criteria, and discrepancies on whether to include a study were resolved by consensus.

Data Extraction

Using a data extracting sheet, two of the authors (A.M. and J.P.) independently retrieved from each of the articles data on study design, age of participants, adjusted odds ratio (OR) with corresponding 95% CI, and covariates adjusted for (Table 1). The adjusted OR for wheeze and/or asthma reported for the highest exposure category compared with the lowest was selected and used for this meta-analysis. If the same population was used in different studies included in the article, data from the most recent one were considered in the analysis.

Disagreements on data extraction were resolved through discussion and, if needed, by consulting a third author (J.G.). Agreement between the reviewers on study selection was determined using the Cohen kappa statistic. This test is an inter-rater agreement measure for categorical data. It evaluates how much agreement is present (observed) discounting how much agreement would be expected by chance alone (expected).

Quality Assessment

The following methodological quality components of the included studies were assessed: size of the population, study design, definition of exposure to endotoxin, assessment of wheeze and asthma, potential confounders' measure and control, statistical methods used in individual papers. The quality of the reporting was evaluated using the Strengthening the Reporting of Observational Studies

in Epidemiology (STROBE) statement checklist for cohort, case-control, and cross-sectional studies, version 4 (35). The studies were classified into the following three categories—A, more than 80% of STROBE criteria fulfilled; B, 50–80% of STROBE criteria fulfilled; and C, if less than 50% of STROBE criteria were fulfilled.

Analysis

OR and 95% CI were presented or computed per category exposure of outcome. The standard error (SE) was estimated based on the formula $[\log(\text{upper limit of the 95\% CI}) - \log(\text{lower limit of the 95\% CI})]/3.92$, deducted from the calculations of $\log(\text{upper limit of the 95\% CI}) = \text{natural logarithm (OR)} + 1.96(\text{SE})$ and $\log(\text{lower limit of the 95\% CI}) = \text{natural logarithm (OR)} - 1.96(\text{SE})$. ORs were combined by means of a weighted average to generate summary estimates using a random effect model. We tested for heterogeneity in results across studies using a Q statistic. The statistical significance was defined as $p < .10$ instead of the conventional level of .05 because of the low test power. The I^2 was determined to quantify the percentage of variation across studies attributable to heterogeneity (36). Studies were identified as outliers if the 95% CI of their effect size did not overlap with the estimate line of the summary estimate on the forest plot (37). Outliers were excluded one by one and the heterogeneity was retested until the p -value was superior to 10%. All analyses were performed using STATA (Version 11; Stata Corporation, College Station, TX, USA).

RESULTS

The steps of our literature search are shown in Figure 1. Briefly, a total of 430 articles were identified. The search yielded 257 articles from PubMed, 27 from Highwire (including 7 workshop poster presentations), 144 from CINAHL, and 2 from The Cochrane Library. No additional article was identified from articles' references screening. After full text screening, 19 studies met our inclusion criteria (11–13, 16, 18, 19, 21, 23–34, 38). There was complete agreement on 196 of the 206 articles after title and abstract screening (inter-reader agreement: $k = 81.6\%$) and on 50 of the 54 articles after full text screening (inter-reader agreement: $k = 84.1\%$).

Studies' Characteristics and Definitions

The studies that were included in our meta-analysis were published between 2001 and 2011. They were conducted mainly in Europe (11, 12, 16, 18, 19, 23, 26, 33) and North America (13, 21, 24, 28, 29, 30, 32), but also in New Zealand (27, 34), Palestine (25), and Latin America (38). Ten studies were cohort (12, 13, 23, 26, 27, 29, 30, 32, 33, 38), eight cross-sectional (11, 16, 18, 19, 21, 24, 25, 34), and one case-control (28). Five studies fulfilled more than 80% of the STROBE criteria (12, 16, 26, 27, 30), and the others, between 50 and 80%.

TABLE 1.—Characteristics of studies on endotoxin and wheeze or asthma included in the meta-analysis.

References	Study design	Sample size	Children age	Endotoxin measured	Partition	Variables adjusted for	Reporting quality
Bolte et al. (16)	Cross-sectional	1942	2 years	Concentration	Quartiles	Age, gender, study region, breastfeeding, number of siblings, parental education, mite allergens, respiratory infections, smoking during pregnancy, parental atopy	A
Braun-Fahrlander et al. (11)	Cross-sectional	812	6–13 years	Load and concentration	Quartiles	Age, sex, study area, family atopy, parental education, number of siblings, farming exposure	B
Carlsten et al. (23)	Cohort	380	7 years	Concentration	Endotoxin units	Sex, family history of asthma, race, firstborn, maternal education, residence	B
Campo et al. (24)	Cross-sectional	532	1 year	Concentration	Quartiles	Age, sex, family atopy, smoker in the house, day-care attendance, number of siblings, pet exposure, cold in past year	B
Douwes et al. (12)	Cohort	810	1–4 years	Load	Tertiles	Age, sex, region, parental education, indoor tobacco, children in household	A
El-Sharif et al. (25)	Cross-sectional	110	6–12 years	Concentration	Tertiles	Age, sex, residence, dust mite, other atopy symptoms	B
Gehring et al. (26)	Cohort	1884	6–12 months	Concentration	Quantiles	Age, sex, residence, family atopy, dust mite, exposure to tobacco, parental education, pet exposure, breastfeeding	A
Gehring et al. (18)	Cross-sectional	1756	5–14 years	Load	Standard deviations	Age, sex, residence, family atopy, parental education, pet exposure	B
Gehring et al. (19)	Cross-sectional	840	9–12	Concentration and load	Quartiles	Age, sex, gender, parental atopy, cat and dust mite allergen, smoking	B
Gillespie et al. (27)	Cohort	881	3–15 months	Concentration	Quartiles	Age, people in house, pet exposure, having a damp and musty smell, dampness or mold in the bedroom, open fireplace; maternal smoking; type of flooring in the bedroom	A
Lawson et al. (28)	Case-control	322	6–18 years	Concentration and load	Mean	Age, sex, environmental tobacco smoke	B
Perzanowski et al. (29)	Cohort	301	12 months	Concentration and load	Mean	Sex, maternal asthma, ethnicity, and tobacco smoke exposure in the house	B
Phipatanakul et al. (30)	Cohort	498	12 months	Concentration	Quartiles	Age, residence, family atopy, household income	A
Rullo et al. (31)	Cohort	104	30 months	Concentration	Mean	Age, sex, breastfeeding, low respiratory infections	B
Ryan et al. (32)	Cohort	483	3 years	Concentration	Mean	Age, family atopy, sex, environmental tobacco smoke, mold, breast feeding, traffic, particles	B
Sordillo et al. (13)	Cohort	377	7 years	Concentration	Median	Age, sex, residence, race/ethnicity, family atopy	B
Thorne et al. (21)	Cross-sectional	557	<18 years	Concentration and load	Quartiles	Age, residence, race/ethnicity, dust mite exposure, presence of smoker in house, number of children in house, household income	B
Tischer et al. (33)	Cohort	690	6 years	Load	Quartiles	Sex, parental atopy, parental education, pet ownership, breastfeeding, season of dust sampling	B
Wickens et al. (34)	Cross-sectional	494	7–10 years	Concentration and load	Mean	Age, sex, residence, race/ethnicity, family atopy, smoking in house, siblings, parental education, pet exposure, open fire place in house, lower respiratory infections, antibiotic use	B

