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RESEARCH

Postnatal exposure to household disinfectants, infant gut microbiota and subsequent risk of overweight in children

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ABSTRACT

BACKGROUND: Emerging links between household cleaning products and childhood overweight may involve the gut microbiome. We determined mediating effects of infant gut microbiota on associations between home use of cleaning products and future overweight.

METHODS: From the Canadian Healthy Infant Longitudinal Development (CHILD) birth cohort, we tested associations between maternal report of cleaning product use and overweight at age 3, and whether associations were mediated by microbial profiles of fecal samples in 3- to 4-month-old infants.

RESULTS: Among 757 infants, the abundance of specific gut microbiota was associated with household cleaning with disinfectants and eco-friendly products in a dose-dependent manner. With more frequent use of disinfectants, *Lachnospiraceae* increasingly became more abundant (highest v. lowest quintile of use: adjusted odds ratio [AOR] 1.93, 95% confidence interval [CI] 1.08 to 3.45) while genus *Haemophilus* declined in abundance (highest v. lowest quintile of use: AOR 0.36, 95% CI 0.20 to 0.65). *Enterobacteriaceae* were successively depleted with greater use of eco-friendly products (AOR 0.45, 95% CI 0.27 to 0.74). *Lachnospiraceae* abundance significantly mediated associations of the top 30th centile of household disinfectant use with higher body mass index (BMI) *z* score ($p = 0.02$) and with increased odds of overweight or obesity ($p = 0.04$) at age 3. Use of eco-friendly products was associated with decreased odds of overweight or obesity independently of *Enterobacteriaceae* abundance (AOR 0.44, 95% CI 0.22 to 0.86), with no significant mediation ($p = 0.2$).

INTERPRETATION: Exposure to household disinfectants was associated with higher BMI at age 3, mediated by gut microbial composition at age 3–4 months. Although child overweight was less common in households that cleaned with eco-friendly products, the lack of mediation by infant gut microbiota suggests another pathway for this association.

Greater emphasis on cleanliness has led to widening use of disinfectants and other cleaning agents in the home.[1] Household cleaning products have been associated with elevated risk of wheeze in persons using these products[2] and in their children.[3] The literature on risk for overweight is more limited and informed by national surveys of ingredients previously common in cleaning products, such as triclosan.[4] According to a new study, high urinary levels of triclosan are evident in US adolescents with greater adiposity.[5]

Although not shown in trials of "usual" home use of cleaning products,[6,7] microbial levels on household surfaces are effectively reduced when the daily dose of disinfectant is standardized.[8] In fact, piglets born in an indoor environment, and then raised under conditions of continuous aerosolization with a disinfectant, have shown perturbed gut microbial composition compared with piglets not reared under these conditions.[9] Indeed, concerns over the potential for antibacterial products to be too effective or even toxic has motivated use of "green" or eco-friendly alternatives.[10,11] When tested, commercial or homemade eco-friendly products have efficacy comparable with bleach[12,13] and other household disinfectants[14] against some microbes but not others.

Central to the hygiene hypothesis of allergic disease is the microbial environment in which we live.[15] According to Hesselmar and colleagues, protection from allergic disease may also involve microbes left on eating utensils after handwashing versus dishwasher use.[16] Environmental microbes may also protect against metabolic disease, as evident from reports of reduced overweight in kindergarten children previously attending daycare.[17] Because infants spend more than 80% of their time indoors,[18] the home microbial environment is especially relevant to the maturation of their gut microbial ecosystem. Direct and indirect evidence of altered gut microbial colonization during infancy has been linked to allergic disease and overweight.[19,20] Short-chain fatty acid metabolites produced by gut microbiota are involved in appetite regulation and in lipid and glucose metabolism; their heightened production is thought to play a role in development of overweight.[21]

Similar to other cohorts,[22] the Canadian Healthy Infant Longitudinal Development (CHILD) study birth cohort was designed to assess the health impact of indoor environmental exposures, including household cleaning products. To date, a detailed evaluation of the impact of household cleaning agents on the gut microbiota of infants or on child overweight is lacking. Herein, we tested associations between household cleaning product use and infant gut microbial composition at 3–4 months of age in the CHILD cohort. Thereafter, we determined the association between cleaning product use and child overweight at age 3 and whether it was mediated by the gut microbial changes observed.

Methods

Study design

The present study includes a subsample of children from the CHILD population-based birth cohort (Figure 1). We recruited women during the second or third trimester of their pregnancy, and enrolled them in the study cohort if their newborns were a singleton live birth at ≥ 35 weeks of gestation with a birth weight of ≥ 2500 g. We excluded in vitro fertilization births because they were more likely to result in multiple gestations or preterm delivery (< 35 wk), which were study exclusion criteria. We excluded home births owing to lack of data on maternal intrapartum antibiotic prophylaxis. We followed the enrolled women throughout pregnancy and their children from birth to age 3 years.

