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Domestic endotoxin exposure and asthma in children: epidemiological studies

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1. ABSTRACT

Homes contain low but measurable concentrations of endotoxin that have been linked to household conditions such as the presence of animals, smoking, crowding, and farm living. While endotoxin exposure in early life appears to have a protective effect for childhood asthma; the evidence from prospective cohort studies of young children suggests that endotoxin exposure contributes to early development of wheeze. Higher domestic endotoxin levels are linked to greater asthma severity in school age children unless children are farm residents where, higher doses of farm-related endotoxin seem to offer some protection against asthma. Currently there are inconsistencies between epidemiological studies examining the role of endotoxin and children's respiratory health that may be due, in part, to selection bias of study populations, timing between measurement of endotoxin levels and the assessment of asthma symptoms. Although there is good evidence to demonstrate that endotoxin exposure in homes is associated with wheeze in children, and less likely to be associated with asthma, understanding the mediating roles of atopy, genetic and other environmental factors requires further and extensive exploration.

2. INTRODUCTION

Asthma is a chronic respiratory disease that is most common in childhood and characterized by such symptoms as cough, wheeze and phlegm resulting from underlying lung inflammation and bronchospasm. Major contributors to the occurrence of asthma in children are allergy and a family history of atopic disease or asthma. While the environment contributes to the development of asthma, timing of an exposure may be extremely important. Childhood asthma can lead to hospitalization, activity limitation, school absenteeism and, in rare cases, death. Early studies of trends in asthma prevalence and incidence provide good evidence that in the latter part of the last century, childhood asthma had increased (1-2) The first large scale global study of childhood asthma prevalence, The International Study of Asthma and Allergies in Childhood (ISAAC), was conducted in 1993 (3) and repeated in 2003 (4) Findings comparing these two time points show that the prevalence of asthma and allergic disorders has continued to increase in many countries (4-5) While increasing or in some cases stabilizing trends in

asthma prevalence have been observed, there continue to be recognized sub-populations of children within countries who are uniquely less predisposed to developing asthma including those living in farming environments where livestock are present (6), those with older siblings (7) or those populations with less observed atopy and increased viral and bacterial infections, primarily fecal in nature (8-10) Researchers, identifying groups of children with lower asthma prevalence and with higher rates of infections, hypothesized that the decrease in asthma prevalence or atopy found in these populations could be a result of the increased exposure to environments high in infectious agents (8, 11) Interest in this hypothesis has since led to a number of population-based investigations examining the role of exposure to microbial agents, and the occurrence of childhood asthma and allergy (6-7, 12)

Endotoxin, composed primarily of lipopolysaccharide (LPS) molecules located in the outer membrane of gram negative bacteria, is one particular microbial-related exposure receiving significant attention because of its potential role in the prevention of asthma and allergy in children and its observed role in the development of acute respiratory symptoms in adults working in livestock (13-15) and cotton operations (16) Pure LPS has been shown to induce airways responsiveness in adults in a laboratory setting. (17) Typically, when endotoxin enters the airways, it initiates a cascade of events at the cellular level of the host defense system inducing the production of inflammatory cytokines responses in the non-allergic Th1 immune pathway (18) Epidemiological studies provide the best evidence to date of the potential role of endotoxin in asthmatic disease in children.

The following review will evaluate the scientific evidence available from recent epidemiologic studies examining associations between asthma and domestic endotoxin exposure among children. We will provide a description of endotoxin, its characteristics and determinants, the scientific evidence for associations between endotoxin and childhood asthma, the strengths and limitations of the current research to date, and future recommendations for research. The literature search for published epidemiological reports was confined to English publications of children 0-17 years of age, where asthma or wheeze were the main outcomes of interest and where endotoxin was objectively measured in the domestic environment. The major search engines used were Medline and PUB Med. The following search terms were included to identify relevant epidemiological studies: “asthma”, or “wheeze” in combination with “child”, “children” “youth”, “adolescent”, we combined these search terms with “endotoxin” and “domestic endotoxin”. We examined the above terms in combination or alone with: “cross-sectional”, “case-control”, “case series”, “cohort”,, “epidemiological study”. Background information for this review was identified through literature searches on, “innate immunity”, “genetics”, “gene-environment interaction”, “lipo-polysaccharide”, “LPS” and “Lipid A”, and “endotoxin”.

3. CHARACTERISTICS OF ENDOTOXIN

3.1. What is endotoxin?

Endotoxin is a component of the cell wall of gram negative bacteria that triggers the release of many host mediators of the immune response (19) Although there are numerous types of toxins that are produced by bacteria, the term endotoxin is properly applied to the LPS components of the cell wall, which are biologically active (20), shown to be among the most powerful classes of immunostimulators, to be highly heat stable and amphiphilic (21) LPS has the capability of inducing non-specific innate immune resistance to microbial and viral infections or causing pathological injury such as septic shock (21) One of the most classic features of LPS is its role as an antigen; hence LPS can activate both Th1 and Th2 immunity pathways. Antigens elicit specific immune responses that result in the ability of mammals and other organisms to become specifically immune to bacteria with that molecular surface, and resist infection following initial exposure or immunization--which is an adaptive, specific immune response. It is the polysaccharide component of the LPS that is responsible for the antigenic function. At the other end of the LPS molecule, are 4, in many cases, Lipid A moieties, which are 3-hydroxy fatty acids (3-OH FA) (22) See Figure 1. However, bacteria are wonderfully diverse and even within a single genus there are enough molecular differences that it is unrewarding to use the 3-OH FA structure in taxonomic classification (23) Unraveling the complex non-specific and specific immune response to LPS is a subject of current research, and the roles of numerous cytokines are being identified (24) Recently, other roles for LPS have been identified, including its role in suppressing the normal production of interferon in response to viral infection (25)

3.2. Endotoxin assay

Biological activity is an inherent property of endotoxin, as is mass, but not all endotoxin molecules have identical potency. Indeed, suitable endotoxin standards have been used that were between 2 and 50 Endotoxin Units (EU)/ng. Early measurement of the activity of endotoxin was based on the observation by W. H. Lowell in the 1880’s that blood from the horseshoe crab, *Limulus polyphemus* coagulates in the presence of what came to be recognized as endotoxin. The great sensitivity of Limulus to endotoxin and the specific nature of the interaction was characterized in the 1960’s (26) In 1971, the routine Limulus Amebocyte Lysate (LAL) test for endotoxin was developed and standardized based on specific endotoxin material recognized by the United States Food and Drug Administration. Because of the importance of endotoxin as a potential contaminant of food and pharmaceuticals, the assay procedures are specified by national regulatory codices, such as the United States Pharmacopea (http://iccvam.niehs.nih.gov/docs/pyrogen/regulatory/28USP85.pdf; accessed July 31, 2010) The LAL test is the acknowledged method of quantifying endotoxin in dust samples.

There are some issues with the methods of measuring endotoxin. Methods that are based on LAL rely

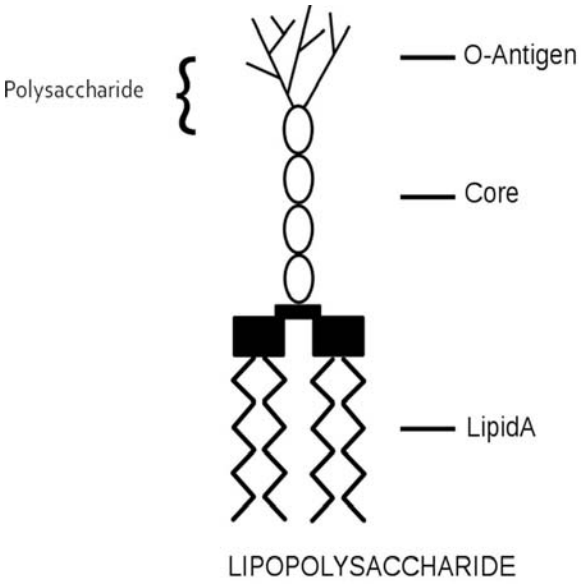

Figure 1. Diagram showing chain length and relation to lipid A

on the biological activity of the endotoxin which has the advantage of not being influenced by chemically-similar materials that lack biologic activity, but has the disadvantages of any biological system including the effect of "interfering substances" and the possible change in activity level of the sample between the time of collection and assay. The chemical analytical methods using Gas Chromatography-Mass Spectroscopy (GC-MS) to quantify total LPS have similar problems with precision and repeatability (27 -29)