In all included studies, endotoxin was extracted from dust collected by vacuuming children's bed, bedroom floor, and/or living room/family room floor. The endotoxin content was measured by kinetic Limulus Amoebocyte Lysate test. Endotoxin results were expressed as endotoxin units per gram or milligram of dust (endotoxin concentration) or endotoxin units per square meter of surface area sampled (endotoxin load). The timing of exposure to endotoxin was classified into early (exposure to endotoxin during the first year of life) and current exposure. Endotoxin concentration and load were partitioned into tertiles, quartiles, or quintiles.

Any wheeze was defined as at least one episode of wheeze in the past 12 months reported or as an answer "yes" to the question "Has your child had wheezing or whistling in the chest in the past 12 months?" from the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire (39). Recurrent wheeze was defined as at least two episodes of wheeze in the past 12 months as for the question "How many attacks of wheezing has your child had in the last 12 months?" Asthma was reported by parents as an answer "yes" to the question "has your child ever had asthma?" from the ISAAC questionnaire or "has a doctor ever told

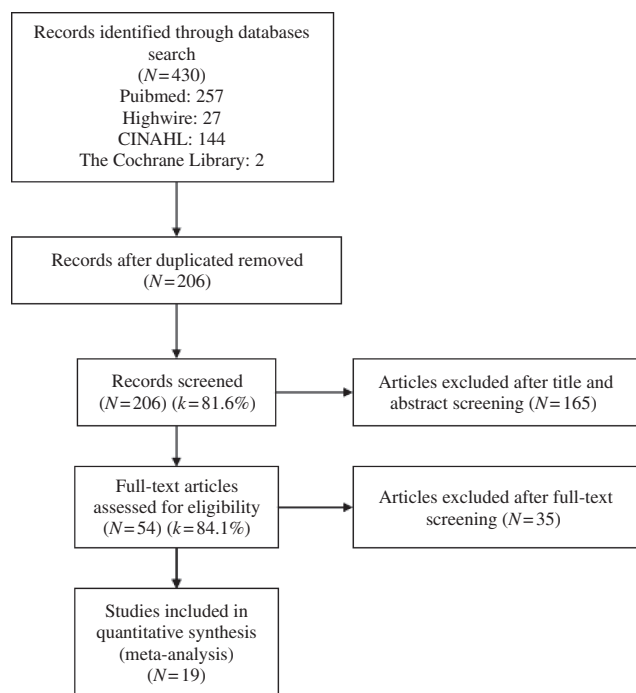


FIGURE 1.—Flow diagram of studies selection.

you your child had asthma?” from the American Thoracic Society—Division of Lung Diseases questionnaire (31).

Regarding age, children were classified as infants between 1 and 12 months of age; as toddlers, between 1 and 3 years of age; as preschoolers, between 3 and 5 years of age; and as school-aged children, if older than 5 years.

Sordillo et al. (13) and Phipatanakul et al. (30) used the same population from The Epidemiology of Home Allergens and Asthma Study, an ongoing birth cohort study recruited in Boston, MA. Campo et al. (24) and Ryan et al. (32) used the same population from the Cincinnati Childhood Allergy and Air Pollution Study, a prospective birth cohort recruited in the greater Cincinnati metropolitan area. Tischer et al. (33) included in their study a population from the birth cohort study on “Influences of lifestyle-related factors on the immune system and the development of allergies in childhood” (LISA) used by Bolte et al. (16) and Gehring et al. (26). However, the study populations were used only once in our analyses.

Endotoxin and Any Wheeze

Eleven studies examined the odds of any wheeze associated with endotoxin exposure (11, 12, 19, 21, 25–30, 34). Among the studies, seven reported an effect size for current endotoxin exposure (11, 19, 21, 25, 28, 29, 34) and five for early endotoxin exposure (12, 26, 27, 29, 30).

On a set of homogeneous studies (Q test p -value = .13, I^2 = 46.2%), exposure during the first year of life to endotoxin, expressed in concentration per amount of dust sampled, was positively associated with any wheeze in infants and toddlers (OR: 1.48, 95% CI: 1.10–1.98) (Figure 2).

Endotoxin and Recurrent Wheeze

Seven studies examined the odds of recurrent wheeze associated with endotoxin exposure (12, 13, 16, 23, 24, 32, 38). Among the studies, four reported an effect size for current endotoxin exposure (13, 16, 23, 24) and four for early endotoxin exposure (12, 13, 32, 38). Current or early endotoxin exposure was not associated with recurrent wheeze in children (Figure 3).