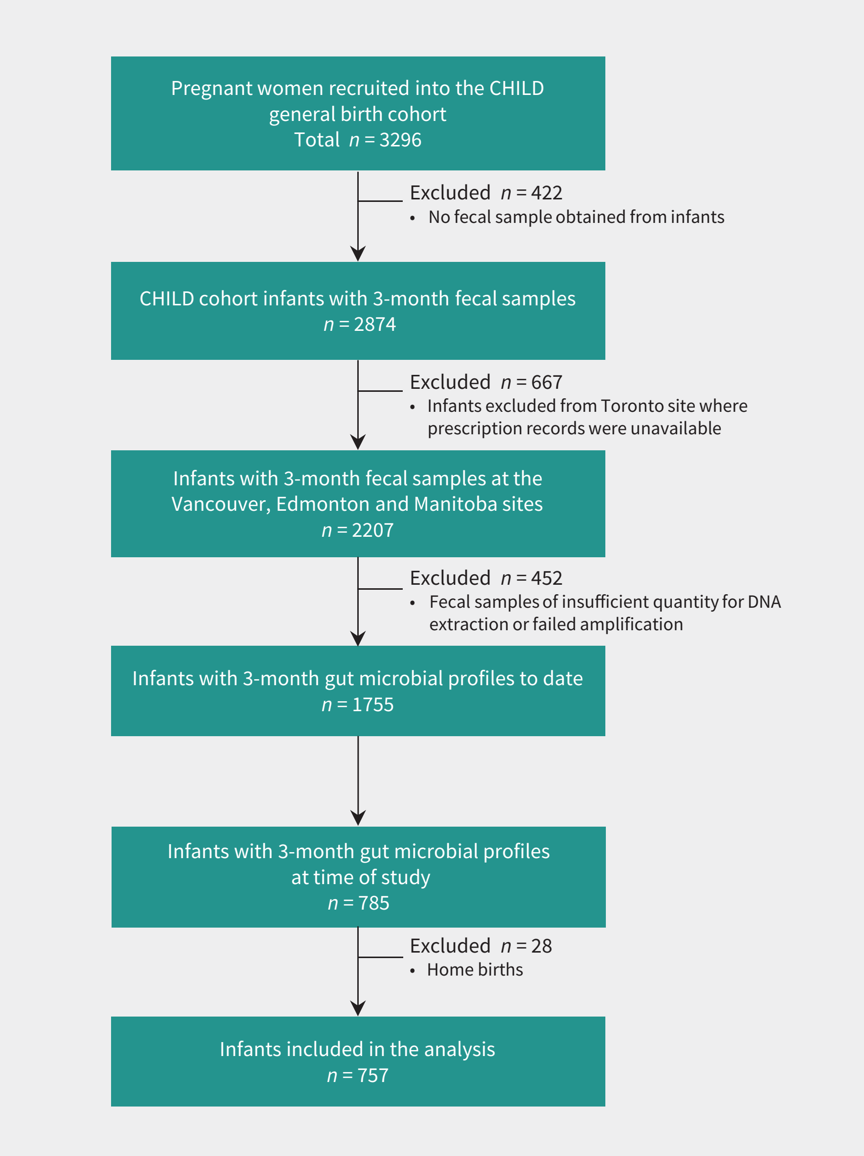

Figure 1: Flowchart of the infants eligible for the study. CHILD = Canadian Healthy Infant Longitudinal Development.

Exposures

At 3–4 months postpartum, mothers completed questionnaires on aspects of their health, home environment and personal use of cleaning products (Appendix 1a, available at www.cmaj.ca/lookup/suppl/doi:10.1503/cmaj.170809/-/DC1). They were asked: "Which of the following products [a list of 31 chemical-based products] do you personally use?" Available responses were daily, weekly, monthly, less than monthly and not used at all. From the 31 queried categories, we grouped cleaning products according to the mechanism of action, namely disinfectant, detergent and other (Appendix 1b). The frequency of use for each queried product was converted into 5 numeric scores: 0 for not used at all, 1 for less than once a month, 2 for monthly, 3 for weekly and 4 for daily usage. We summed the scores to produce a total score that was divided at the median into higher or equivalent versus lower (reference category) exposure groups. We also tested higher centile cut-off values for cleaning product variables. Eco-friendly products comprised one of the queried products, for which we created a separate variable. Based on original response values, categories for eco-friendly products were no use (reference), less than monthly use or at least monthly use. We included questions on the home environment, such as indoor smoking (parents and visitors), number of siblings, household pets and maternal history of asthma and allergy. Maternal body mass index (BMI) was calculated from measured height and pre-pregnancy weight in the perinatal section of the hospital birth record; overweight was defined as BMI ≥ 25.[23]

Fecal microbiota profiles

Fecal samples were collected at 3–4 months of age. Full details of fecal microbiota profiling can be found in Appendix 1c.