The inconsistency of endotoxin assay results over time and between laboratories is a problem that is encountered when trying to study the role of endotoxin in the etiology or prevention of asthma. One is left wondering whether the observed differences are due to actual changes in the amount of endotoxin that is present in the environment, or are due to variations in the performance of the assays themselves (27) The use of assay kits containing reference amounts of internationally recognized standardized units has facilitated a high level of within laboratory precision, and pooled coefficients of variation for replicate samples ranging from 1 to 11% have been reported. However, the variation is greater among laboratories; and significant differences in assay results among laboratories may be related to both the extraction and analytical methods (28) The problems with variation in results among laboratories are not removed by switching to GC-MS assays of total LPS. Randomly allocated side-by-side filter samples of various agricultural dust submitted to 5 laboratories, produced significant differences in results between laboratories ($p < 0.01$) whether they were using either GC-MS (total LPS) or LAL (free bioactive endotoxin) assays (27) Although the GC-MS technique for measuring 3-OHFA, which has been referred to as total endotoxin, and the use of recombinant factor C (rFC) to measure free bioactive endotoxin are both sophisticated, currently-used methods to measure endotoxin, the Pearson correlations between the two methods, within a single laboratory, were low and ranged from 0.72 to 0.11 in 134 samples of agricultural airborne dust (29) Hence, the problem of obtaining a precise estimate of the amount of endotoxin in a sample of organic dust remains.

3.3. Endotoxin and the response in the lung

As mentioned previously, the biologically active component of endotoxin is LPS. It should be noted that the lung response to LPS is variable and reflective of dosage, genetic and environmental conditions (30) Most studies of lung response to endotoxin have been conducted using in-vitro techniques and animal models, mainly with sheep or rodents. Animal models provide insight into the cellular response to LPS, but the structures of LPS receptors may be significantly different between species (23)

The response to LPS depends on the dose and duration of exposure as well as the individual's previous exposure history. The typical early lung response to LPS is neutrophilic and mononuclear recruitment with an increase in CD4+ T lymphocytes and macrophages, to a lesser extent. The response is amplified by the release of cytokines from these cells accompanied by an up regulation of ICAM-1 expression (31) LPS has been found to induce an immediate constriction of the airways that is transient and not associated with either lung edema or vascular changes. While the dosage of LPS seems to be important in the response by the lung, the length of exposure may also be important for occurrence of lung injury (32) The physical effects of endotoxin exposure can include coughing, dyspnea and reduction in FEV_1 (33) while the immunological response can include the release of anti-inflammatory cytokines including TNF-α, IL-6, IL-1 and IL-8 as well as a neutrophilic response (17, 33-34)

3.4. Domestic endotoxin

3.4.1. Gram-negative sources

Endotoxin exposure is ubiquitous in both the indoor and outdoor environments (35-36), and can be found in soil, water and air. It has been measured in homes and industry, with high airborne endotoxin levels found in agricultural processing plants and animal confinement buildings and much lower levels found in homes. Airborne endotoxin exposure levels range from below the threshold of detection (< 1 EU/m^3) in clean laboratory or office settings to more than 10,000 EU/m^3 in poultry facilities (37) Airborne levels of endotoxin in the home have been found to range from 0.01 to 30.23 EU/ m^3 (38-40) While predominant species of gram-negative bacteria found responsible for household endotoxin have not been reported, species identified in the air of daycares and schools frequented by children include *Acinetobacter lwoffi, A. calcoaceticus, Enterobacter sp. and Klebsiella sp* (41)

3.4.2. Collection of domestic endotoxin

Sampling methods for endotoxin in domestic environments include collection of airborne dust particles or settled dust. Currently, the most common method for collecting domestic endotoxin is by vacuuming settled dust.

Dust is often collected from high traffic areas such as the living room and kitchen floors or from prominent exposure sites of the child such as floors of the bedroom, play area, and the sleeping surface of the child's mattress. Endotoxin level is often reported as endotoxin units (EU)/mg of dust or as EU/area of sampling. Levels of endotoxin vary between locations in the home with floor samples typically having higher levels than mattress samples (38, 42-44) although mattress endotoxin levels appear to have better reproducibility on repeated sampling (45) Household endotoxin levels are also known to vary by season. 46) Endotoxin levels also vary by site (47) and over time (46) There is less variation between bedroom floor samples and higher variation with kitchen floor samples. Within home variability has been shown to be less than between home variation (48) Sampling of airborne endotoxin has demonstrated a weak correlation with overall vacuumed dust from the same home and better correlation with vacuumed dust from family rooms (38)

3.4.3. Environmental determinants of domestic endotoxin

Predictors of domestic endotoxin levels from vacuumed dust have been well described and are sometimes used as surrogates for endotoxin exposure when objective measurements of endotoxin are unavailable. Notable predictors of high domestic endotoxin levels are domestic pets, vermin and current living conditions including presence of dogs (38, 47, 49-52), cats (38, 42-43, 49-50), cockroaches (47) (51), mice (49), environmental tobacco smoke (43, 53-54), larger family size (42, 52, 55), animal husbandry (43) and farm or rural living (56-57) Higher levels of domestic endotoxin have been reported to occur with variation in housekeeping and housing qualities including poor housekeeping (47), and home characteristics associated with farming (42) presence of carpets (52), less floor vacuuming (49, 52), and higher relative humidity of the indoor air (52, 55) Conversely, lower levels of endotoxin can be found in homes using central air conditioners (58) or those using forced air heating by natural gas (43) While airborne sampling of domestic endotoxin levels is less common, associations between higher ambient endotoxin levels and the presence of dogs have been reported in one study (38)

4. EPIDEMIOLOGICAL EVIDENCE

4.1. Background to epidemiological evidence

As the prevalence of childhood asthma increased in the 1980's and 90's, a number of epidemiological studies, primarily those of cross-sectional design, were initiated during that time to identify the importance of the household environment and other factors for asthma etiology. A limitation of the findings of earlier studies was the difficulty establishing causal relationships between significant domestic risk factors and childhood asthma using a cross-sectional design approach. Consequently, several birth/infant cohort studies were established in the 1990's to provide better evidence of the etiological importance of certain indoor environmental conditions for childhood asthma. Several cohort studies, primarily consisting of newborn offspring of atopic mothers, were undertaken to examine the effects of early life exposure to certain domestic factors potentially responsible for the later development of asthma and wheeze in children. These cohort studies included objective measurement of the child's home environment in early life using household dust for assessment of indoor air contaminants. These findings led to important recognition of the protective role of Th1 response of innate immunity for childhood asthma (7) Although domestic dust collected as part of these studies was used mainly to assess allergen levels in the home, collected dust was also available to examine other potential domestic agents including endotoxin and molds. While cohort studies provide our best evidence to date of the etiology of childhood asthma and the role of endotoxin, both case-control and cross-sectional studies continue to add important information regarding the importance of domestic endotoxin for acute asthma and wheeze. A limited number of panel studies provide useful information about the temporal effects of endotoxin for asthma severity. Tables 1, 2 and 3 provide the major design information of the cohort, case-control and cross-sectional studies found where indoor endotoxin was assessed as a potential risk factor for asthma. The tables provide information about the characteristics of the study populations used for a particular assessment. Often the prospective analyses did not include the full study population but a proportion of the cohort under study where endotoxin was measured. With the exception of one study (40) the endotoxin levels reported are vacuumed dust collected from various floor surfaces of the home or from the mattress where the child slept. We have provided the odds ratios or beta coefficients adjusted for significant covariates when reported; however, adjusted findings were not always available.

