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Characterization of Lipopolysaccharides Present in Settled House Dust

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Endotoxin is biologically active lipopolysaccharide (LPS), a family of macromolecules with similar chemical structures that are the major lipid of the outer membrane of gram-negative bacteria. Environmental endotoxin is ubiquitous and has been detected in settled house dust and home air (7, 10, 15, 22). Thus, everyone is constantly exposed to at least low levels of environmental endotoxin.

House dust endotoxin has been associated with asthma severity in adults and children (14, 15, 25). Park et al. reported that early-life exposure to house dust endotoxin is associated with an increased risk of repeated wheezing during the first year of life (20). These observations indicate that exposure to low levels of home endotoxin induces airway inflammation and may aggravate or contribute to the development of airway disease in susceptible individuals. Recently, a possible protective effect of early-life endotoxin exposure on the risk of childhood asthma has also been attracting considerable attention (3, 13, 29, 30). Several reports suggest that early-life endotoxin exposure may induce immune polarization toward a Th1 cytokine profile that may reduce the risk of atopic diseases in later life. However, more information on the critical timing and routes of exposure, the necessary dose, and a characterization of the LPS encountered in the environment may still be required to understand the biological impacts of endotoxin exposure.

LPS consists of polysaccharide and lipid A components. Lipid A, the endotoxic component, shows a unique structure with bisphosphorylated β-(1-6)-N-glucosamine disaccharide as a backbone. This backbone structure typically carries 4 mol equivalents of 3-hydroxy fatty acids (3-OHFA) with nonhydroxylated fatty acids ester linked to one or more of the 3-hydroxy groups (24). The 3-OHFA are a unique component of the lipid A molecule, making them well suited as a chemical marker for LPS (11, 12). Gram-negative bacteria from different genera may contain 3-OHFAs of differing chain lengths (31). Furthermore, the biological activity of endotoxin is dependent on the structure of lipid A (19, 23, 26–28). Takada et al. (28) demonstrated that the presence of 3-OHFA groups on the bisphosphorylated β-(1-6)-N-glucosamine disaccharide backbone is required for endotoxin activity with Limulus amoeboocyte lysate. Qureshi et al. (23) showed that the fatty acid composition determines, in part, the endotoxin activity of lipid A in mammals, and recent observations suggest that lipid A structure may determine specificity for Toll-like receptors 2 and 4 (19). Therefore, data about the quantity and quality of LPS in environmental samples, in addition to their activity in the Limulus assay, may be critical to understanding the biological effects of environmental endotoxin exposure.

Our objective in this study was to characterize the LPS in house dust samples. We analyzed the quantity and distribution of different chain lengths of 3-OHFA, determined by gas chromatography-mass spectrometry (GC-MS), in house dust samples collected in the Boston area. We used the 3-OHFA distribution as an indicator of variations in microbial flora characteristic of differing environments and examined the specific activity (endotoxin units [EU] per nanomole of LPS) of dust samples by comparing the Limulus assay activity of the
samples with their LPS content determined by assay for 3-OHFA. We hypothesized that each type of dust sample (bed, bedroom, family room, and kitchen), samples from different seasons, and samples associated with certain home characteristics (e.g., pets) would have characteristic flora and that this would be reflected in differences in 3-OHFA distribution and specific activity among the samples.

MATERIALS AND METHODS

Origin of house dust samples. We analyzed settled house dust collected for two observational studies: a birth cohort study of home allergens and endotoxin and development of childhood asthma (study 1) (9, 20) and a longitudinal study of home allergens and endotoxin (study 2) (4, 21). Study 1 is an ongoing, longitudinal, closed birth cohort study of children born to parents with histories of allergens or asthma. Recruitment criteria and methods have been previously described (9). Study 2 was designed to characterize seasonal variation in home allergens, fungus, and endotoxin levels. We recruited 20 subjects from the faculty, staff, and students at the Harvard School of Public Health who lived in the greater Boston, Mass., area. Each participant in the 20 homes answered a home characteristic questionnaire and collected three dust samples (bedroom, bed, bedroom, and kitchen floor) on prescheduled days every month from April 1995 through July 1996 as previously described (4, 21). Thus, we had four types of samples (bed, bedroom floor, family room, and kitchen) from study 1 and three types (bed, bedroom floor, and kitchen) from study 2.

