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# Characterization of Lipopolysaccharides Present in Settled House Dust

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**The 3-hydroxy fatty acids (3-OHFAs) in lipopolysaccharides (LPS) play an important role in determining endotoxin activity, and childhood exposure to endotoxin has recently been associated with reduced risk of atopic diseases. To characterize the 3-OHFAs in house dust (HD), we used gas chromatography-mass spectrometry to assay 190 HD samples. Dust from beds, bedroom floors, family rooms, and kitchen floors was collected as part of a birth cohort study of childhood asthma (study 1) and a longitudinal study of home allergen and endotoxin (study 2). We also measured endotoxin activity with a *Limulus* assay and computed specific activity (endotoxin activity per nanomole of LPS). Longer-chain ( $C_{16:0}$  and  $C_{18:0}$ ) 3-OHFAs were predominant in HD compared with short-chain ( $C_{10:0}$ ,  $C_{12:0}$ , and  $C_{14:0}$ ) acids. Endotoxin activity was positively correlated with short-chain 3-OHFAs in both studies. In study 2, 3-OH  $C_{16:0}$  was negatively correlated and 3-OH  $C_{18:0}$  was not correlated with endotoxin activity, consistent with previous findings that the *Limulus* assay responds preferentially to LPS containing short-chain 3-OHFAs. Kitchen dust contained the highest concentrations of 3-OH  $C_{10:0}$ , the highest endotoxin activities, and the highest specific activities ( $P < 0.03$ ). Bed dust contained the largest amounts of long-chain 3-OHFAs, the highest concentrations of LPS, and the lowest specific activities. Apartments had significantly different types of LPS ( $P = 0.03$ ) compared with single-family homes in study 2. These data suggest that the *Limulus* assay may underestimate exposure to certain types of LPS. Because nontoxic LPS may have immune modulating effects, analysis of 3-OHFAs may be useful in epidemiologic studies.**

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  $\beta(1-6)$ -D-glucosamine disaccharide as a backbone. This backbone structure typically carries 4 mol equivalents of 3-hydroxy fatty acids (3-OHFAs) with nonhydroxylated fatty acids ester linked to one or more of the 3-hydroxyl groups (24). The 3-OHFAs 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  $\beta(1-6)$ -D-glucosamine disaccharide backbone is required for endotoxin activity with *Limulus* amoebocyte 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-OHFAs, 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

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samples with their LPS content determined by assay for 3-OHFAs. 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 allergies and/or asthma. Recruitment criteria and methods have been previously detailed (9). Between September 1994 and June 1996, families were recruited within 24 to 48 h of the birth of the index child. We collected dust samples from the baby's bed, the bedroom floor, the family room, and the kitchen floor in each home and administered a home characteristic questionnaire within the first 3 months of life of cohort members as previously described (9). Study 2 was designed to characterize seasonal variation in home allergen, 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 floor, 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 m<sup>2</sup> 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 m<sup>2</sup> 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- $\mu$ m mesh sieve, reweighed 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* amoebocyte 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 and 0.05 M potassium phosphate). Dust samples were serially diluted without centrifugation and with frequent vortexing to maintain particulates in suspension. The response parameter for the LAL reaction was the maximum rate of optical density change ( $V_{max}$ ). The log potency and its variance were computed by using the method developed by Milton et al. (17). Results were reported in endotoxin units with reference to EC5 or EC6 reference standard endotoxin (U.S. Pharmacopoeia, Inc.; 1 ng of EC5 and EC6 = 10 EU) after adjustment for lot-to-lot differences in LAL sensitivity to environmental endotoxin as previously described (22).

**Assay for 3-OHFA content of dust samples.** Preparation of dust samples for 3-OHFA analysis was done essentially as described previously (16). Briefly, samples were hydrolyzed with 4 M methanolic HCl at 100°C for 18 h, methyl esters of the released fatty acids were extracted with heptane, nonhydroxylated esters were separated from hydroxylated esters by using a silica gel column, and finally, hydroxylated fatty acid esters were derivatized with *N,O*-bis-(trimethylsilyl)trifluoroacetamide and pyridine to form trimethylsilyl derivatives. The deri-

vated 3-OHFAs were analyzed by selected ion monitoring of  $m/z$  175 and  $m/z$  (M-15) (16, 26); deuterated 3-OH C<sub>14</sub> was used as an internal standard (monitoring  $m/z$  178). The amount of LPS (nanomoles) was calculated as the sum of the number of nanomoles of 3-OHFAs over all measured carbon chain lengths divided by four. The limit of detection (LOD) was 0.5 pmol/mg of dust for each of the 3-OHFAs. Samples with a 3-OHFA concentration below the LOD were, for statistical analyses, assigned a concentration for that particular 3-OHFA equal to the LOD divided by the square root of 2.