4.2. Cohort studies

Table 1 lists the ten cohort studies where the relationship between domestic endotoxin exposure and later development of childhood asthma and wheeze has been reported. While nine of the ten cohort investigations began with the birth of a healthy index case, one study examined associations between domestic endotoxin and the later development of respiratory symptoms in siblings of enrolled infants (59)

The earliest reports of the natural history of asthma and the role of domestic endotoxin surfaced in 2001. Park *et al.* reported that a 55% increase in repeated wheeze in the first year of life was associated with endotoxin levels greater than or equal to 100 EU/mg (median value) measured when the child was 3 months (60) In this study of inner city Boston children, adjustment for family size did not appear to affect the positive associations between domestic endotoxin levels in the home and persistent wheezing. Celedon *et al.* examined children in this cohort when they were 7 years of age and found no association between exposure to household endotoxin at 3 months and later development of asthma at 7 years (61) However, there were significant findings in this study for wheeze. There was a positive association between endotoxin found in the floor samplings of the family room and persistent wheeze at 7 years of age and whereby as endotoxin levels increased, the risk of persistent and

Table 1. Cohort studies examining associations between domestic endotoxin and childhood asthma or wheeze

Location, reference	Study population	Outcome (s) measured	Endotoxin measurement (source and time)	Findings[1]
1. Olso, Norway, 62	Birth cohort 42% (n=206) studied at 10 years	*Asthma:*2 of: asthma symptoms, doctor diagnosed asthma or use of asthma education < 10 years of age *Current asthma:* asthma in past 12 months or symptoms or positive exercise test *Atopy*: SPT[5] positive or specific IgE≥0.35 kU/L FEV1, FVC[6], PEF[7]	Continuous EU^2/mg (living area floor collected by parents at 2 years of age)	*Asthma:*NS[3] *Current Asthma*: NS *Atopy*: NS *Spirometry*:↓$FEV1^4$ with ↓endotoxin exposure (β= -0.12; -0.20 to -0.03) No interaction between endotoxin, dust allergens or β glucan for asthma
2. Cinncinatti, USA, 64	Infant cohort (~ 6 months old) - Parent SPT positive to at least 1/15 75% (n=468) studied at 36 months	*Recurrent wheeze:* ≥ 2 episodes/12 months *Persistent wheeze*: ≥ 2 episodes/year for both 2 and 3 year or diagnosis of asthma by 36 month *Asthma Predictive Index*	Quartiles of EU/mg: categorized as high and low-cutoffs not defined (floor of infant's primary activity room at ~ 8 months of age)	*Persistent wheezing*: Significant interaction between elemental carbon attributable to traffic and high levels of domestic endotoxin: aOR: 5.85 (1.89-18.13) No interaction for recurrent wheeze or Asthma Predictive Index in adjusted models
3. Boston, USA, 61	Birth cohort At least one parent with hay fever, asthma or allergies 88.4% (n=440) studied at 7 years	*Asthma*: Physician diagnosed asthma and ≥ wheezing in past 12 months *Persistent wheezing*: an episode of wheeze before 3 yrs and 1 episode in 4^{th} year *Transient wheeze*: ≥1 episode of wheeze before age 3 but not afterwards	Quartiles of EU/mg: Q1: <52.48 Q2: 52.49-79.99 Q3: 80.48-125.59 Q4: >125.59 (Family room at 2-3 months)	*Asthma*: NS Persistent wheeze: aOR_{4thQ}: 3.5 (1.3-9.8) *Transient wheeze*: Linear trend for association with increased endotoxin (p < 0.05)
4.Wellington and Christchurch, New Zealand, 68	Birth cohort 80% (n=884) studied at 15 months	*Wheezing:* any history of wheezing *Allergy*: SPT to 9 allergens measured at 15 months	Quartiles of EU/g Q1: <4621 Q2: 4621-10,749 Q3: 10,750-23,672 Q4: >23.673 (child's bedroom floor at 3 months)	*Wheezing*: aOR_{4thQ}:154 (1.03-2.30) *Allergy:* NS Effect modification: Wheezing among infants with parental history of allergic disease: aOR_{4thQ}: 1.67 (1.07-2.60)
5. The Netherlands, 65	Prenatal intervention and birth cohort of children of atopic mothers 64% (n=447) studied at 4 years	*Asthma:* doctor diagnosed asthma in last 4 years. *Persistent wheeze*: any episode in 1^{st} 3 years and ≥1 episode in 4^{th} year. *Transient wheeze:* Wheeze in first 3 years: *Atopy:* IgE concentration >0.35IU/mL to ≥ 1 allergen	Tertiles of EU/m^2: Low < 142.3 Medium= 142.3-1657.1 High= ≥ 1657.2 (living room dust for 696 collected in first 3 months)	*Asthma*: Medium exposure: aOR: 0.47 (0.26-0.86); High exposure: aOR: 0.40 (0.21-0.77) *Persistent wheeze*: NS No interaction between endotoxin and atopy for asthma
6. Cincinnati, USA, 63	Infant cohort (~ 6 months) -Parent SPT positive to at least 1/15 100% (n=532) studied at 12-15 months,	*Recurrent wheeze*: ≥ 2 episodes/12 months *Recurrent wheeze with event:* Recurrent wheeze requiring medication *Any wheeze*: ≥ 1 episode /12 months *Allergic wheezing*: Any wheeze and positive SPT	Quartiles of EU/mg: Q1: <40.9 Q2: 40.9-78.6 Q3: 78.7-160.9 Q4: >160.9 (floor of infant's primary activity room before 15 months)	*Recurrent wheeze*: and endotoxin aOR_{3rdQ}: 0.4 (0.1-0.9) with 2 or more dogs in the home; *Any wheeze*: aOR_{3rdQ}: 0.3 (0.1-0.8) with ≥ 2 dogs in home. *Allergic wheeze*: NS
7. Inner city New York, USA, 69	Birth cohort Prenatal non-smoking mothers Studied at: 24 months 67.4% (n=203) 36 months 54.2% (n=163)	Report of wheeze (child) at least once 13-24 mo, 25-36 mo	EU/mg and EU/m^2 (bedroom floor dust collected at 12 months. Dust collected at 36 months for 47 children)	24 months: *Wheeze:* aOR: 1.3 (1.01-1.8); 36 months: NS Interaction at 24 and 36 months with higher prevalence of wheeze associated with ↑EU/mg in children with maternal history of asthma (p = 0.29and 0.38, respectively)
8. Prince Edward Island, Canada, 46	Birth cohort full term children 94.5% (n=332) Studied 0-24 months	Daily symptom diary for 2 years *Illness episode*: Stuffy nose, cough, wheeze or SOB for 2 days x number of days x number of times/year *Illness days*: Number of days with illness /year	Airborne endotoxin EU/m3 (5-7 days in first 12 months)	*Illness days with wheeze:* ↑EU/m3 adjusted beta (SE): 4.68 (1.66), p< 0.01. *Illness episodes*: ↑EU/m3 adjusted beta: 0.46 (0.13); p< 0.001)
9. Leipzig, Germany, 67	Birth cohort of full term, normal weight infants 79% (1942) studied at 2 yrs of age	*Asthma:* Diagnosed by physician. *Repeated wheeze:* ≥ 2 episodes since birth *Atopy*: specific IgE≥0.35kU/L	Quartiles of EU/g Q1: 57-1028 Q2: 1029-2987 Q3: 2988-7751 Q4: >7751 (mother and child's mattress at 3 months of age)	*Asthma:* NS *Repeated wheezing*: associated with endotoxin: aOR_{4thQ}: 1.52 (1.08-2.14) *Atopy:* NS Interaction: endotoxin with infants with parental history of atopy (p < 0.008 for trend) *Repeated wheezing*: aOR_{4thO}: 1.77 (1.14-