Overweight outcomes

Trained research assistants measured weight and height at 1 and 3 years of age, and generated age- and gender-adjusted BMI *z* scores from World Health Organization growth charts.[24] We categorized weight status at 1 and 3 years of age into overweight or obese (> 97th centile) from generated BMI *z* scores.

Statistical analysis

We conducted statistical analyses using SPSS software (version 23; IBM SPSS Statistics). The distribution of putative covariates by category of cleaning product was assessed by χ^2. The Mann–Whitney *U* test was employed to compare the median relative abundance, richness and diversity of dominant bacterial taxa. We corrected multiple comparisons by converting crude *p* values to false discovery rate values (*q* values).

We determined associations between household cleaning product categories and infant gut microbial composition and diversity with logistic regression analysis, testing bivariate and quintile categories of cleaning product use. We selected potential confounding variables based on the literature[25] and on *p* values (< 0.2) after univariate comparisons. We adjusted models for covariates such as fecal sample age, birth mode, breastfeeding status and antibiotic exposure (infant and maternal intrapartum antibiotic prophylaxis) and presence of the "other" product (for example, models for disinfectant use were adjusted for detergent use).

We also conducted multiple logistic regression modelling to test associations between cleaning product use and overweight or obesity at age 1 and 3 years. When the outcome was BMI *z* score, we employed multiple linear regression analysis. We adjusted models for the above covariates when relevant. Lastly, using the R package for mediation, we employed causal mediation analysis to test whether cleaning product use (X) affected the overweight outcome (Y) through a microbiota intermediate variable (M).[26] Full details of the mediation analysis can be found in Appendix 1c.

Ethics approval

The study was approved by the Human Ethics Boards of the Universities of Alberta, Manitoba and British Columbia; written, informed consent was obtained from all participants.

Results

Data on fecal microbial profiles and prescription records were analyzed in 757 hospital-born infants selected from 3296 infants recruited into the CHILD general birth cohort (Figure 1). Our study sample was representative of the sociodemographics of the larger CHILD cohort (Appendix 1d), apart from small differences in breastfeeding and use of antibiotics in infants, which were adjusted for in models of cleaning product use. High correlations between exclusivity of breastfeeding with maternal education level and study site precluded their addition to models but enabled adjustment for these 2 covariates through the strong proxy measure of breastfeeding status. We excluded maternal pre-pregnancy overweight from the final model for disinfectants and excluded household pet exposure from the final model for eco-friendly products because these covariates did not change the adjusted odds ratio (AOR) estimate > 15%.[27,28]

Patterns of household cleaning product use

Parental self-report of use of cleaning products was highly correlated with the research assistants' visual assessment of the presence or absence of those cleaning products in the home ($r = 0.32$, $p = 0.0001$). The most common household disinfectant was a multisurface cleaner (22%), whereas handwashing detergents (26%) and spray air fresheners (18%) were the most commonly used in their categories. Close to 80% of households used multisurface cleaners on a weekly to daily basis, and this usage rose to 90% when the cut-off score for high use was the top 30th centile (Appendix 1e). Frequency of household disinfectant usage was significantly correlated with detergent usage ($r = 0.450$, $p = 0.0001$), but weakly and inversely with use of eco-friendly products ($r = –0.032$, $p = 0.047$). Significant correlations were found between pre- and postnatal use of eco-friendly cleaning products ($r = 0.62$, $p = 0.0001$), and pre- and postnatal use of disinfectants ($r = 0.60$, $p = 0.0001$).

Disinfectant use was higher at the Edmonton study site and among households with infants delivered by cesarean, who received intrapartum antibiotic prophylaxis, or who were exposed to cigarette smoke, but lower among households with breastfed infants (Table 1). Eco-friendly products were used more often by mothers who had allergies, breastfed their infants, or had higher education, and at the Vancouver study site, and less often in women who were overweight pre-pregnancy or whose infants were admitted to hospital after birth. Associations with use of detergents or other chemicals are shown in Appendix 1f. Exclusive breastfeeding, a covariate related to several cleaning product types, was much more likely among women who were university educated ($p < 0.0001$) or who lived in Vancouver ($p < 0.0001$).