Endotoxin and Asthma

Nine studies examined the odds of asthma associated with endotoxin exposure (11–13, 18, 19, 21, 23, 33, 34). Among the studies, eight reported an effect size for current endotoxin exposure (11, 13, 18, 19, 21, 23, 33, 34) and one for early endotoxin exposure (12).

On a set of homogeneous studies (Q test p -value = .46, I^2 null), current endotoxin exposure, expressed in concentration per amount of dust sampled, was negatively associated with asthma in school-aged children (OR: 0.82, 95% CI: 0.69–0.97) (Figure 2).

The studies that examined current endotoxin exposure, expressed in load per surface area sampled, and asthma were significantly heterogeneous (Q test p -value = .04, I^2 = 60.2%) (Figure 3). After exclusion of the outlier study by Tischer et al. (33) (Dutch) (which effect size and 95% CI did not overlap with the summary estimate line in Figure 4), the homogeneity significantly improved (Q test p -value = .49, I^2 null) and current endotoxin load was negatively associated with asthma in school-aged children (OR: 0.68, 95% CI: 0.50–0.93) (Figure 5).

DISCUSSION

The results of our meta-analysis indicate that endotoxin exposure is a risk factor for wheeze in infants and toddlers, but protects against asthma in school-aged children. Consistent with our findings with regard to endotoxin and wheeze in infants and toddler, endotoxin has been found to induce airway inflammation and upregulation of pro-inflammatory cytokines, resulting in bronchoconstriction and consequently in wheeze. The pro-inflammatory properties of endotoxin have been described in both asthmatic and non-asthmatic children and among adults in occupational settings (10, 17, 27, 40, 41). However, several studies did not find an association between endotoxin and wheeze in children older than 5 years (11, 19, 28, 34). For their part, El-Sharif et al. (25) surprisingly found an inverse correlation for both atopic and non-atopic wheezes.

We did not find a relationship between endotoxin and recurrent wheeze, like most of the literature which fails to report significant results (12, 13, 16, 17, 24, 32, 38). Only Park et al. (42) and Litonjua et al. (13, 23, 33, 43, 44), consistent with endotoxin inflammatory properties, found endotoxin associated with the risk for repeated wheeze during the first year of life.

Coherent with the negative relationship of endotoxin with asthma in school-aged children found in our study, an increasing number of articles published in the recent

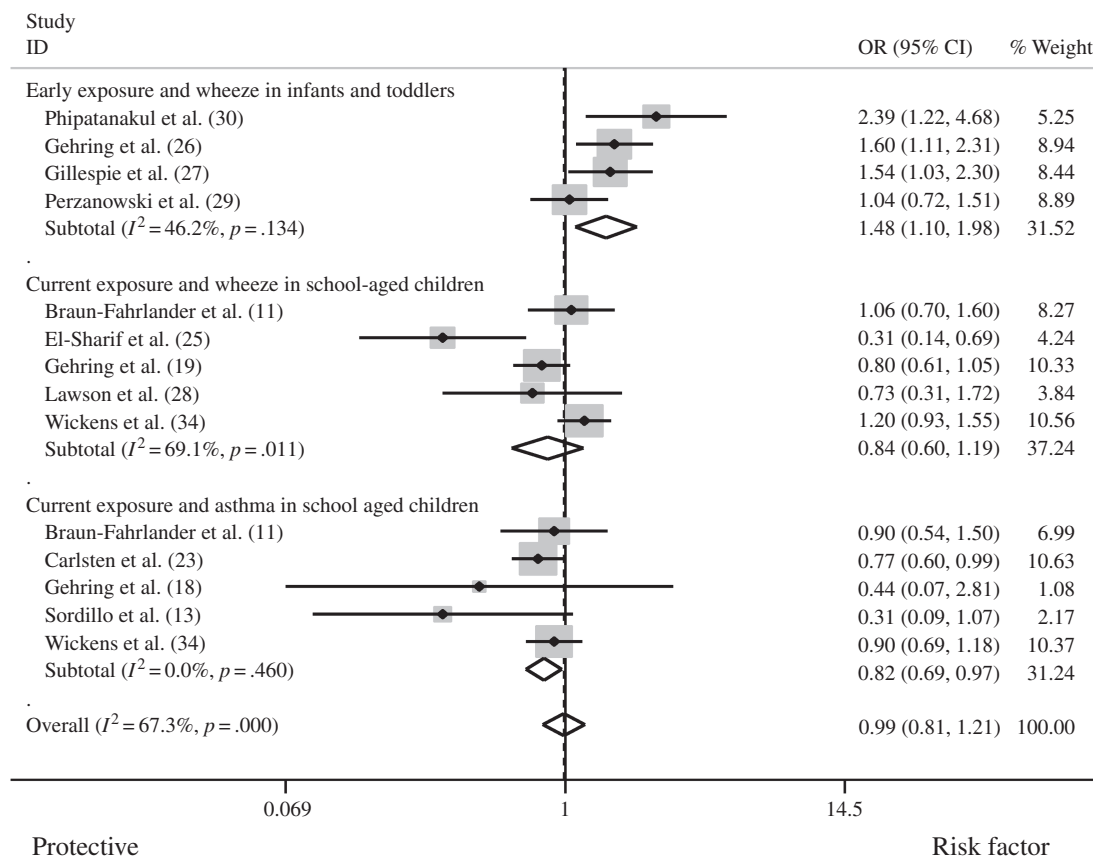


FIGURE 2.—Forest plot for the association of endotoxin exposure, expressed in endotoxin concentration per amount of dust sampled, with any wheeze and asthma in toddlers, infants, and school-aged children using a random effect model.