				2.73)
10. Boston, USA, 59	226 siblings (< 5 years old) of birth cohort. At least one parent with hay fever, asthma or allergies 4 year followup	*Wheeze*: Wheezing in the past 12 months	Median cutoff for EU/mg High:: >81.3 (living room floor for 173 assessed at various ages for siblings: 14 months, 22 months, 34 months, 36 months	14 months Wheeze*:* aOR: 3.40 (1.70-6.80) Overall: *Wheeze:* aOR: 1.52 (1.07-2.14) Interaction: As time from endotoxin exposure increased, the proportion of children who wheezed with high endotoxin exposure decreased.
11. Munich and Leipzig, Germany, 66	Birth cohort of full term, normal weight infants 77% (1884) assessed at 6 and 12 months	*Wheeze*: Report of wheeze or whistling in chest past 6 months *Cough with respiratory infection*: Cough associated with cold or bronchitis past 6 months	Quintiles of EU/g Q1:≤824 Q2: 824-1973 Q3: 1973-4365 Q4: >4365-10,163 Q5: >10163 (mother and child's mattress at 3 months of age)	6 months: *Wheeze*: aOR_{5thQ}: 2.37 (1.40-4.03); *Cough with respiratory infection*: 1.73 (1.28-2.33) 12 months: *Wheeze*: aOR_{5thQ}: 1.60 (1.10-2.30)
12. Boston, USA, 60	499 full term infants Parents with asthma or allergy Assessed at 12 months	*Any wheeze:* Any report of wheeze *Repeated wheeze*: ≥2 reports of wheeze in first 12 months	Endotoxin EU/mg Geometric mean cutoff < 100 and ≥ 100 (family room floor at 2-3 months)	*Repeated wheeze* : aOR: 1.55 (1.00-2.42) *Any wheeze*: NS *Repeated wheeze*:1.53 (1.01-2.30) Not adjusted for living in apartment with ≥3 families

Adjusted odds ratios (aOR) and 95% confidence intervals (CI) are presented whenever reported.[1] Abbreviations: [2]endotoxin units, [3]non significant, [4]forced expiratory volume at 1 second, [5]skin prick test, [6]forced vital capacity, [7]peak expiratory flow rate, [8] standard error

Table 2. Case control studies assessing relationship between asthma/wheeze and domestic endotoxin

Location, reference	Study population	Outcome (s) measured	Endotoxin measurement (source)	Findings[1]
1. Estevan, Canada, 43	Cases: 6-13 years and Non-sibling Controls: Matched on age and sex (n=197) Controls were not related to cases	*Cases*: Physician diagnosed asthma and asthma episode, physician or ER visit in past 12 months or wheeze in past 12 months *Atopic asthma*: Any positive SPT	Endotoxin EU^2/mg (floor of child's play area and the child's mattress.0)	*Asthma*: NS[3] for either play area or mattress endotoxin. Association between endotoxin in mattress and absenteeism from school in past 12 months with chest illness in atopic cases adjusted beta (SE)[4] 1.05 (0.36), ($p < 0.05$)
2. Palestine, 70	Cases: 6 to 12 years Controls: Matched 1:1 on grade, school and sex (n=132)	*Cases*: Report of wheeze in past 12 months *Atopy*: Any positive SPT[5] or IgE>0.35 kIU/l	Endotoxin: EU/mg categorized by tertiles Low: < 16.02 Medium 16.02-41.75 High >41.75 EU/mg (living room floor dust and mattress)	*Wheezing*: NS. *Atopic case:* Living room dust: OR_{2ndT}: 0.02 (0.00-0.26) and OR_{3rdT} 0.04 (0.01-0.35) *Non-atopic cases*: Mattress: OR_{2ndT}: 0.13 (0.02-0.71)
3. Manchester, UK, 44	Cases 4-17 years and Non-sibling Controls: Matched on age, sex, sib-ship size (n=200) Controls were not related to cases	*Cases* Physician diagnosed asthma with validation of probable asthma	Endotoxin: EU/mg (living room floor, bedroom floor, child's mattress)	*Cases:* ↑ EU/mg in living room dust: aOR: 1.88 (1.11-3.18) More cases (9.7%) exposed to endotoxin >100 EU/mg dust compared to controls (1.3%)

Adjusted odds ratios (aOR) and 95% confidence intervals (CI) are presented whenever reported.[1] Abbreviations: [2]endotoxin units, [3]non significant, [4]standard error, [5]skin prick test

transient wheeze also increased (61) In a study from Sweden that also assessed the health of school age members of the cohort, endotoxin concentrations in living room dust were not associated with a history of asthma or current asthma but were associated with lower forced expiratory volumes at one second (FEV1) These significant findings were not present later when an outlier in the data was removed and the analysis re-run (62)

Campo *et al.* (63) found opposite results between endotoxin and respiratory outcomes for children living in Cincinnati. In homes where endotoxin levels exceeded 100 EU/mg exposures and where there were two or more dogs, any wheeze or recurrent wheeze at 12 months of age was less common. In this study any positive association between wheeze and endotoxin concentration appeared to peak around 100 EU/mg (75^{th} percentile) and then drop sharply with higher endotoxin levels. Examining the same birth cohort, Ryan *et al.* identified effect modification between endotoxin levels in the home and particulate levels from outdoor traffic with persistent wheeze and asthma by 3 years of age (64) Elemental carbon attributable to traffic (ECAT) was measured as time weighted average of daily exposure for periods 7-12, 13-24, and 25-36 months. Children who were also exposed to the high particulate counts from traffic in the first year of life in the presence of high domestic endotoxin exposure in the home measured around 8 months of age had increased prevalence of persistent wheeze (OR: 5.85) at age 3. Endotoxin levels in the home were not associated at 36 months with a predictive index for asthma (recurrent wheeze at 36 months and one of: parental asthma history, allergic sensitization to one or more aeroallergens and eczema; or two of wheeze without a cold, physician diagnosed allergic rhinitis and allergic sensitization to milk or egg) (64) In another birth cohort from the Netherlands of children with atopic mothers (65), endotoxin levels in vacuumed dust measured in the first 3 months of life was inversely related to asthma

at 4 years with the strongest protective associations for the highest levels of endotoxin exposure (≥1657.2 EU/m^2)

There were three birth cohorts where the atopic status of the mother or parent was not considered in the enrollment criteria of the study. Results from these studies indicate that atopic/asthma status of the parent could influence the presence of wheezing in their young children upon high exposure to domestic endotoxin. In the " Influence of Lifestyle Related Factors on the Immune System and the Development of Allergies in Childhood" (LISA) study from Germany, Gehring *et al* found that the highest level of endotoxin (5th quintile) collected at 3 months was associated with wheezing at 6 and 12 months (66) Conversely, physician diagnosed atopic eczema wan less like to occur with higher levels of endotoxin (adjOR: 0.50; 95% CI o.28-0.88): Bolte examined children in this same cohort when they were 2 years of age and found that endotoxin levels in the 4th quartile continued to be associated with increased wheezing. Furthermore, any wheeze was increased in those children with a history of parental atopy (67) Interestingly, atopic dermatitis at this age was now associated with increased endotoxin exposure. Gillespie *et al* also identified an effect of a family history of atopy in the relationship between endotoxin and wheezing in a birth cohort of 881 children living in New Zealand (68) Wheezing was increased in those children who had a family history with allergic disease when endotoxin levels in the bedroom floor dust were ≥ 3rd quartile. In this latter study, there was also a dose response relationship between endotoxin levels and increased wheezing. Perzanowski *et al.* enrolled non-smoking prenatal women into a US inner city birth cohort study. Higher domestic endotoxin levels were associated with a modest increase in wheeze at 2 years of age of the index child. By 36 months the association between endotoxin and a higher prevalence of wheeze was significant only for children of mothers with a history of asthma (69) In this study increasing endotoxin concentration was associated with lower adjusted risk of eczema in first 12 months of life but not at 36 months.

Perhaps one of the most comprehensive early assessments of the health effects of domestic endotoxin in children that did not focus on a positive atopic history of the parent as study eligibility criteria was a Canadian study conducted by Dales et a.l in Atlantic Canada This geographic area of Canada has one of the highest rates of childhood asthma (40) Airborne endotoxin was measured over a 5 day period in the first year of life. For two years parents also recorded illness episodes of at least 2 days duration of any of wheeze, cough, stuffy nose or shortness of breath. Although the effect size of the association between domestic endotoxin and wheeze decreased over the two years, there continued to be a strong association between early endotoxin exposure and frequency of wheezing episodes. Of the household characteristics assessed, only increased indoor humidity was associated with higher endotoxin levels in the home.

In the inner city Boston cohort study the health of older siblings was studied (59) Siblings were younger than 5 years of age at the time of the home interview in the first year of life for the index sibling. Initially, increased wheezing in siblings was associated with endotoxin levels greater than or equal to 83.1 EU/mg but the association decreased over time so that by 46 months after the initial assessment of endotoxin levels in the home, the association was no longer significant. In this case, timing of the exposure with the age of the child appeared to be an important factor for the occurrence of respiratory symptoms in children.