Association of cleaning product use with whole gut microbial community measures

The impact of cleaning products (median or higher v. below) on microbial community composition (beta diversity) was significant for disinfectant use ($p = 0.03$) but not for use of eco-friendly products ($p = 0.1$), detergents ($p = 0.10$) or other cleaning products ($p = 0.1$). However, gut microbiota richness (Chao1 Index)

Table 1: Distribution of status of exposure to disinfectant and eco-friendly products at 3–4 months, according to study covariates*

Characteristic	**No. of infants with higher exposure to disinfectant, *n* (%)† *n* = 404 (53.4)**	***p* value‡**	**No. of infants with higher exposure to eco-friendly products, *n* (%)† *n* = 361 (47.7)**	***p* value‡**
Infant sex (*n* = 757)				
Male (*n* = 415)	224 (54.0)	0.7	191 (46.0)	0.3
Female (*n* = 342)	180 (52.6)		170 (49.7)	
Maternal pre-pregnancy overweight (*n* = 737)				
No (*n* = 455)	231 (50.8)	0.06	235 (51.6)	**0.01**
Yes (*n* = 282)	163 (57.8)		118 (41.8)	
Maternal allergy (*n* = 742)				
No (*n* = 270)	146 (54.1)	0.7	114 (42.2)	**0.02**
Yes (*n* = 472)	250 (53.0)		241 (51.1)	
Term (37+ wk) delivery (*n* = 748)				
No (*n* = 30)	18 (60.0)	0.5	15 (50.0)	0.8
Yes (*n* = 718)	381 (53.1)		343 (47.8)	
Birth mode (*n* = 749)				
Vaginal (*n* = 549)	275 (50.1)	**0.02**	272 (49.5)	0.3
Cesarean-elective (*n* = 83)	51 (61.4)		37 (44.6)	
Cesarean-emergency (*n* = 117)	73 (61.4)		50 (42.7)	
Maternal intrapartum antibiotic prophylaxis (*n* = 738)				
No (*n* = 368)	183 (49.7)	**0.047**	178 (48.4)	0.8
Yes (*n* = 370)	211 (57.0)		175 (47.3)	
Hospital admission after birth (*n* = 757)				
No (*n* = 224)	111 (49.6)	0.2	125 (55.8)	**0.004**
Yes (*n* = 533)	293 (55.0)		236 (44.3)	
Hospital readmission (*n* = 757)				
No (*n* = 721)	386 (53.5)	0.7	339 (47.0)	0.1
Yes (*n* = 36)	18 (50.0)		22 (61.1)	
Newborn antibiotic use (*n* = 673)				
No (*n* = 649)	346 (53.3)	0.2	306 (47.1)	0.4
Yes (*n* = 24)	16 (66.7)		9 (37.5)	
Infant antibiotic use by 3 mo (*n* = 720)				
No (*n* = 322)	160 (49.7)	0.1	152 (47.2)	0.9
Yes (*n* = 398)	222 (55.8)		189 (47.5)	
Infant diet (*n* = 755)				
Not breastfed (*n* = 115)	73 (55.8)	**0.02**	37 (32.2)	**0.0001**
Partially breastfed (*n* = 233)	131 (56.2)		109 (46.8)	
Exclusively breastfed (*n* = 407)	200 (49.1)		215 (52.8)	
Older siblings (*n* = 751)				
No (*n* = 389)	203 (52.2)	0.4	183 (47.0)	0.7
Yes (*n* = 362)	199 (55.0)		175 (48.3)	
Furry household pets (*n* = 755)				
No (*n* = 208)	101 (48.6)	0.1	91 (43.8)	0.2
Yes (*n* = 547)	303 (55.4)		270 (49.4)	
Household smoking (*n* = 755)				
No (*n* = 646)	335 (51.9)	**0.03**	312 (48.3)	0.5
Yes (*n* = 109)	69 (63.3)		49 (45.0)	
Maternal education (*n* = 737)				
High school (*n* = 38)	23 (60.5)	0.1	7 (18.4)	**0.0001**
College (*n* = 176)	106 (60.2)		75 (42.6)	
University (*n* = 523)	265 (50.7)		270 (51.6)	
Study centre (*n* = 757)				
Edmonton (*n* = 246)	160 (65.0)	**0.0001**	108 (43.9)	**0.0001**
Winnipeg (*n* = 167)	83 (49.7)		60 (35.9)	
Vancouver (*n* = 344)	161 (46.8)		193 (56.1)	
Overweight or obesity at 3 yr (*n* = 675)				
No (*n* = 609)	311 (51.5)	0.1	301 (49.4)	**0.0001**
Yes (*n* = 66)	42 (63.6)		17 (25.8)	

*Comparisons by χ^2 test.
† Higher = higher frequency of use of disinfectant or eco-friendly products (≥ median score).
‡ Significant *p* values are indicated in boldface type. Exact *p* values were reported if *n* < 30 in each cell.

and diversity (Shannon or Simpson) did not differ between higher and lower frequency of use for any of the cleaning product groups (Appendices 1g–1j).