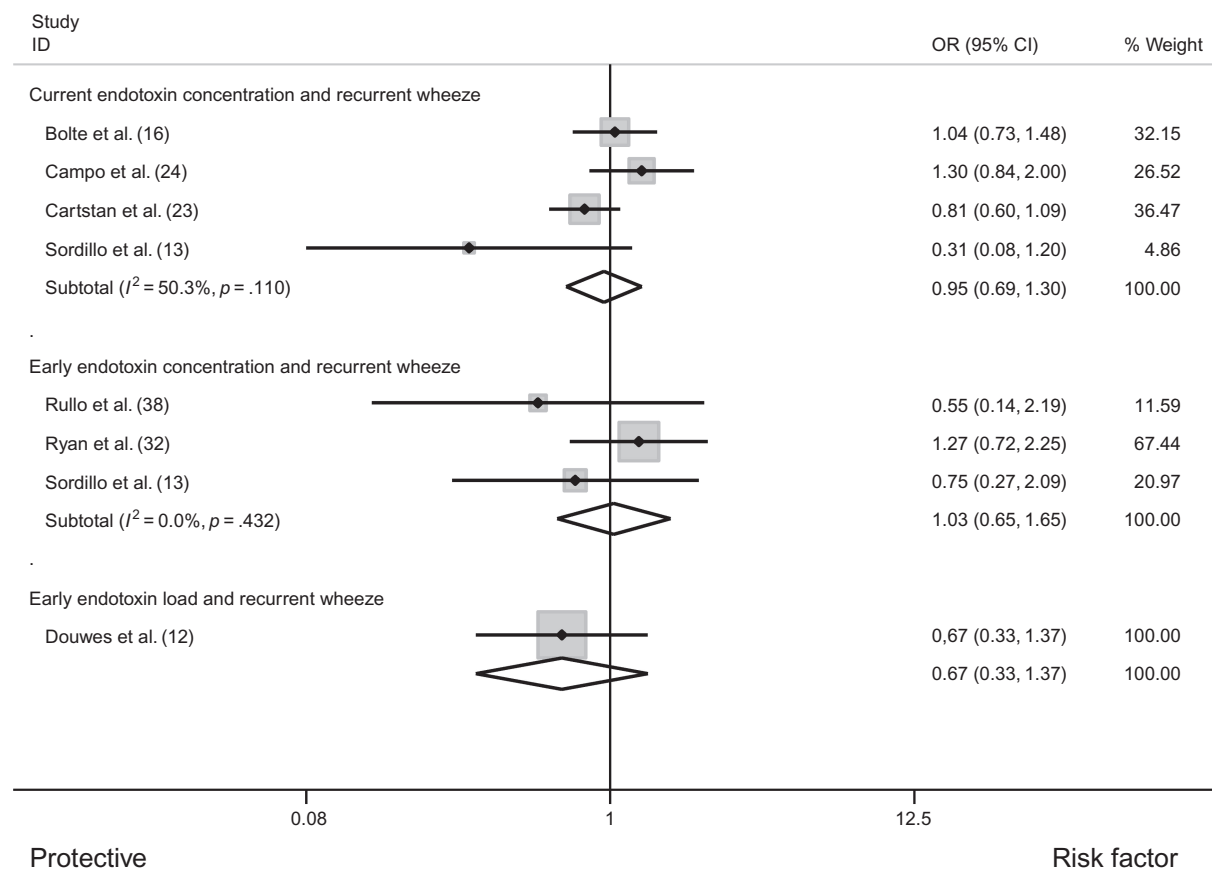


FIGURE 3.—Forest plot for the association of current and early endotoxin exposure with recurrent wheeze in children using a random effect model.