A VG (Manchester, United Kingdom) Trio-1 S system was used for GC-MS. The gas chromatograph, a model 5890 (Hewlett-Packard, Avondale, Pa.), was equipped with a fused-silica capillary column (30 m by 0.25 mm [inside diameter]) coated with CP-Sil 8 CB-MS, 0.25- $\mu$ m film thickness (Chrompack, Middelburg, The Netherlands). Injections were made with a Hewlett-Packard 7673 autosampler in the splitless mode; the split valve was opened 1 min after injection. Helium was used as the carrier gas, at an inlet pressure of 7 lb/in<sup>2</sup>, and the temperature of the oven was programmed to increase from 90 to 280°C at 20°C/min. The ion source temperature was 200°C, and electron impact ionization was used at 70 eV. Analyses were made in the selected ion monitoring mode.

**Data analysis.** Specific activity of LPS in house dust samples was computed by dividing the endotoxin activity level (endotoxin units per milligram) by the LPS concentration (nanomoles per milligram) for each sample. The distributions of measured endotoxin activity levels and 3-OHFA and LPS concentrations within each study were positively skewed. Thus, we performed log transformations to obtain symmetrical distributions.

We computed the Spearman correlation coefficient (SAS Proc Corr; SAS Institute Inc., Cary, N.C.) to examine the correlation between endotoxin activity (endotoxin units per milligram of dust) and nanomoles of 3-OHFA per milligram of dust for each carbon chain length (C<sub>10:0</sub>, C<sub>12:0</sub>, C<sub>14:0</sub>, C<sub>16:0</sub>, or C<sub>18:0</sub>). We used multivariate mixed models with a random effect of home to examine the fixed effect of the study (controlled for fixed effects of season and sample type) on the concentrations of 3-OHFAs and LPS, the levels of endotoxin activity, and specific activity of LPS in dust. We used separate mixed models for each study to examine fixed effects of season and sample type (SAS Proc mixed; SAS Institute Inc.). The least-squares means option was used to compute adjusted geometric means and make comparisons between seasons and sample types with a Tukey-Kramer correction for multiple comparisons.

## RESULTS

We analyzed a total of 190 dust samples from the two studies for both endotoxin activity and 3-OHFA concentrations. There were no samples that fell below the LOD of the *Limulus* assay; four samples were below the LOD for C<sub>10:0</sub> 3-OHFA, and none were below the LOD for the remaining 3-OHFAs in the GC-MS analysis. The numbers of homes and samples in each study by sample type and season are shown in Table 1. One hundred thirty-seven dust samples were from study 1, and 53 were from study 2. Study 1 included four types of dust samples (bed, bedroom floor, kitchen, and family room floor); however, because only one bed dust sample from study 1 was assayed for 3-OHFAs, the results for that sample are not included in the remaining tables. Of the 120 homes in study 1, 19 (15.8%) had dogs at the time of sample collection and 20 (16.7%) with no dog at the time of sample collection reported having had dogs previously. Twenty-six homes (21.7%) were apartments in buildings with three or more units, and none of the apartments had dogs at the time of sample collection or previously. Study 2 included only three types of dust samples since we did not collect dust samples from family room floors; 13 homes (65.0%) were apartments, and none of the homes in study 2 kept a dog.

Table 2 shows the coefficients of correlation between the concentrations of 3-OHFAs (nanomoles per milligram) and endotoxin activity (endotoxin units per milligram of dust). In study 1, we observed positive, statistically significant ( $P < 0.05$ ) correlations between endotoxin activity and the various

TABLE 1. Number of homes and samples by sample type, season, and study

Sample type or season	Study 1 <sup>a</sup>		Study 2 <sup>b</sup>		Total	
	Homes	Samples	Homes	Samples	Homes	Samples
Bedroom floor	52	53	15	23	67	76
Kitchen floor	21	22	7	12	28	34
Bed	1	1	8	18	9	19
Family room	60	61			60	61
Spring	17	18	10	11	27	29
Summer	44	51	7	7	50	58
Fall	38	40	6	9	44	49
Winter	28	28	15	26	43	54
Total <sup>c</sup>	120	137	19	53	139	190

<sup>a</sup> Study 1 is a Boston-based longitudinal birth cohort study of childhood asthma.

<sup>b</sup> Study 2 is a longitudinal study of home allergens and endotoxin in 20 homes of Harvard faculty, students, and staff.

<sup>c</sup> The sum of the number of homes by sample type or season does not equal the total number of homes because some homes contributed samples of more than one type and/or samples from more than one season.