Disinfectants

The gut microbiota of infants living in homes with higher (≥ median) use of disinfectant products were enriched in *Lachnospiraceae* (3.320% v. 1.197%, AOR 1.34, 95% confidence interval [CI] 1.02 to 1.90) and its genus *Ruminococcus* (0.039% v. 0.008%, AOR 1.55, 95% CI 1.10 to 2.17), as well as *Coriobacteriaceae* (0.047% v. 0.031%, AOR 1.47, 95% CI 1.05 to 2.06) (Figure 2, Table 2, Appendix 1g). They had reduced abundance of fecal *Pasteurellaceae* (0.015% v. 0.046%, AOR 0.67, 95% CI 0.48 to 0.95) and its genus *Haemophilus* (0.015% v. 0.046%, AOR 0.69, 95% CI 0.49 to 0.98), as well as genus *Clostridium* (0.015% v. 0.054%, AOR 0.61, 95% CI 0.43 to 0.86). We did not see these associations with other cleaning products (Appendix 1k).

Quintile of household disinfectant use and fecal abundance of *Lachnospiraceae* were positively associated in a dose-dependent manner (Figure 2), strongest at the highest level of use (AOR 1.93, 95% CI 1.08–3.45) (Figure 3A). An inverse association with genus *Haemophilus* was strongest at the highest level of household disinfectant use (AOR 0.36, 95% CI 0.20 to 0.65) (Figure 2 and Figure 3B). The abundance of *Lachnospiraceae* and *Pasteurellaceae* was negatively correlated ($r = -0.194$, $p = 0.00001$, Spearman test). At the genus level, abundance of *Ruminococci* (of *Lachnospiraceae*), was negatively correlated with genus *Haemophilus* (of *Pasteurellaceae*) ($r = -0.157$, $p = 0.002$) or *Clostridium* ($r = -0.122$, $p = 0.01$).

Eco-friendly products

Infants residing in homes with more frequent use of eco-friendly products had reduced fecal abundance of *Enterobacteriaceae* (16.364% v. 20.335%, AOR 0.62, 95% CI 0.45 to 0.87) and its genus *unclassified Enterobacteriaceae* (16.043% v. 20.131%, AOR 0.60,