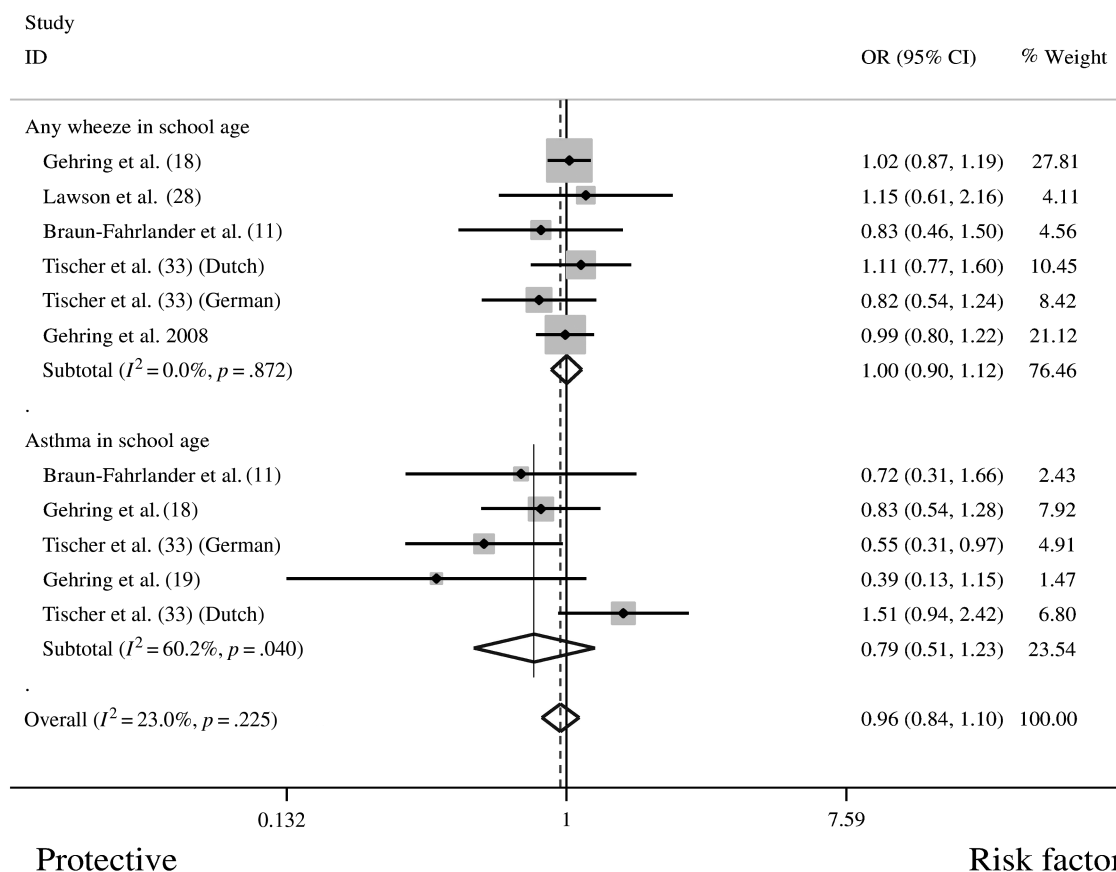


FIGURE 4.—Forest plot for the association of endotoxin exposure, expressed in endotoxin load per surface area sampled, with any wheeze and asthma in school-aged children using a random effect model. The effect size reported by Tischer et al. (33) (Dutch), in which 95% confidence interval does not overlap with the summary estimate line, was identified as outlier for the relationship between endotoxin and asthma.

years are suggesting a protective effect of endotoxin against the allergic disease (13, 23, 33, 43, 44). However, the mechanism is still not fully understood. Possible explanations indicate that endotoxin is a potent inducer of interleukin-12 and interferon gamma, which downregulate the production of T-lymphocyte helper 2 (Th2) cells involved in the development of allergic diseases (45). Yet, it is still unclear whether asthma is simply a Th2 disease or if Th1 also plays a role in its development (46, 47). The stronger protective effect against asthma found for current endotoxin load per area sampled in comparison to current endotoxin concentration per amount of dust sampled can be explained by the fact that endotoxin load is a better predictor of total endotoxin exposure. It indicates both the component concentration and the amount of dust in the sampled area (48). Our meta-analysis did not include many longitudinal studies on asthma in school-aged children exposed to endotoxin in their first year of life, but it is possible that the children with current endotoxin exposure have had a prolonged exposure prior to the assessment.

It has been proposed that the effect of endotoxin on the development of wheeze and asthma is the result of an interaction between genetic determinants and environmental factors (49). Single nucleotide polymorphisms (SNP) in genes *CD14* and Toll-like receptors

(especially *TLR4*) are known to modulate the response to endotoxin. A recent study (50) reported that the association between endotoxin and wheeze is significantly modified by SNPs -260 C/T (rs2569190), -1247 T/C (rs2569191), -1721 A/G (rs2915863) in gene *CD14*, and SNP (rs10808798 T/C) in gene *MD2* (v-myb-dependent gene 2, which variably regulates the lipopolysaccharides binding and clustering to TLR 4) among farmers (50, 51). Other investigators (52) examined the relationship between a promoter polymorphism in gene *CD14* (*CD14* -159 C to T), and noted the CC genotype at -159 of *CD14* correlated with an increased risk of non-atopic wheeze but reduced risk of allergic sensitization. Zambelli-Weiner et al. (53) evaluated the role of *CD14* C-260T polymorphism in the association between house dust endotoxin and asthma or other allergic disease in a population of African descent in the Barbados. They found the *CD14* TT genotype is protective against asthma for low house dust endotoxin ($<44,000$ EU/m²) but it is a risk factor for asthma in individuals with high house dust endotoxin exposure ($>44,000$ EU/m²). A protective effect of missense mutations in gene *TLR4* (TLR4-A896G and TLR4-C1196T) on severe asthma due to a decrease in endotoxin response has also been suggested (54).

In relation to the methodological quality of studies included in our meta-analysis, they all included large

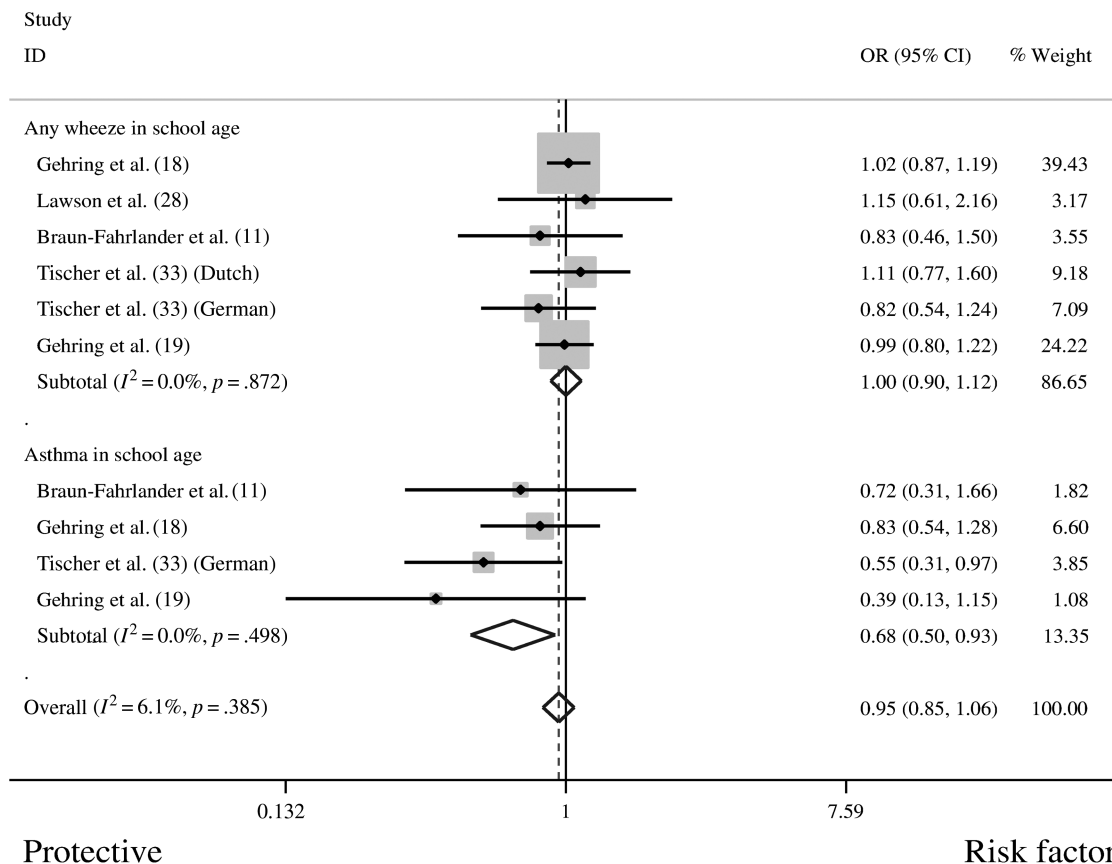


FIGURE 5.—Forest plot for the association of endotoxin exposure, expressed in endotoxin load per surface area sampled, with any wheeze and asthma in school-aged children using a random effect model after exclusion of outlier effect size reported by Tischer et al. (33) (Dutch).