All samples were collected by using the previously described protocol (5, 9). We used a Eureka Mighty-Mite vacuum cleaner (model 3621; The Eureka Co., Bloomington, Ind.) modified to hold cellulose extraction thimbles (19 by 90 mm) to collect house dust. In the bedroom, 2 m² of the bedroom floor surrounding the bed was vacuumed for 5 min. For bed dust, all layers of bedding were vacuumed for 5 min. In the family room, the seat cushion, arms, and back of the upholstered chair where the baby spent the most time were vacuumed (for 2.5 min) along with 2 m² of the surrounding floor (for 2.5 min). In the kitchen, the edges of the floor under cabinets, around the refrigerator, and under the sink were vacuumed for 5 min. Within 24 h after collection, we weighed and sifted dust through a 425-μm mesh sieve, weighed the fine dust, and made aliquots for various analyses—allergens, culturable fungi, and endotoxin. Dust samples were stored at −20°C until analysis. An assay for endotoxin activity in the Limulus assay was done only if there was sufficient dust remaining after all of the other assays had been performed. Of the samples with dust remaining after completion of the Limulus assay, 203 were selected at random and sent to Lund, Sweden, for 3-OHFA analysis by GC-MS.

Assay for endotoxin activity of dust samples. The endotoxin activity in dust samples was measured by the kinetic Limulus assay with the resistant-parallel-line estimation method as previously described (17, 18). Limulus amebocyte lysate (LAL) was obtained from BioWhittaker (Walkersville, Md.); reference standard endotoxin was obtained from the U.S. Pharmacopoeia, Inc. (Rockville, Md.); and control standard endotoxin was obtained from Associates of Cape Cod (Woods Hole, Mass.). Control and reference standards and field samples were serially diluted for endotoxin analysis in a standard buffer (0.01% triethylamine (Woods Hole, Mass.). Control and reference standards and endotoxin activity in dust samples from study 1, we observed positive, statistically significant (P < 0.05) correlations between endotoxin activity and the various
3-OHFs (range, 0.20 to 0.55). The correlation was strongest for the C_{12:0} and C_{14:0} 3-OHFs. In study 2, positive correlations of endotoxin activity with short-chain 3-OHFs were also observed, but the strongest and only significant positive correlation was with the C_{10:0} 3-OHFA at α = 0.05. However, the C_{14:0} 3-OHFA was significantly (P = 0.08) and positively correlated with endotoxin activity at α = 0.1. The C_{16:0} 3-OHFA was significantly (P = 0.04) negatively correlated with endotoxin activity, and the C_{18:0} 3-OHFA was not correlated with endotoxin activity. In general, the correlations of short-chain 3-OHFs with endotoxin activity were consistently positive while those of long-chain 3-OHFs were more weakly positive or negative.

There was no difference in the mean levels of C_{10:0} to C_{14:0} 3-OHFs between studies in the mixed model with random effect of home and fixed effects of season and sample type.

### TABLE 2. Correlation between concentrations of 3-OHFs and endotoxin activity

<table>
<thead>
<tr>
<th>Endotoxin and carbon length of 3-OHFA parameter</th>
<th>Correlation coefficienta</th>
<th>Study 1 (n = 137)</th>
<th>Study 2 (n = 53)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_{10:0} (nmol/mg of dust)</td>
<td>0.27b</td>
<td>0.39b</td>
<td>0.35b</td>
</tr>
<tr>
<td>C_{12:0} (nmol/mg of dust)</td>
<td>0.62b</td>
<td>0.35b</td>
<td>0.30b</td>
</tr>
<tr>
<td>C_{14:0} (nmol/mg of dust)</td>
<td>0.68b</td>
<td>0.50b</td>
<td>0.52b</td>
</tr>
<tr>
<td>C_{16:0} (nmol/mg of dust)</td>
<td>0.66b</td>
<td>0.63b</td>
<td>0.77b</td>
</tr>
</tbody>
</table>

a Spearman correlation.

b P < 0.05.

c P = 0.08.

Samples from study 2 had significantly greater concentrations of C_{10:0} (P = 0.015) and C_{18:0} (P = 0.003) 3-OHFs and of total LPS (P = 0.03) than did those from study 1. There was no study-by-sample type interaction for C_{16:0} and C_{18:0} 3-OHFs and total LPS. The study-by-sample type interaction for endotoxin activity was marginally significant (P = 0.052). However, there was a significant (P = 0.02) study-by-sample type interaction for LPS specific activity, suggesting that the effect of sample type on specific activity was different by study. Therefore, we performed the analyses of season and sample type with separate models for each study.