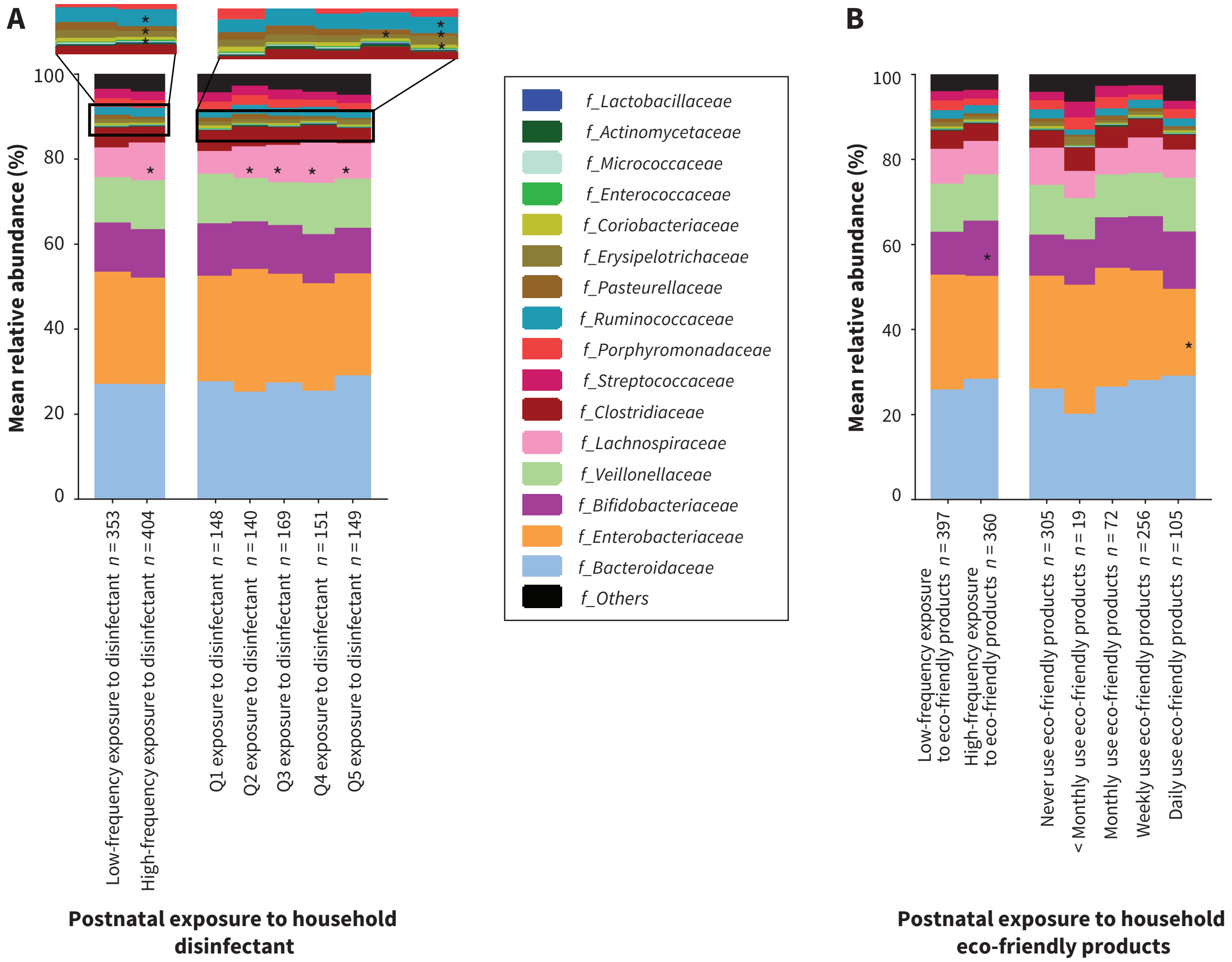

Figure 2: Composition of key gut microbiota at the family level, by exposure to A) household disinfectant and B) eco-friendly products in all infants ($n = 757$). The stacked bar charts show mean relative abundance of gut microbiota populations at the family level in infant feces at 3 months of age. A) Left to right, binary category of exposure to disinfectant (≥ median score) and disinfectant exposure in quintiles. B) Left to right, binary category of exposure to eco-friendly products (≥ median score) and questionnaire category of use of eco-friendly products. Asterisks show *p* values < 0.05 from median relative abundance comparisons from Appendices 1g–1h (median relative abundance with interquartile range comparisons can be found in Appendices 1g–1h).

95% CI 0.43 to 0.83) (Figure 2, Table 3, Appendix 1h). An inverse dose–response was apparent between frequency of use of eco-friendly products and fecal abundance of *Enterobacteriaceae*, with daily use being associated with the greatest depletion of these microbiota (AOR 0.45, 95% CI 0.27 to 0.74) (Figure 2, Figure 3C). We did not see this association with other cleaning products.

Detergents and other cleaning products

Infants living in homes with higher use of detergents had higher abundance of *Erysipelotrichaceae* (0.031% v. 0.007%, AOR 1.63, 95% CI 1.16 to 2.29) (Appendices 1i and 1l), but there was no significant dose–response. We did not see this association with other cleaning products (Appendix 1k). We saw no changes in taxon median abundance in infant gut microbiota with frequency in use of other cleaning products, after adjustment for other covariates (Appendices 1j and 1m).

Associations of use of cleaning products and gut microbiota with overweight or obesity

Overweight in children aged 3 years was more prevalent after maternal overweight before pregnancy, cesarean delivery, intrapartum antibiotic prophylaxis, household tobacco exposure and infant antibiotic treatment; it was less prevalent among infants who were exclusively breastfed, whose mothers were more highly educated, or who were at the Vancouver study site (Appendix 1n).

In unadjusted analyses, use of household disinfectant greater than the median was not significantly associated with BMI *z* score (difference in *z* score 0.12, 95% CI −0.03 to 0.26) or overweight or obesity at age 3 years (OR 1.66, 95% CI 0.98 to 2.80) (Table 4). However, the top 30th centile of disinfectant use was associated with higher BMI *z* score (difference in *z* score 0.17, 95% CI 0.01 to 0.33), although not with overweight or obesity (OR 1.32, 95% CI 0.78 to 2.25) (Table 4). Household use of eco-friendly products greater than the median was associated with a lower BMI *z* score (difference in *z* score −0.25, 95% CI −0.40 to −0.11) and reduced odds of overweight or obesity at age 3 years (OR 0.36, 95% CI 0.20 to 0.63). We observed no significant associations between use of household detergents and BMI *z* score, overweight or obesity.