In summary, cohort studies to date provide significant information about the early occurrence of wheeze in children exposed to domestic endotoxin and provide less support of the importance of endotoxin in the development of asthma. Very little asthma was reported in these birth cohorts who at the time of reporting, except for one cohort (61), children were still preschoolers. Although asthma was unlikely to be associated with domestic endotoxin exposure in these studies, persistent wheezing, reported to be an early signal of later asthma was associated with endotoxin in two studies (61, 64) and protective for persistent wheeze in another (65) Findings of associations for wheeze are for the most part, modest and inconsistent across studies, perhaps biased by the requirement of a positive atopic history of a parent (in most cases, the mother) for entry into the study cohort. Some studies experienced notable time lapses between when endotoxin measurements were taken in the home and when symptoms were measured. In most studies domestic endotoxin was often measured early in the children's life and measured only once. The time between exposure assessment and measurement of outcomes could influence findings observed. For example, the Boston birth cohort study, that also assessed the relationship between wheezing and endotoxin in siblings of the cohort, found that high endotoxin exposure in siblings was associated with an increased risk for wheezing during the study period and that this level of risk decreased over time. (59) In the three studies where endotoxin collection was within the year of assessment of respiratory symptoms, higher domestic endotoxin was associated with increased wheezing (60, 66, 69)

4.3. Case control studies

While longitudinal studies provide information about the etiology of early exposure to domestic endotoxin, case-control studies provide better information of associations between current asthma or wheeze status and current endotoxin exposure. From a public health perspective these studies provide merit for environmental control but are less informative regarding causal associations.

There were three case-control studies reported by the time of this review (Table 2) One study focused on atopic wheeze in the past 12 months (70) and two others assessed associations between endotoxin and symptomatic asthma (44) or asthma/wheeze within 12 months (43) In all studies, cases were matched to controls on potential confounders such as age and sex. Higher endotoxin levels were associated with decreased wheezing in atopic cases in

the study from Palestine whereas, in the study of homes of English children, Tavernier *et al.* (44) reported an association between higher endotoxin levels in the dust from the living room floor area and asthma. The study of Canadian children found no association between endotoxin levels in either the child's mattress or play area dust with the presence of asthma. Endotoxin levels in this latter study however, were very low and could contribute to the non-significant findings reported. As well, the study of children in England excluded controls of siblings with asthma (44) while the Canadian study did not (43) The designs of the English and Canadian case control studies were very similar although the English study did not use a conditional analysis (70) approach to account for the matched design. In a post hoc analysis conducted in the Canadian study (43), skin prick test positive asthmatic cases and those whose homes had endotoxin levels greater than 80EU/mg were more likely to miss school because of chest illness. Similar associations were not found in this study for non-atopic asthmatic children or for controls regardless of atopic status.

As with prospective studies modest positive associations were noted with higher endotoxin levels in domestic environments. The matching of cases and controls in these studies limit the usefulness of examining age and sex as potential effect modifiers or confounders of associations between endotoxin and asthma.

4.4. Cross-sectional studies

Cross-sectional studies that examined associations between asthma and domestic endotoxin are by far the most frequently reported studies. Table 3 provides information about cross-sectional analyses that began in the late 1990's. There are 3 studies from North America, several multicentre studies from Europe and one study from Asia.

In a multicenter, large urban study conducted in the US a significant association was found between wheeze and domestic endotoxin from bedroom floors and bedding in homes for adults and but not for children. A 1999 study of 812 rural German, Austrian and Swiss farming and non-farming children, found that higher endotoxin load (EU/m^2) measured in mattress dust was associated with a reduced risk of hay fever, atopic sensitization, atopic wheeze and atopic asthma among 6 to 13 year old farming children (12) As well, the investigators of this study reported an increased risk of non-atopic wheeze associated with endotoxin load among children from non-farming environments. However, in another cross-sectional study (71) that measured domestic endotoxin, in the homes of farmer's children and a reference population (5-13 years), endotoxin measured from vacuumed mattress dust was not associated with asthma (physician diagnosed asthma or obstructive bronchitis more than twice in their lifetime) or current wheeze (wheeze in the past 12 months) Endotoxin levels were however. inversely related to atopic status measured as allergen specific IgE to at least one allergen. A recent cross-sectional study of a clinic population in Asia found a positive association between endotoxin in mattress dust and the number of wheezing episodes in the past 12 months in school age children (54)

Three cross-sectional studies have examined the symptom burden associated with childhood asthma. In a Canadian study, conducted with rural children with asthma from farming and non-farming backgrounds, increased endotoxin levels in the home were associated with decreased school absenteeism. This relationship was not modified by farm living (72) In the second study, children with asthma living in Puerto Rico were less likely to have visited physicians for their asthma and report less asthma symptoms. Interestingly, 89% of children in this study were exposed to high domestic endotoxin concentrations greater than 100EU/mg. Leung *et al.* recently examined the association between domestic endotoxin and respiratory symptoms, exhaled nitric oxide values and percent predicted lung function (forced vital capacity, forced expiratory value at 1 second and peak expiratory flow rate) in asthmatic children living in Hong Kong (54) Endotoxin levels were not associated with lung function or exhaled nitric oxide levels. Results from their analyses assessing the association between endotoxin and specific respiratory symptoms (wheeze, cough, and sleep disturbance, exercise or speech limitation) found that only the number of wheeze attacks was associated with domestic endotoxin in the child's mattress.

While cross-sectional studies to date have the advantage of providing information about current endotoxin exposure; results from these studies identify both a positive and negative relationships between asthma and endotoxin exposures. Consequently, the limited consistency of findings across studies limits their usefulness in identifying the overall role of endotoxin in childhood asthma.

4.5. Panel studies

Panel studies of asthma provide information concerning the severity of disease in children with asthma who may be exposed to higher levels of domestic endotoxin. Two studies enrolled both asthmatic and non-asthmatic children as subjects (73-74) A 12 month study from Brazil, completed in 1993/4, included 10 dust mite sensitized cases with asthma and 10 non allergic controls without respiratory symptoms between the ages of 6 and 16 years (73) Monthly endotoxin measurements were collected from dust of the child's mattress and bedroom floor. Increased severity, assessed by an overall clinical score based on symptoms and medication use, was associated with higher endotoxin levels in dust samples. Airways obstruction was also assessed in a 1993/4 study from the Netherlands where peak flow variability was measured over 16 weeks in a group of 74 asymptomatic and 74 children with chronic respiratory symptoms including recent wheeze, shortness of breath with wheezing, dry cough and/or ever diagnosed with asthma (74) Higher levels of endotoxin from living room floor dust were associated with higher peak flow variability in the unadjusted analysis of this school age population but not once the analysis was adjusted for pets, use of carpets and the presence of dust mites. One other study assessed the role of endotoxin in presenting asthma symptoms in children aged 6-13 years attending a U.S. school

specifically designed for children with asthma (75)

Personal samplers were used to evaluate endotoxin levels in the ambient environment. Observations were conducted over two consecutive school days and repeated 3 times. Children with higher endotoxin levels from personal monitoring were more likely to experience asthma symptoms and lowered FEV_1 related to sleep but not to play exposures.

5. STRENGTHS AND LIMITATIONS OF EPIDEMIOLOGICAL STUDIES

5.1. Measurement of domestic endotoxin

5.1.1. Methods of sampling

The methods of sampling used in studies may be a major factor in obscuring the strength and consistency of associations between domestic endotoxin and childhood asthma. Endotoxin can be measured by different methods including airborne sampling of home areas, personal sampling of the subject, and vacuum sampling from home locations such as the floor or child's mattress. However, these measures may not be correlated. One study investigated the levels of endotoxin in homes measured endotoxin from sampled dust from the bedroom bed, the bedroom floor and the kitchen floor as well as indoor airborne samples collected over a 24 hour period from the bedroom (38) None of the correlations between endotoxin measured by vacuumed dust and by airborne samplers were statistically significant. Spearman's correlation coefficients between endotoxin from airborne monitoring and floor or mattress sampling were low (0.23 and 0.33)

In a sub-study of 23 % of homes where both floor dust and air were sampled for endotoxin, one group re-examined their initial positive findings of an association (OR: 1.45) between wheeze and endotoxin found in floor dust (39) Believing that the level of endotoxin in airborne dust was the exposure of main interest and that the floor dust endotoxin was a surrogate for airborne endotoxin, they corrected for measurement error between the two sources of dust to determine the risk for wheeze if endotoxin was measured from airborne samples. Results of their reanalysis using this correction factor showed that there was a nearly 6-fold increase in the prevalence of wheeze for every interquartile range increase in airborne endotoxin, suggesting that the magnitude of the effect of endotoxin for wheeze may be underestimated in studies where settled dust is used.