3-OHFAs (range, 0.20 to 0.55). The correlation was strongest for the C<sub>12:0</sub> and C<sub>14:0</sub> 3-OHFAs. In study 2, positive correlations of endotoxin activity with short-chain 3-OHFAs were also observed, but the strongest and only significant positive correlation was with the C<sub>10:0</sub> 3-OHFA at  $\alpha = 0.05$ . However, the C<sub>14:0</sub> 3-OHFA was significantly ( $P = 0.08$ ) and positively correlated with endotoxin activity at  $\alpha = 0.1$ . The C<sub>16:0</sub> 3-OHFA was significantly ( $P = 0.04$ ) negatively correlated with endotoxin activity, and the C<sub>18:0</sub> 3-OHFA was not correlated with endotoxin activity. In general, the correlations of short-chain 3-OHFAs with endotoxin activity were consistently positive while those of long-chain 3-OHFAs were more weakly positive or negative.

There was no difference in the mean levels of C<sub>10:0</sub> to C<sub>14:0</sub> 3-OHFAs 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-OHFAs and endotoxin activity

Endotoxin and carbon length of 3-OHFA parameter	Correlation coefficient <sup>a</sup>				
	C <sub>10:0</sub>	C <sub>12:0</sub>	C <sub>14:0</sub>	C <sub>16:0</sub>	C <sub>18:0</sub>
Study 1 ( $n = 137$ )					
Endotoxin activity (EU/mg of dust)	0.27 <sup>b</sup>	0.39 <sup>b</sup>	0.55 <sup>b</sup>	0.20 <sup>b</sup>	0.27 <sup>b</sup>
C <sub>10:0</sub> (nmol/mg of dust)		0.62 <sup>b</sup>	0.35 <sup>b</sup>	0.30 <sup>b</sup>	0.30 <sup>b</sup>
C <sub>12:0</sub> (nmol/mg of dust)			0.68 <sup>b</sup>	0.50 <sup>b</sup>	0.52 <sup>b</sup>
C <sub>14:0</sub> (nmol/mg of dust)				0.66 <sup>b</sup>	0.63 <sup>b</sup>
C <sub>16:0</sub> (nmol/mg of dust)					0.77 <sup>b</sup>
Study 2 ( $n = 53$ )					
Endotoxin activity (EU/mg of dust)	0.28 <sup>b</sup>	0.16	0.24 <sup>c</sup>	-0.29 <sup>b</sup>	0.03
C <sub>10:0</sub> (nmol/mg of dust)		0.73 <sup>b</sup>	0.35 <sup>b</sup>	0.10	0.28 <sup>b</sup>
C <sub>12:0</sub> (nmol/mg of dust)			0.67 <sup>b</sup>	0.30 <sup>b</sup>	0.61 <sup>b</sup>
C <sub>14:0</sub> (nmol/mg of dust)				0.46 <sup>b</sup>	0.71 <sup>b</sup>
C <sub>16:0</sub> (nmol/mg of dust)					0.56 <sup>b</sup>

<sup>a</sup> Spearman correlation.

<sup>b</sup>  $P < 0.05$ .

<sup>c</sup>  $P = 0.08$ .

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

Sample type or season <sup>a</sup>	Adjusted geometric mean no. of nmol/mg of dust				
	C <sub>10:0</sub>	C <sub>12:0</sub>	C <sub>14:0</sub>	C <sub>16:0</sub>	C <sub>18:0</sub>
Study 1					
Bedroom floor	0.020	0.052	0.047	0.146	0.091
Kitchen floor	0.033	0.045	0.048	0.134	0.070
Family room	0.022	0.055	0.052	0.186	0.109
Spring	0.016	0.038 <sup>b</sup>	0.049	0.149	0.078 <sup>b</sup>
Summer	0.020	0.058 <sup>b</sup>	0.058	0.146	0.085 <sup>b</sup>
Fall	0.029	0.053 <sup>b</sup>	0.055	0.156	0.091 <sup>b</sup>
Winter	0.023	0.034 <sup>b</sup>	0.035	0.121	0.054 <sup>b</sup>
Study 2					
Bedroom floor	0.018	0.049	0.057	0.217 <sup>b</sup>	0.126 <sup>b</sup>
Kitchen floor	0.036	0.062	0.050	0.131 <sup>b</sup>	0.080 <sup>b</sup>
Bed	0.016	0.056	0.062	0.292 <sup>b</sup>	0.164 <sup>b</sup>
Spring	0.030	0.053	0.055	0.195	0.114
Summer	0.029	0.065	0.052	0.187	0.101
Fall	0.016	0.061	0.065	0.235	0.144
Winter	0.016	0.045	0.054	0.196	0.119

<sup>a</sup> 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.