In unadjusted analyses, higher fecal levels of *Lachnospiraceae* at age 3–4 months were significantly associated with increased BMI *z* score at age 1 (difference in *z* score 0.29, 95% CI 0.13 to 0.45) and at age 3 (difference in *z* score 0.28, 95% CI 0.14 to 0.43) (Appendix 1o). In addition, higher fecal levels of *Coriobacteriaceae, Erysipelotrichaceae* and *Ruminococcaceae* were associated with increased BMI *z* score at age 1 and age 3, and higher fecal levels of *Enterococcaceae* and *Clostridiaceae* were associated with increased BMI *z* score at age 3 but not age 1. However, only higher fecal levels of *Lachnospiraceae* were associated with overweight or obesity at 3 years (OR 1.79, 95% CI 1.06 to 3.04) (Appendices 1o and 1p).

After adjustment for higher fecal abundance of *Lachnospiraceae* and other covariates, the association between top 30th centile of disinfectant use and BMI *z* score was no longer significant (difference in BMI *z* score 0.11, 95% CI −0.02 to 0.25) (Table 4, Appendix 1q). Results were unchanged with the addition of fecal *Coriobacterial, Erysipelotrichiaceal* and *Ruminococcaceal* abundance to this model (data not shown). Together, association of disinfectant use with greater *Lachnospiraceae* abundance, association of *Lachnospiraceae* abundance with BMI *z* score or overweight at age 3, and diminishment of the association of disinfectant use with BMI *z* score after adjustment for *Lachnospiraceae* abundance suggested a mediation effect by this family of microbiota. Mediation analysis confirmed that *Lachnospiraceae* abundance had a

Table 2: Crude and adjusted odds ratios for higher abundance (≥ median) of key infant gut microbiota at 3–4 months with frequent household use of disinfectants

Ref: Lower use of disinfectants	Crude model (*n* = 757) OR (95% CI)	Adjusted model* (*n* = 699) AOR (95% CI)
Bifidobacteriaceae (below v. above median)	0.89 (0.67 to 1.19)	1.00 (0.73 to 1.39)
Coriobacteriaceae (below v. above median)	1.40 (1.05 to 1.86)	1.47 (1.05 to 2.06)
Lachnospiraceae (below v. above median)	1.67 (1.26 to 2.23)	1.34 (1.02 to 1.90)
Genus *Ruminococcus* (below v. above median)	1.47 (1.11 to 1.96)	1.55 (1.10 to 2.17)
Ruminococcaceae (below v. above median)	1.42 (1.07 to 1.89)	1.15 (0.80 to 1.64)
Genus *Oscillospira* (below v. above median)	1.39 (1.05 to 1.85)	1.04 (0.74 to 1.47)
Erysipelotrichaceae (below v. above median)	1.25 (0.94 to 1.67)	0.90 (0.61 to 1.22)
Genus *Clostridium* (below v. above median)	0.74 (0.55 to 0.98)	0.61 (0.43 to 0.86)
Genus *Veillonella* (below v. above median)	0.88 (0.66 to 1.17)	0.73 (0.52 to 1.02)
Enterobacteriaceae (below v. above median)	0.81 (0.61 to 1.07)	0.82 (0.58 to 1.15)
Pasteurellaceae (below v. above median)†	0.65 (0.49 to 0.87)	0.67 (0.48 to 0.95)
Genus *Haemophilus* (below v. above median)†	0.68 (0.51 to 0.90)	0.69 (0.49 to 0.98)

Note: AOR = adjusted odds ratio, CI = confidence interval, OR = odds ratio.
*Adjusted for mode of delivery, breastfeeding status, direct and indirect exposure to antibiotics, and household detergent in first 3 months, and fecal sampling age.
†Adjusted for mode of delivery, breastfeeding status, direct and indirect exposure to antibiotics, household smoking and household detergent in first 3 months, and fecal sampling age (*n* = 698).