Conflicting results in the associations between endotoxin and respiratory health outcomes could be a result of the sampling method used to collect dust. In a study of 6-13 year old children with asthma both personal and stationary air samplers were used to measure endotoxin (75) Statistically significant inverse associations between endotoxin dust measured by personal samplers and evening FEV_1 from daily monitoring were reported. However, in this same study positive but non-significant associations between endotoxin from stationary airborne monitors and children's FEV_1 were found. Limitations of study findings were noted as stationary air sampling was only measured in the child's school and not in the home.

A limitation with personal sampling, which is more likely to represent contaminants in the breathing zone of the child, is the potential inconvenience of monitoring for families and the child. Personal monitoring requires cooperation from children and their families which may be difficult to obtain. Loss of data can influence findings. Floor samples allow a relatively quick method of assessing endotoxin exposure in a home. However, it must be considered a surrogate of actual exposure as the associations may be conservative and underestimated as noted above (39) While standards for collection of settled dust have been proposed and evaluated, (76) these are not consistently employed across studies.

The length of time between endotoxin assessment and evaluation of symptoms could result in measurement bias regarding the exposure. It seems quite likely that increased exposures to endotoxin are associated with concurrent respiratory symptoms in children with a diagnosis of asthma as noted by the findings of the few panel studies conducted to date. However, in cohort studies where the exposure preceded the development of disease, the findings for the role of endotoxin in a diagnosis of asthma are less convincing of a causal relationship. Often there was a large time gap between when the endotoxin assessment occurred and when respiratory symptoms were measured in these studies. Time lapses between dust collection and outcome assessment were as little as a few months or as long as 8 years. Although analysis from two dust collections six months apart in the homes of one cohort (48) showed good reliability between assessment times, findings from the same cohort study also showed an attenuation of associations as time from exposure assessment increased. (59) Endotoxin levels in the home are known to vary over seasons (36, 46) In some studies reviewed there was no evidence that assessment or adjustment in the analyses for timing of sampling had occurred.

5.1.2. Endotoxin expression

Comparing levels of endotoxin between studies can be difficult because of the inconsistently applied reporting of the endotoxin found in domestic dust. In the studies reviewed here, endotoxin measured from settled dust has been expressed as a concentration (EU/mg), a load (EU/m^2), as both or as other units of measurements including ng/mg, $\mu g/m^2$ and EU/ml. Most often, endotoxin is reported as a concentration. What makes the evaluation of endotoxin results difficult across studies is the lack of sufficient information to convert load to concentration or vice versa. It has been suggested that expressing endotoxin as a concentration does a poor job of characterizing its total burden and an alternative expression, endotoxin load (EU/m^2 of vacuumed area), should also be reported as well as it may more accurately describe the burden of exposure (77)

Table 3. Cross-sectional studies examining associations between domestic endotoxin and childhood asthma or wheeze

Location, reference	Study population	Outcome (s) measured	Endotoxin measurement (source)	Findings[1]
1. Hong Kong ,54	Asthma clinic population 5-18 years (n=144)	*Wheeze* (past 12 months); *wheezing attacks* (0, 1-3, 4-12, >12); Exhaled *nitric oxide*; Spirometry: FEV_1[4] FVC[5], PEF[6]	EU^2/mg and EU/m^2 measured (dust from child's mattress, bedroom floor and living room	*Wheezing attacks* associated with mattress EU/mg (p for trend =0.044) Spirometry: NS Exhaled nitric oxide: NS
2. Humboldt, Canada, 72	Children, 6-18 years with asthma or wheeze in past 12 months (n=98) Recruited as part of a case control study	*Wheeze episodes* 0, 1-3, ≥ 4 *Medication use* 0, bronchodilator or inhaled steroid, multiple medication use *Sleep disturbance with wheeze School absenteeism*: ≥ 3 days for chest illness *Tobacco smoke exposure*: ≥ 1.24 ng/ml in salivary cotinine	EU/mg and EU/m^2 measured (dust from play area and child's mattress)	School Absenteesim; Effect modification by tobacco smoke exposure ($p<0.05$) and age ($p<0.05$) Children <12 years of age: ↑ mattress EU/m^2 had less school absenteeism. aOR: 0.27 (0.09-0.81) ↑ EU/m^2 in play dust and low smoke exposure associated with less absenteeism
3. Albania, Italy, New Zealand, Sweden, United Kingdom, 83	Children 9 to 12 years (n=840)	*Asthma:* Physician diagnosed asthma *Current wheeze*: Any report of wheeze or whistling noise in chest in past 12 months *Atopy*: specific IgE > 0.35kU/L ≥1 allergen	EU/mg and EU/m^2 (living room floor dust)	*Overall Asthma*: ↑EU/m^2 aOR: 0.53 (0.29-0.96) *Current Wheeze*: ↑EU/mg aOR: 0.77 (0.64-0.93 *Atopy* :NS after adjusting for covariates
4. Austria, Germany, The Netherlands, Sweden, and Switzerland, 71	Children 5-13 years of farmers and non farmers (n ~ 440)	*Asthma:* Physician diagnosed asthma or obstructive bronchitis *Current wheeze*: Wheezing in the past 12 months	EU/g measured (dust from the child's mattress)	*Asthma:* NS *Current wheeze*: NS
5. Multi-city, USA, 55	All ages (n = 831 homes, 2456 people)	*Asthma:* Physician diagnosed asthma *Asthma past 12months Asthma medication* past year *Ever wheeze* *Current wheeze:* - past 12 months - past month	EU/mg measured as dichotomous variables Bedroom floor = >16.6 Mattress= >19.6 Family room floor = >33.9	*Wheezing ever*: Bedroom floor: aOR: 2.27 (1.28-4.00): *Wheezing past month*: Bedroom floor: aOR: 1.95 (1.05-3.61) Associations with endotoxin driven by adults with no statistically significant associations among children <18 years. Interactions between atopy and endotoxin for outcomes tested (NS)
6. Puerto Rico, 82	Children 1 to 17 years with asthma (n=226)	*Asthma symptoms*: frequency (none/ a lot) *Visit to physician for asthma: (*yes/ no)	EU/mg measured (dust from bedroom floor)	*Asthma Symptoms*: ↑EU/mg with no symptoms (p = 0,003) *Physician visits*: ↑EU/mg with less than 3.5 visits (p=0.02)
7. Saxony Anhalt Germany, 85	Children 5-14 years from 2 cross-sectional surveys (n= 444) 50% atopic	*Asthma:* physician diagnosed asthma *Wheeze*: Report of wheeze in past 12 months *Atopy*: allergen specific IgE 0.35 kU/L ≥1 allergen	EU/m^2 measured (dust from living room floor)	*Asthma:* NS *Wheeze:* NS *Atopy:* ↑EU/m^2 associated with ≥ 2 specific IgE allergen, aOR: 0.80 (0.67-0.97)
8. Austria, Germany, and Switzerland, 12	6-13 years (n=812) Farming and non-farming children	*Atopic asthma or wheeze:* Report of asthma or wheeze and allergen specific IgE 0.35 kU/L ≥1 allergen	Quartiles (not defined) of EU/mg and EU/m^2 (dust from child's mattress)	*Overall Atopic asthma*: ↑ EU/m^2 aOR 0.48 (0.28-0.81) *Atopic wheeze*: ↑ EU/m^2 aOR = 0.62 (0.39-0.99) *Non-atopic wheeze*: ↑ EU/m^2 aOR = 1.82 (1.04-3.18) among children from non farming households