Table 3 shows the adjusted geometric mean concentration of the 3-OHFs (nanomoles per milligram) in house dust samples. The concentration of C_{10:0} was consistently among the lowest, and the concentration of C_{16:0} was consistently the highest, of the five measured 3-OHFs. The level of C_{10:0} 3-OHFs was highest in kitchen dust in both studies, but the difference was not statistically significant. In study 2, both bed and bedroom floor dust samples had significantly higher concentrations of 3-OH C_{16:0} (P < 0.02) than did kitchen dust; bed dust had significantly greater and bedroom floor dust had borderline significantly greater (P = 0.06) 3-OH C_{18:0} concentrations than did kitchen dust. The concentrations of C_{16:0} and C_{18:0} were not significantly different between bed dust and bedroom floor dust. These results indicate that there is within-home variation in the concentrations of different chain length 3-OHFs in house dust, which suggests that the type of LPS, and thus the microbial flora, in house dust varies with the area sampled within a home.

The mixed model for study 1 also showed significant overall seasonal variation in 3-OH C_{12:0} (P = 0.04) and 3-OH C_{18:0}

### TABLE 3. Nanomoles of 3-OHFs in house dust samples from two studies

<table>
<thead>
<tr>
<th>Sample type or season</th>
<th>Adjusted geometric mean no. of nmol/mg of dust</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C_{10:0}</td>
</tr>
<tr>
<td>Study 1</td>
<td></td>
</tr>
<tr>
<td>Bedroom floor</td>
<td>0.020</td>
</tr>
<tr>
<td>Kitchen floor</td>
<td>0.033</td>
</tr>
<tr>
<td>Family room</td>
<td>0.022</td>
</tr>
<tr>
<td>Spring</td>
<td>0.016</td>
</tr>
<tr>
<td>Summer</td>
<td>0.020</td>
</tr>
<tr>
<td>Fall</td>
<td>0.029</td>
</tr>
<tr>
<td>Winter</td>
<td>0.023</td>
</tr>
<tr>
<td>Study 2</td>
<td></td>
</tr>
<tr>
<td>Bedroom floor</td>
<td>0.018</td>
</tr>
<tr>
<td>Kitchen floor</td>
<td>0.036</td>
</tr>
<tr>
<td>Bed</td>
<td>0.016</td>
</tr>
<tr>
<td>Spring</td>
<td>0.030</td>
</tr>
<tr>
<td>Summer</td>
<td>0.029</td>
</tr>
<tr>
<td>Fall</td>
<td>0.016</td>
</tr>
<tr>
<td>Winter</td>
<td>0.016</td>
</tr>
</tbody>
</table>

a Results shown are the least-squares means from analysis of log-transformed data, with the sample type means adjusted for season and the seasonal means adjusted for sample type.

b P < 0.05.

c P = 0.08.
3-OHFA (P = 0.03) concentrations. Adjusted for multiple comparisons, the 3-OH C12:0 3-OHFAs concentration in summer was marginally significantly (P = 0.053) higher than that in the winter, and dust collected in the fall contained significantly (P = 0.03) more, and dust collected in the summer contained borderline significantly (P = 0.056) more, 3-OH C18:0 than did dust collected in the winter (Table 3). The seasonal pattern of 3-OH C12:0 and 3-OH C18:0 was similar in study 2 but did not reach statistical significance, possibly because of the smaller sample size.

Mean endotoxin activity in dust (Table 4) varied significantly with sample type in both studies. Endotoxin activity was significantly (P < 0.021) lower in bedroom floor dust than in kitchen floor dust in both studies and was significantly (P < 0.0001) lower in bed dust than that in kitchen floor dust in study 2. Bedroom floor and family room dust samples had similar activity levels (P = 0.63) in study 1, while bedroom floor dust had marginally (P = 0.049) higher endotoxin activity than did bed dust in study 2. Seasonal variation was marginally significant (P = 0.05) for study 1, and adjusted multiple comparison suggested that the summer level was significantly higher than the winter level (P = 0.04). In study 2, seasonal variation was significant (P = 0.01); spring, summer, and fall had similar endotoxin activity levels, but only spring had significantly (P = 0.01) greater endotoxin activity than winter.

The amount of total LPS (nanomoles per milligram of house dust) also varied significantly (P = 0.01) with sample type in study 2. However, the order of the total LPS level was reversed from that of endotoxin activity. Both bed and bedroom floor dust samples had greater amounts of total LPS than did kitchen dust. Adjusted multiple comparisons showed that the total amount of LPS was significantly (P = 0.008) higher in bed dust than in kitchen floor dust while endotoxin activity was significantly (P < 0.0001) lower in bed dust than in kitchen floor dust (Table 4). LPS concentration variation with season was borderline significant in study 1 (P = 0.059), with fall greater than winter (P = 0.054). LPS concentration did not vary significantly with season in study 2 (P = 0.76).