sample sizes, in many cases representative of a city population. With regard to the study design, 8 of the 19 were cohort studies, allowing the assessment of temporality between the respiratory diseases and endotoxin exposure. Exposure to endotoxin was measured in one single day in almost all the studies, and may have not been representative of the household. The sampling period was not specified in many studies, and it has been suggested that fall and winter seasons could be associated with lower endotoxin level (55). The outcomes of wheeze and asthma were reported based on the ISAAC and American Thoracic Society—Division of Lung Diseases questionnaires, thus were not physician-confirmed. The potential biases in individual studies may have affected the summary effect size in our meta-analysis. Combining effect sizes adjusted for different covariates or for exposure partitioned differently (tertiles, quartiles, quintiles) may have reduced the consistency across studies and influenced the precision of the summary estimates. This article also included only articles published in English which may not be representative of all the studies conducted on the topic (publication and language bias).

CONCLUSION

The present meta-analysis supports previous studies suggesting that endotoxin exposure protects children

against asthma but exacerbates inflammatory symptoms on the respiratory system. These findings are consistent with the “hygiene hypothesis” suggesting that endotoxin exposure early in life induces airway symptoms and subsequently promotes a modulation of the immune response that protects against asthma (46, 56, 57). Further research needs to investigate the underlying mechanisms for a better understanding of childhood asthma and the development of appropriate preventive measures.

DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

REFERENCES

1. World Health Organization. WHO factsheet 307: Asthma. Available at: <http://www.who.int/mediacentre/factsheets/fs307/en/index.html>. Accessed May 9, 2011.
2. Centers for Disease Control and Prevention. National Health Interview Survey data, Table 1–1, lifetime asthma population estimates—in thousands—by age, United States: National Health Interview Survey, 2007. US Department of Health and Human Services, CDC. 2010. Available at: <http://www.cdc.gov/asthma/nhis/07/table1-1.htm>. Accessed May 9, 2011.
3. Centers for Disease Control and Prevention. National Health Interview Survey data, Table 2–1 lifetime asthma prevalence percent by age,