Adjusted odds ratios (aOR) and 95% confidence intervals (CI) are presented whenever reported.[1] Abbreviations: [2]endotoxin units, [3]non significant, [4]forced expiratory volume at 1 second, [5]forced vital capacity, [6]peak expiratory flow rate, [8] standard error

5.1.3. Selection of site in home for collection of vacuumed dust for endotoxin

The findings of associations between asthma and endotoxin do not appear to be specific to any one location in the home. The two most common sites for sampling of settled dust are high activity areas such as play area or living room floors and the child's mattress. Endotoxin levels have been shown to vary between areas in the home (50, 55, 65, 78) with mattress endotoxin levels tending to be lower than floor endotoxin levels (55, 65, 78) Although the levels of endotoxin are lower in mattress dust, the importance of reporting mattress endotoxin, which is in close proximity to the breathing area of the child, requires further evaluation particularly if personal sampling is not available. Predictors of endotoxin have been shown to vary for different locations in the home (42-43) and results from those sites most frequently used by the child should receive important consideration when planning the study design,

Finally, endotoxin from different sources may be of different molecular composition and associations between endotoxin and respiratory outcomes may be dependent on the heterogeneity and diversity of LPS in the collected dust (23) A study conducted to characterize LPS within homes showed that there were qualitative differences regarding the type of LPS depending on the location of sampling with different areas of the home containing different types of bacteria (78) Endotoxin activity can be different among the types of LPS (79) These variations in molecular structure could have the potential to affect human health differentially as demonstrated in a study that assessed the nature of endotoxin in schools (80)

Total LPS in this study was associated with an increased risk of attacks of breathlessness in students and upon characterization of the types of LPS by 3-OH-GC-MS techniques, the shorter lengths of LPS were inversely associated with attacks of breathlessness.

5.2. Measuring respiratory outcomes

One explanation for the inconsistencies in results among studies may be the use of different definitions of the outcomes assessed in studies. This is especially noteworthy when differences in the results of studies between preschool children and elementary school children are evaluated. In studies of preschool children, wheeze was typically the outcome considered, with increased risk of wheeze associated with higher endotoxin levels. The use of wheeze in this population reflects the difficulty in obtaining an accurate diagnosis of asthma in preschool children. However, it has been fairly well documented that the majority of children will out grow wheeze (early transient wheeze) without developing asthma (81) When persistent or recurrent wheeze (marker of future asthma) was used as outcomes, researchers in one study found a protective effect for endotoxin (63) while researchers in four other studies found direct associations (60-61, 64, 67) Perhaps the most conflicting information comes from cross-sectional studies where current wheeze (a good indicator for current respiratory symptoms) assessed at the same time as domestic endotoxin showed both positive (54-55) negative (82-83) or no associations (84-85) with increased endotoxin levels in the home. In schoolchildren, diagnosed asthma is usually considered to be the outcome of interest. Similarly to wheeze increased risk, no association, and protective risks for asthma have all been reported with this age group. It may be that early wheeze, later wheeze, and asthma later in life are three different phenotypes of obstructive lung disease in children (81) Asthma diagnosis by questionnaire however, has shown a high positive predictive value (0.89) with physician assessment (86) where as wheezing that is identified by parents may be quite different from wheezing assessed by a physician. Parents of children 4 months to 15 years agreed with clinicians on 45% of occasions if wheeze was present in the child. Reliance on a questionnaire or interview report of doctor diagnosed asthma without further confirmation of the condition could contribute to further misclassification of asthma in study subjects (87)

5.3 Assessing interactions

The variability of the findings between studies assessing the effects of domestic endotoxin on the development and severity of childhood asthma suggest a role of other factors or mediators that when present could influence the very nature of this relationship. Specifically, allergic status of the child or parent may influence the respiratory response of children to household endotoxin. Certain airborne exposures (indoor and outdoor) occurring in conjunction with endotoxin could have an effect on the associations between childhood asthma and domestic endotoxin exposure. As well, the response to endotoxin could be ultimately dependent on the inherited genetic make-up and the adaptive immunity of the individual.

5.3.1 The role of atopy

The association between endotoxin and asthma may be dependent on the allergic status of the child or parent. Allergy was a major consideration of most investigators with many of the studies assessing the development of atopy in the index cases and/or subsequently assessing its role as a mediator in the relationship between asthma and endotoxin (64-65, 67-69) Many of the cohort studies reported here enrolled children at birth or infancy if the parent had an atopic history or asthma (60-61, 63-65) Assessing interactions between endotoxin exposure and allergic status of child or their parental history of allergic disease resulted in either no interaction observed (64-65) or if interaction was present, endotoxin was found to be significantly associated with wheezing in those children with an allergic history (67-69) In one case control study evaluating school absenteeism due to a chest illness in cases and controls, found an increased risk of school absenteeism with higher endotoxin levels in the home, but only among those children with atopic asthma (43)

Protective associations between endotoxin with asthma or wheeze have been reported when atopic status was considered. One study identified inverse relationships with high endotoxin concentrations and asthma wheeze in a farming population with generally high endotoxin exposures. Results from this cross-sectional study from Germany, Switzerland and Austria found differences in the association between mattress endotoxin for both atopy and atopic asthma versus non-atopic asthma and atopic wheeze. (12) Among the overall study population, there were statistically significant inverse associations between endotoxin and atopic asthma and wheeze. (12) However, when considering children from non-farming households, there was a positive association between non-atopic wheeze and endotoxin. (12) In a case control study of a semi-rural population in Palestine (70) where cases were defined by the presence of wheeze in the past 12 months, there was a statistically significant inverse association between medium endotoxin levels from the mattress and being a non-sensitized case compared to a non-sensitized control. (70) Thus, it seems that atopy does affect the relationship between endotoxin levels in the home and asthma or wheeze and requires consideration for effect modification in all studies of asthma and endotoxin exposure.

5.3.2 Other environmental exposures

As the household dust collected from sampling is a mixture of multiple contaminants and therefore, other dust components besides endotoxin may also be directly associated with asthma or may indirectly interact with endotoxin to affect the presence of asthma or asthma symptoms. It is possible that the combinations of microbial components in the environment could have a modulating effect on the immune response for atopy and asthma (18, 88) Several studies have assessed other components of collected dust, including β (1,3)- glucan, allergens, and gram-negative bacteria (40, 54, 62, 74, 85, 89) Modest or no correlations with endotoxin have been found with these co-existing contaminants of dust. As well, interactions between early exposure to dust components β (1,3)- glucan,

allergens and endotoxin were found not to be important when asthma and allergies were assessed at 10 years of life in the study of children in Oslo (62) A possible limitation of studies to date is that not all of the major components in house dust, which can also include outdoor contaminants, have been characterized simultaneously.

There is however, some information about other components of indoor dust in the environments of children that should be further evaluated. Airborne and settled dust from schools and daycares has been shown to consist almost exclusively of gram-positive bacteria. The levels of endotoxin containing gram-negative bacteria were markedly less abundant in these environments (10^2-10^4 fold less density) and overall levels of endotoxin were much less when compared with five animal sheds (41) In one study of Polish dwellings, over 160 different species of bacteria and fungi were isolated from the air of examined homes (90) Van Strein *et al.* found that muramic acid, a major component of bacterial peptidoglycan found in both gram positive and gram negative cell walls, is associated independently from endotoxin with lower wheeze in the past 12 months (91) In this study endotoxin and muramic acid were correlated. Smit *et al.* provide supporting evidence for a role for mycobacterium in the prevention of asthma and allergic disease (88) Another example of the importance of considering other exposures is found in a study of children from the Netherlands (74) In this study that investigated the association between endotoxin, (1-3) β-D Glucan, and peak flow variability in children, univariate analysis showed statistically significant associations between endotoxin and peak flow variability. However, after adjusting for *Der* p 1, pets and carpet, the association became non-significant. Independent of endotoxin, (1-3) β-D Glucan continued to have statistically significant associations in the adjusted analysis among subjects who were symptomatic and atopic as well as asthmatic and atopic. To date, the importance of interrelationship of the myriad of biologically active components in household dust and their effect with endotoxin in the development of asthma or for affecting asthma severity requires further evaluation.