The specific activity of LPS in house dust (Table 4) varied significantly with sample type in both studies, with kitchen dust significantly more active per nanomole of LPS than any other dust (study 1, comparison with bedroom floor [P = 0.03] and family room [P = 0.02]; study 2, comparisons with bed dust and bedroom floor dust [P < 0.001]). Also in study 2, LPS in bed dust had significantly (P = 0.01) lower specific activity than that in bedroom floor dust. LPS in family room and bedroom floor dust had similar specific activities. Seasonal variation of the specific activity of LPS in dust was not significant in study 1 and was borderline significant in study 2 (P = 0.08, spring > winter).

In study 2, 3-OH C18:0 was significantly (P = 0.03) lower in apartments (0.09 nmol/mg) than in other homes (0.16 nmol/mg), controlling for season and sample type. Presence of dogs or cats at home was not associated with significant changes in the amount of specific 3-OHFAs.

**DISCUSSION**

We found that LPS in bed dust had a predominance of longer-chain 3-OHFAs, while kitchen floor dust was characterized by increased amounts of short-chain 3-OHFAs. Bedroom floor and family room dust resembled bed dust more closely than kitchen dust. Similarly, kitchen dust was more active in the Limulus assay than was bed dust, and bedroom floor and family room dust samples were intermediate. These data demonstrate that LPS in house dust varies qualitatively by location within homes.

We observed that concentrations of longer chain length 3-OHFAs and of total LPS were highest in the fall. This finding indicates that LPS in house dust may vary qualitatively across seasons, suggesting different microbial flora in dust from different seasons.

Our results confirm our previous observation (27) that different chain lengths of 3-OHFAs in LPS are differently correlated with endotoxin activity detected by the Limulus assay. Shorter-chain (C10:0, C12:0, and C14:0) 3-OHFAs are positively correlated with endotoxin activity, while longer-chain (C16:0 and C18:0) 3-OHFAs tend to have lower, no, or even negative correlations with endotoxin activity in the Limulus assay. The predominance of short-chain fatty acids in kitchen dust therefore accounts for the otherwise paradoxical finding that kitchen dust contained the smallest amounts of LPS but the largest amounts of endotoxin bioactivity.

The observation that kitchen samples had significantly more endotoxin activity and higher LPS specific activities and had the highest fraction of C10:0 relative to those from other rooms suggests that the kitchen may be different from other environments within the house so that it supports different microbial
flora. It is likely that the increase in C_{10:0} is an indication of increased organisms that grow in pooled water or plumbing, such as pseudomonas-like organisms that are rich in C_{10:0} and C_{12:0} (1).

We did not observe that the presence of pets such as dogs and cats at home changes the microbial flora in house dust, as we had expected on the basis of previous reports of higher endotoxin levels in the presence of pets. Andersson et al. (1) demonstrated that dust collected from cattle barns and swine confinement buildings had different microbial flora from that collected from schools and day care centers, suggesting that animal and human sources have characteristic flora. However, our failure to find different gram-negative flora between homes with and without pets may result from the small number of homes with pets in the data analyzed. On the other hand, our data showed that apartments in buildings with three or more units had significantly decreased amount of 3-OH C_{18:0} compared with single-family or duplex houses. These data suggest that apartment dwellers may be exposed to different types of LPS compared with people living in single-family or duplex homes.

It is known that biological activities of LPS from different species of bacteria may vary qualitatively. For example, *Rhodopseudomonas sphaeroides* LPS is nontoxic but retains significant immunostimulatory activity and is capable of inactivating suppressor T cells and of preventing tolerance to polysaccharide antigens (2, 23). Thus, nontoxic, as well as toxic, LPS may be able to modulate the response to inhaled allergens (6). The unique immunological properties of nontoxic LPS appear to be determined by the 3-OHFA composition of *R. sphaeroides* lipid A. Merely determining the endotoxic activity of house dust with an extract from horseshoe crab blood may miss important biological activities in humans. Andersson et al. (1) showed that 3-OH C_{14:0} correlated better with acute irritant reactions than did other 3-OHFA in a variety of environmental dust samples. However, endotoxin exposure has been implicated as a factor that may modulate immune system development, especially by affecting polarization of Th1 and Th2 cells (3, 8). It is a factor that may modulate immune system development, especially by affecting polarization of Th1 and Th2 cells (3, 8).

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