- United States: National Health Interview Survey, 2007. US Department of Health and Human Services, CDC. 2010. Available at: <http://www.cdc.gov/asthma/nhis/07/table2-1.htm>. Accessed May 9, 2011.
4. National Center for Health Statistics. Health, United States, 2006 Chart book on trends in the health of Americans. Available at: <http://www.cdc.gov/nchs/data/atus/atus06.pdf>. Accessed May 9, 2011.
5. Masoli M, Fabian D, Holt S, Beasley R. Global Initiative for Asthma (GINA) Program. The global burden of asthma: executive summary of the GINA dissemination committee report. *Allergy* 2004; 59(5): 469–478.
6. Baena-Cagnani CE. The global burden of asthma and allergic diseases: the challenge for the new century. *Curr Allergy Asthma Rep* 2001; 1(4):297–298.
7. Metsala J, Kilkkinen A, Kaila M, Tapanainen H, Klaukka T, Gissler M, Virtanen SM. Perinatal factors and the risk of asthma in childhood—a population-based register study in Finland. *Am J Epidemiol* 2008; 168(2):170–178.
8. Radon K. The two sides of the “endotoxin coin”. *Occup Environ Med* 2006; 63(1):73–78.
9. Murakami D, Yamada H, Yajima T, Masuda A, Komune S, Yoshikai Y. Lipopolysaccharide inhalation exacerbates allergic airway inflammation by activating mast cells and promoting Th2 responses. *Clin Exp Allergy* 2007; 37(3):339–347.
10. Smit LA, Heederik D, Doekes G, Lammers JW, Wouters IM. Occupational endotoxin exposure reduces the risk of atopic sensitization but increases the risk of bronchial hyperresponsiveness. *Int Arch Allergy Immunol* 2010; 152(2):151–158.
11. Braun-Fahrlander C, Riedler J, Herz U, Eder W, Waser M, Grize L, Maisch S, Carr D, Gerlach F, Bufe A, Lauener RP, Schierl R, Renz H, Nowak D, Von Mutius E. Allergy and Endotoxin Study Team. Environmental exposure to endotoxin and its relation to asthma in school-age children. *N Engl J Med* 2002; 347(12):869–877.
12. Douwes J, van Strien R, Doekes G, Smit J, Kerkhof M, Gerritsen J, Postma D, de Jongste J, Travier N, Brunekreef B. Does early indoor microbial exposure reduce the risk of asthma? The prevention and incidence of asthma and mite allergy birth cohort study. *J Allergy Clin Immunol* 2006; 117(5):1067–1073.
13. Sordillo JE, Hoffman EB, Celedon JC, Litonjua AA, Milton DK, Gold DR. Multiple microbial exposures in the home may protect against asthma or allergy in childhood. *Clin Exp Allergy* 2010; 40(6):902–910.
14. Bertelsen RJ, Carlsen KC, Carlsen KH, Granum B, Doekes G, Haland G, Mowinkel P, Lovik M. Childhood asthma and early life exposure to indoor allergens, endotoxin and beta(1,3)-glucans. *Clin Exp Allergy* 2010; 40(2):307–316.
15. Yilmaz M, Altintas DU, Bingol-Karakoc G, Inal A, Kilic M, Sutolukta Z, Guneser-Kendirli S. Exposure to house dust endotoxin and allergic sensitization in allergic and non-allergic children living in Adana, Turkey. *Turk J Pediatr* 2009; 51(3):225–231.
16. Bolte G, Bischof W, Borte M, Lehmann I, Wichmann HE, Heinrich J, LISA Study Group. Early endotoxin exposure and atopy development in infants: results of a birth cohort study. *Clin Exp Allergy* 2003; 33(6):770–776.
17. Celedon JC, Milton DK, Ramsey CD, Litonjua AA, Ryan L, Platts-Mills TA, Gold DR. Exposure to dust mite allergen and endotoxin in early life and asthma and atopy in childhood. *J Allergy Clin Immunol* 2007; 120(1):144–149.
18. Gehring U, Bischof W, Fahlbusch B, Wichmann HE, Heinrich J. House dust endotoxin and allergic sensitization in children. *Am J Respir Crit Care Med* 2002; 166(7):939–944.
19. Gehring U, Strikwold M, Schram-Bijkerk D, Weinmayr G, Genuneit J, Nagel G, Wickens K, Siebers R, Crane J, Doekes G, Di Domenicantonio R, Nilsson L, Priftanji A, Sandin A, El-Sharif N, Strachan D, van Hage M, von Mutius E, Brunekreef B, ISAAC Phase Two Study Group. Asthma and allergic symptoms in relation to house dust endotoxin: phase two of the international study on asthma and allergies in childhood (ISAAC II). *Clin Exp Allergy* 2008; 38(12):1911–1920.
20. Rennie DC, Lawson JA, Kirychuk SP, Paterson C, Willson PJ, Senthilselvan A, Cockcroft DW. Assessment of endotoxin levels in the home and current asthma and wheeze in school-age children. *Indoor Air* 2008; 18(6):447–453.
21. Thorne PS, Kulhankova K, Yin M, Cohn R, Arbes Jr, SJ, Zeldin DC. Endotoxin exposure is a risk factor for asthma: the national survey of endotoxin in United States housing. *Am J Respir Crit Care Med* 2005; 172(11):1371–1377.
22. Tavernier G, Fletcher G, Gee I, Watson A, Blacklock G, Francis H, Fletcher A, Frank T, Frank P, Pickering CA, Niven R. IPEADAM study: indoor endotoxin exposure, family status, and some housing characteristics in English children. *J Allergy Clin Immunol* 2006; 117(3):656–662.
23. Carlsten C, Ferguson A, Dimich-Ward H, Chan H, Dybuncio A, Rousseau R, Becker A, Chan-Yeung M. Association between endotoxin and mite allergen exposure with asthma and specific sensitization at age 7 in high-risk children. *Pediatr Allergy Immunol* 2011; 22(3):320–326.
24. Campo P, Kalra HK, Levin L, Reponen T, Olds R, Lumms ZL, Cho SH, Khurana Hershey GK, Lockey J, Villareal M, Stanforth S, Lemasters G, Bernstein DI. Influence of dog ownership and high endotoxin on wheezing and atopy during infancy. *J Allergy Clin Immunol* 2006; 118(6):1271–1278.
25. El-Sharif N, Douwes J, Hoet P, Nemery B. Childhood asthma and indoor aeroallergens and endotoxin in Palestine: a case-control study. *J Asthma* 2006; 43(3):241–247.
26. Gehring U, Bolte G, Borte M, Bischof W, Fahlbusch B, Wichmann HE, Heinrich J, LISA study group. Lifestyle-related factors on the immune system and the development of allergies in childhood. Exposure to endotoxin decreases the risk of atopic eczema in infancy: a cohort study. *J Allergy Clin Immunol* 2001; 108(5):847–854.
27. Gillespie J, Wickens K, Siebers R, Howden-Chapman P, Town I, Epton M, Fitzharris P, Fishwick D, Crane J, New Zealand Asthma and Allergy Birth Cohort Study Group. Endotoxin exposure, wheezing, and rash in infancy in a New Zealand birth cohort. *J Allergy Clin Immunol* 2006; 118(6):1265–1270.
28. Lawson JA, Dosman JA, Rennie DC, Beach J, Newman SC, Senthilselvan A. Relationship between indoor environment and asthma and wheeze severity among rural children and adolescents. *J Agromed* 2009; 14(2):277–285.
29. Perzanowski MS, Miller RL, Thorne PS, Barr RG, Divjan A, Sheares BJ, Garfinkel RS, Perera FP, Goldstein IF, Chew GL. Endotoxin in inner-city homes: associations with wheeze and eczema in early childhood. *J Allergy Clin Immunol* 2006 May; 117(5):1082–1089.
30. Phipatanakul W, Celedon JC, Sredl DL, Weiss ST, Gold DR. Mouse exposure and wheeze in the first year of life. *Ann Allergy Asthma Immunol* 2005; 94(5):593–599.
31. Ferris BG. Epidemiology standardization project (American Thoracic Society). *Am Rev Respir Dis* 1978; 118(6 Pt 2):1–120.
32. Ryan PH, Bernstein DI, Lockey J, Reponen T, Levin L, Grinshpun S, Villareal M, Hershey GK, Burkle J, LeMasters G. Exposure to traffic-related particles and endotoxin during infancy is associated with wheezing at age 3 years. *Am J Respir Crit Care Med* 2009; 180(11):1068–1075.
33. Tischer C, Gehring U, Chen CM, Kerkhof M, Koppelman G, Sausenthaler S, Herbarth O, Schaaf B, Lehmann I, Kramer U, Berdel D, Von Berg A, Bauer CP, Koletzko S, Wichmann HE, Brunekreef B, Heinrich J. Respiratory health in children and indoor exposure to (1,3)-{beta}-D-glucan, EPS mould components, and endotoxin. *Eur Respir J* 2010.
34. Wickens K, Lane JM, Fitzharris P, Siebers R, Riley G, Douwes J, Smith T, Crane J. Farm residence and exposures and the risk of allergic diseases in New Zealand children. *Allergy* 2002; 57(12):1171–1179.
35. Von Elm E, Altman DG, Egger M, Pocock SJ, Gotsche PC, Vandenbroucke JP, STROBE Initiative. The strengthening the reporting of observational studies in epidemiology (STROBE) statement: guidelines for reporting observational studies. *J Clin Epidemiol* 2008; 61(4):344–349.
36. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ* 2003; 327(7414):557–560.
37. Gumedze FN, Jackson D. A random effects variance shift model for detecting and accommodating outliers in meta-analysis. *BMC Med Res Methodol* 2011; 11:19.
38. Rullo VE, Arruda LK, Cardoso MR, Valente V, Zampolo AS, Nobrega F, Nasipitz CK, Sole D. Respiratory infection, exposure to mouse