<sup>b</sup> In study 1, there was no significant effect of sample type on 3-OHFA and there was a significant effect of season on 3-OH C<sub>12:0</sub> ( $P = 0.042$ ) and C<sub>18:0</sub> ( $P = 0.034$ ). In study 2, there was a significant effect of sample type on 3-OH C<sub>16:0</sub> ( $P = 0.001$ ) and C<sub>18:0</sub> ( $P = 0.008$ ) but no significant effect of season.

Samples from study 2 had significantly greater concentrations of C<sub>16:0</sub> ( $P = 0.015$ ) and C<sub>18:0</sub> ( $P = 0.003$ ) 3-OHFAs and of total LPS ( $P = 0.03$ ) than did those from study 1. There was no study-by-sample type interaction for C<sub>16:0</sub> and C<sub>18:0</sub> 3-OHFAs 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-OHFAs (nanomoles per milligram) in house dust samples. The concentration of C<sub>10:0</sub> was consistently among the lowest, and the concentration of C<sub>16:0</sub> was consistently the highest, of the five measured 3-OHFAs. The level of C<sub>10:0</sub> 3-OHFAs 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<sub>16:0</sub> ( $P < 0.02$ ) than did kitchen dust; bed dust had significantly greater and bedroom floor dust had borderline significantly greater ( $P = 0.06$ ) 3-OH C<sub>18:0</sub> concentrations than did kitchen dust. The concentrations of C<sub>16:0</sub> and C<sub>18:0</sub> 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-OHFAs 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<sub>12:0</sub> ( $P = 0.04$ ) and 3-OH C<sub>18:0</sub>



TABLE 4. Endotoxin activity, nanomoles of LPS, and LPS specific activity in house dust from two studies

Sample type or season <sup>a</sup>	Adjusted geometric mean		
	Endotoxin (EU/mg)	LPS (nmol/mg)	LPS sp act (EU/nmol)
Study 1			
Bedroom floor	68.8 <sup>b</sup>	0.095	725 <sup>b</sup>
Kitchen floor	120 <sup>b</sup>	0.092	1,303 <sup>b</sup>
Family room	79.8 <sup>b</sup>	0.112	715 <sup>b</sup>
Spring	91.5	0.086	1,068
Summer	122	0.098	1,240
Fall	106	0.104	1,021
Winter	76.5	0.069	1,105
Study 2			
Bedroom floor	77.7 <sup>b</sup>	0.120 <sup>b</sup>	643 <sup>b</sup>
Kitchen floor	215 <sup>b</sup>	0.085 <sup>b</sup>	2,123 <sup>b</sup>
Bed	49.3 <sup>b</sup>	0.155 <sup>b</sup>	319 <sup>b</sup>
Spring	123 <sup>b</sup>	0.114	1,015
Summer	98.9 <sup>b</sup>	0.107	853
Fall	98.7 <sup>b</sup>	0.132	705
Winter	64.7 <sup>b</sup>	0.116	541

<sup>a</sup> 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.

<sup>b</sup> In study 1, there were significant effects of sample type on endotoxin activity ( $P = 0.03$ ) and on specific activity ( $P = 0.02$ ) but there was no significant seasonal effect. In study 2, there were significant effects of sample type on endotoxin activity ( $P < 0.001$ ), total LPS ( $P = 0.01$ ), and specific activity ( $P < 0.001$ ) and also a significant effect of season on endotoxin activity ( $P = 0.014$ ).

3-OHFA ( $P = 0.03$ ) concentrations. Adjusted for multiple comparisons, the 3-OH C<sub>12:0</sub> 3-OHFA 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 C<sub>18:0</sub> than did dust collected in the winter (Table 3). The seasonal pattern of 3-OH C<sub>12:0</sub> and 3-OH C<sub>18:0</sub> 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 C<sub>18:0</sub> 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 (C<sub>10:0</sub>, C<sub>12:0</sub>, and C<sub>14:0</sub>) 3-OHFAs are positively correlated with endotoxin activity, while longer-chain (C<sub>16:0</sub> and C<sub>18:0</sub>) 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 C<sub>10:0</sub> 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-OHFAs 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 not known whether the immunological modulating effects of LPS important to the development of atopy and asthma are restricted to those structures that strongly activate the *Limulus* assay or even those structures specific for Toll-like receptor 4 (19). Thus, understanding the biology of early-life endotoxin exposure may require information about the types of lipid A to which children are exposed. Given that the endotoxin activity measured by the *Limulus* assay is largely determined by the amount of shorter-chain fatty acids, the predominance of longer-chain 3-OHFAs in bed dust, in dust from single family homes, and in dust sampled during the fall suggests that exposure may be underestimated by the *Limulus* assay. Since bed dust endotoxin is likely to be an important source of exposure, analysis of 3-OHFAs in bed dust may be useful in future studies.

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