While components of the dust may not be fully characterized and evaluated, potential interaction of some other exposures have been studied, including particulate matter from traffic (64), presence of dogs (63), and smoking (72) Among infants with a parental history of atopy, dog ownership modified the effect of endotoxin for both recurrent wheeze and any wheeze (63) Higher endotoxin levels in the home were associated with a reduced risk of wheeze, but only in the presence of dogs. When at least two dogs were present, the associations were statistically significant while when one dog was present, the associations were at least of borderline statistical significance, indicating a dose-response effect. A separate study using a high risk birth cohort identified a synergistic effect between home endotoxin and traffic-related particles on the outcome of persistent wheezing at 36 months (64) When both high levels of traffic related particulate and endotoxin were present, there was an increased risk of persistent wheeze. In a third study the effect of endotoxin on school absenteeism was modified by exposure to tobacco smoke (72) Among children with asthma or wheeze, there was a reduced risk of school absenteeism associated with endotoxin but only when there was low tobacco smoke exposure. As noted in the study by Braun-Fahrlander *et al.*, living or not living on a farm also modified the effect of endotoxin exposure in atopic and non-atopic subjects with asthma (12)

5.3.3. Gene environment interactions

Finally, another consideration when looking at the results from studies examining the association between endotoxin and respiratory outcomes is gene-environment interactions. More than 100 genes have been associated with asthma or allergic phenotypes in recent studies (92) Environmental factors including endotoxin are capable of modifying these associations. The genes *CD14*, *TLR4* and *MD2* play an important role in the human immune response to endotoxin exposure. CD14 is the receptor for the lipopolysaccharide (LPS) and is required for the LPS-induced response in human (93) TLR4 is the signaling receptor for LPS (94) with TLR4 is required for intra-molecular signaling. (95-96) Effects of polymorphisms in the genes including *CD14*,and *TLR4* on the association between endotoxin and allergic phenotypes (equivalently gene-environment interaction) have been investigated in several studies (97) Only a few studies have investigated the effect of these polymorphisms in the association between endotoxin and asthma phenotypes. A polymorphism in the *TLR2* gene (*TLR2*/−16934) has been shown to be associated with asthma and current asthma symptoms in farmers' children. (98) This association was not observed in non-farmers' children. In addition, this association was not significant in children exposed to high (median: 41.3 EU/mg) and low (median: 17.4 EU/mg) endotoxin concentrations (98) Gene-environment interaction between the *CD14* polymorphism (C-260T) and household endotoxin levels (EU/m^2) on current asthma was reported in the Barbados Asthma Genetics Study (99) In a case-control analysis, the TT genotype in the C-260T polymorphism was protective against current asthma compared to CC/CT genotypes for low household endotoxin levels ($\leq$ 44,000 EU/m^2) and was a risk factor of current asthma for high endotoxin levels greater than 44,000 EU/m^2 (99) In this study (99), household endotoxin levels were much higher than those reported in studies conducted in Canada, Europe, and the USA indicating that higher levels of endotoxin might be required to observe a statistically significant gene-environment interaction in the association between endotoxin and asthma phenotypes.

6. CONCLUSIONS

The results of associations between domestic endotoxin and respiratory symptoms in the epidemiological studies reviewed vary such that there appears to be an increase in risk of wheeze in early childhood, and limited evidence to support an association with asthma later in childhood.

In many of the cohort studies, cohorts are too young to identify a relationship between early endotoxin exposure and later development of asthma, although for the

two cohort studies examining children at 4-7 years, endotoxin is a risk factor for wheeze but the association with asthma is not present (59, 61) The relationship between the measurement of endotoxin and the development of wheeze appears to diminish over time. Thus it seems likely that the importance of exposure to endotoxin for developing asthma or wheeze is age dependent with weakening of the association with early exposure as the child ages.

Both protective and adverse effects are reported with higher endotoxin levels in the home. At present, associations with asthma are inconclusive and would suggest that while a case can be made for an inverse association between high levels of domestic endotoxin and atopy, domestic endotoxin has modest importance for asthma in the absence of atopy or genetic predisposition. For children who have asthma, it may be a different matter. High doses of endotoxin in the home are generally noxious and can result in increased asthma severity as evidenced by increased emergency room visits, increased asthma medication and missing school because of chest illnesses in the past year. The cut off levels of domestic endotoxin in vacuumed dust that are associated with asthma-related symptoms are not known but it appears that exposures greater that 80 EU/mg of settled dust may evoke symptoms in children. However, median levels of endotoxin load in most homes are markedly lower than this, suggesting that the responsibility of domestic endotoxin for asthma symptoms in children may not be of the most concern except for those asthmatic children living in farming environments where endotoxin levels are typically much higher. Currently, there are no reports from intervention studies where remediation of endotoxin levels in homes of asthmatic children has been undertaken.

A major limitation of current studies is the imprecise measurement of indoor endotoxin. Vacuuming settled dust provides very crude estimates of what is "at the nose" of the child. At best we can only hypothesize that these exposures have some importance for childhood asthma. As well, the lack of simultaneous investigation of other respirable particles in the home including molds and gram-positive bacteria that have the potential to act in an additive or synergistic manner with endotoxin limits our understanding of the role of domestic air in asthma development and management for children.

The dose and quality of endotoxin and the timing of the endotoxin exposure in the home may be critical in determining whether or not endotoxin is protective for asthma. The findings by Ege *et al. (*84) and Douwes *et al. (*100) would suggest that we need to look much earlier in the child's life at the effect of endotoxin exposure than we have currently. It is possible that epigenetic effects during the prenatal period or in newborn life may in fact play a role in the protective effects for asthma that are found later for school age children. The lack of information about prenatal exposures to endotoxin and the measurement of endotoxin levels prenatally may in fact be very important in determining the role of bacterial components in preventing or stimulating asthma illness. As well multiple assessments of exposures are required. Many of the studies reported here measured endotoxin at only one time point and a decision as to the important "exposure window" is thus far, unknown.

It is possible that there is a particular level of exposure that is relevant for protection or exacerbation and that these exposures need to be of certain strength and of sufficient duration to create an effect. There is no doubt that asthmatic children will experience the effects of endotoxin when they are exposed later in life but it may be the epigenetic effect of high endotoxin exposure in early life that protects some children from developing asthma.

7. RECOMMENDATIONS FOR FUTURE RESEARCH

Asthma continues to be a major reason for hospitalization, medical expenditures by families and poorer quality of life for children. Endotoxin has been highlighted as a contributing environmental exposure that can either limit disease occurrence or result in poorer health. To date, the findings are confusing and somewhat contradictory. Based on this review of the literature we have highlighted several areas for focus in future epidemiological research:

1. Characterize the types of endotoxin at a molecular level and examine the specific molecular characteristics that protect against asthma or exacerbate asthma. Identifying the particular species of gram-negative bacteria in homes may be important in determining which bacteria may be primarily responsible for both the protective and inflammatory effects seen in studies of children. Detection of antibodies against Enterobacteria in sera of children may be useful in determining the nature of protective and adverse effects of endotoxin accompanying certain domestic environments (101)

2. Provide more precise measurement of endotoxin exposure in children using personal sampling. What may be in the floor dust or found in stationary monitoring systems is not necessarily what is inhaled by the child. Important cutoff values for endotoxin levels that are associated with wheeze and asthma should be validated and used consistently in epidemiological studies. Complete studies with endotoxin measured at several time points in childhood including during the prenatal period. Lung development occurs prior to birth and objective studies of the influence of environmental factors of the child's home prior to birth are required.

3. Future epidemiological studies must consider investigating potential interactions between other environmental exposures and endotoxin in the investigation of the role of endotoxin in childhood asthma. Generally other bacterial species and molds commonly found in household environments should be assessed concomitantly with endotoxin. As well, potential gene-environment interactions with endotoxin require consideration. The role of atopic disease in the relationship between domestic endotoxin exposures and asthma requires clarification.

4. Use of other methods to validate asthma besides questionnaire report of physician diagnosed asthma is warranted. However, this is expensive, time-consuming and may be difficult in large epidemiological studies. More convincing evidence of the validity of questionnaire reports of diagnosis in studies is needed. For cohort studies, which appear to be the most useful in evaluating the importance of endotoxin exposure for childhood asthma, objective confirmation of diagnosis and symptoms is paramount.

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