- allergen and breastfeeding: role in recurrent wheezing in early life. *Int Arch Allergy Immunol* 2009; 150(2):172–178.
39. Asher MI, Keil U, Anderson HR, Beasley R, Crane J, Martinez F, Mitchell EA, Pearce N, Sibbald B, Stewart AW. International study of asthma and allergies in childhood (ISAAC): rationale and methods. *Eur Respir J* 1995; 8(3):483–491.
 40. Jagielo PJ, Thorne PS, Watt JL, Frees KL, Quinn TJ, Schwartz DA. Grain dust and endotoxin inhalation challenges produce similar inflammatory responses in normal subjects. *Chest* 1996; 110(1):263–270.
 41. Keman S, Jetten M, Douwes J, Borm PJ. Longitudinal changes in inflammatory markers in nasal lavage of cotton workers. Relation to endotoxin exposure and lung function changes. *Int Arch Occup Environ Health* 1998; 71(2):131–137.
 42. Park JH, Gold DR, Spiegelman DL, Burge HA, Milton DK. House dust endotoxin and wheeze in the first year of life. *Am J Respir Crit Care Med* 2001; 163(2):322–328.
 43. Hu D, Zhang RB, Wu J. Study on the relations between concentration of endotoxin in dwelling and atopic asthma in school-age children. *Zhonghua Liu Xing Bing Xue Za Zhi* 2007; 28(4):354–357.
 44. Ober C, Vercelli D. Gene-environment interactions in human disease: nuisance or opportunity? *Trends Genet* 2011; 27(3):107–115.
 45. Gereda JE, Leung DY, Thatayatikom A, Streib JE, Price MR, Klinnert MD, Liu AH. Relation between house-dust endotoxin exposure, type 1 T-cell development, and allergen sensitisation in infants at high risk of asthma. *Lancet* 2000; 355(9216):1680–1683.
 46. Custovic A. Hygiene hypothesis for asthma: pros and cons. Program and abstracts of the American academy of allergy. *Asthma and Immunology 60th Anniversary Meeting*; March 7–12, 2003, Denver, CO, USA.
 47. Von Mutius E. Hygiene hypothesis for asthma: pros and cons. Program and abstracts of the American Academy of Allergy, Asthma and Immunology 60th Anniversary Meeting; March 7–12, 2003; Denver, CO, USA.
 48. Park JH, Cox-Ganser J, Rao C, Kreiss K. Fungal and endotoxin measurements in dust associated with respiratory symptoms in a water-damaged office building. *Indoor Air* 2006; 16(3):192–203.
 49. Doreswamy V, Peden DB. Modulation of asthma by endotoxin. *Clin Exp Allergy* 2011; 41(1):9–19.
 50. Smit LA, Heederik D, Doekes G, Koppelman GH, Bottema RW, Postma DS, Wouters IM. Endotoxin exposure, CD14 and wheeze among farmers: a gene-environment interaction. *Occup Environ Med* 2011.
 51. Kobayashi M, Saitoh S, Tanimura N, Takahashi K, Kawasaki K, Nishijima M, Fujimoto Y, Fukase K, Akashi-Takamura S, Miyake K. Regulatory roles for MD-2 and TLR4 in ligand-induced receptor clustering. *J Immunol* 2006; 176(10):6211–6218.
 52. Simpson A, John SL, Jury F, Niven R, Woodcock A, Ollier WE, Custovic A. Endotoxin exposure, CD14, and allergic disease: an interaction between genes and the environment. *Am J Respir Crit Care Med* 2006; 174(4):386–392.
 53. Zambelli-Weiner A, Ehrlich E, Stockton ML, Grant AV, Zhang S, Levett PN, Beaty TH, Barnes KC. Evaluation of the CD14/260 polymorphism and house dust endotoxin exposure in the Barbados asthma genetics study. *J Allergy Clin Immunol* 2005; 115(6):1203–1209.
 54. Sackesen C, Karaaslan C, Keskin O, Tokol N, Tahan F, Civelek E, Soyer OU, Adalioglu G, Tuncer A, Birben E, Oner C, Kalayci O. The effect of polymorphisms at the CD14 promoter and the TLR4 gene on asthma phenotypes in Turkish children with asthma. *Allergy* 2005; 60(12):1485–1492.
 55. Abraham JH, Gold DR, Dockery DW, Ryan L, Park JH, Milton DK. Within-home versus between-home variability of house dust endotoxin in a birth cohort. *Environ Health Perspect* 2005; 113(11):1516–1521.
 56. Racila DM, Kline JN. Perspectives in asthma: molecular use of microbial products in asthma prevention and treatment. *J Allergy Clin Immunol* 2005; 116(6):1202–1205.
 57. Eder W, von Mutius E. Hygiene hypothesis and endotoxin: What is the evidence? *Curr Opin Allergy Clin Immunol* 2004; 4(2):113–117.

Copyright of Journal of Asthma is the property of Taylor & Francis Ltd and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.