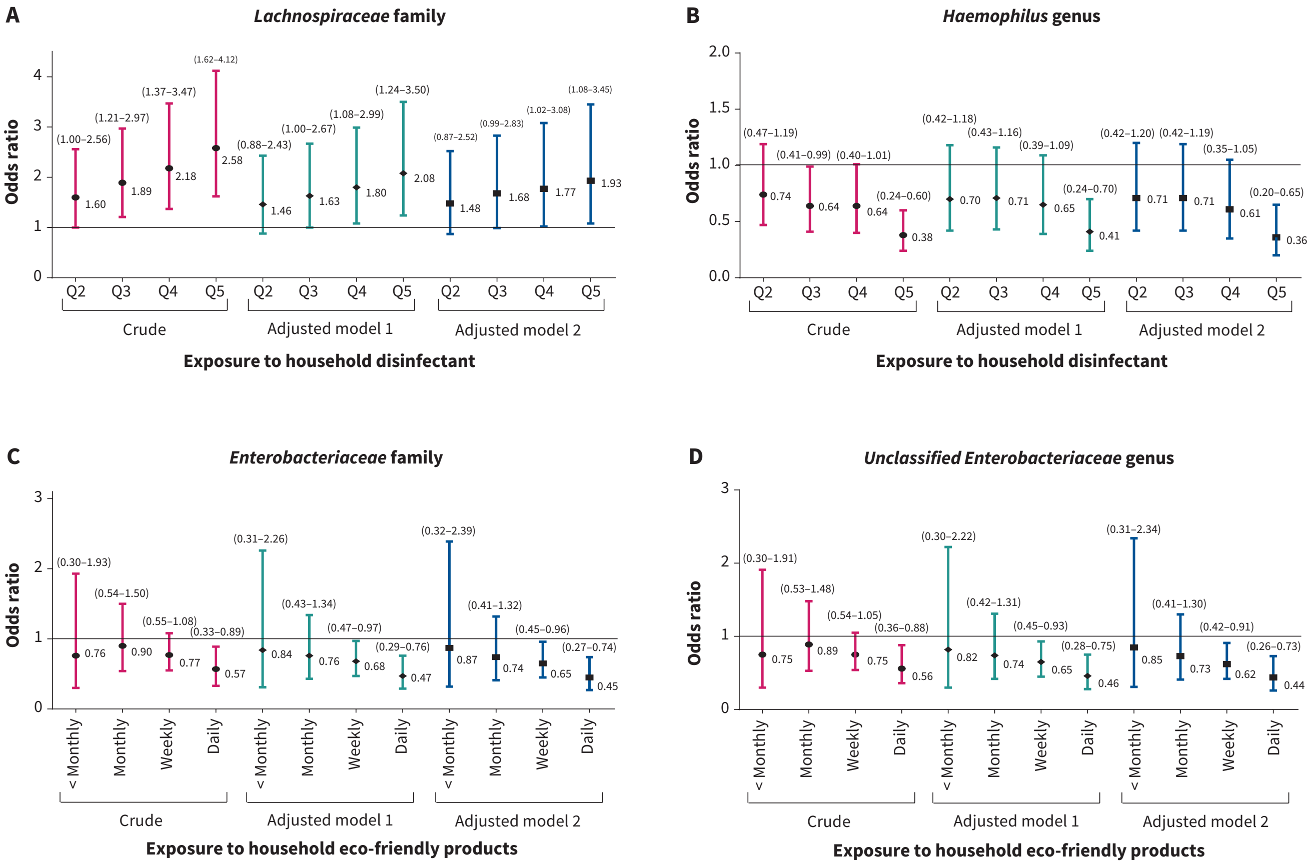

Figure 3: Crude and adjusted likelihood ratios for higher abundance (≥ median) of infant gut microbiota: A) *Lachnospiraceae* family, B) *Haemophilus* genus at 3–4 months with frequent household use of disinfectants, C) *Enterobacteriaceae* family, and D) unclassified *Enterobacteriaceae* genus at 3–4 months with frequent household use of eco-friendly products. A) and B): Model 1: Adjusted for mode of delivery, breastfeeding status, direct and indirect exposure to antibiotics in first 3 months. Model 2: Adjusted for mode of delivery, breastfeeding status, direct and indirect exposure to antibiotics, exposure to household detergent in first 3 months and fecal sampling age. Q2, Q3, Q4, Q5 = quintiles of household disinfectant exposure (ref: Q1 = lowest quintile). C and D): Model 1: Adjusted for mode of delivery, breastfeeding status, direct and indirect exposure to antibiotics in first 3 months. Model 2: Adjusted for mode of delivery, breastfeeding status, direct and indirect exposure to antibiotics, exposure to household detergent and disinfectant in first 3 months, maternal allergy during pregnancy, maternal overweight and fecal sampling age. Daily, weekly, monthly, < monthly = frequency of use of household eco-friendly products (ref: do not use eco-friendly products).

Table 3: Crude and adjusted odds ratios for higher abundance (≥ median) of key infant gut microbiota at 3–4 months with frequent household use of eco-friendly products

Ref: Lower use of eco-friendly products	Crude model (*n* = 757) OR (95% CI)	Adjusted model* (*n* = 685) AOR (95% CI)
Bifidobacteriaceae (below v. above median)	1.35 (1.02 to 1.80)	1.11 (0.81 to 1.52)
Coriobacteriaceae (below v. above median)	0.99 (0.74 to 1.31)	1.05 (0.76 to 1.45)
Lachnospiraceae (below v. above median)	0.86 (0.65 to 1.14)	1.09 (0.77 to 1.53)
Genus *Ruminococcus* (below v. above median)	0.88 (0.66 to 1.17)	0.95 (0.69 to 1.32)
Erysipelotrichaceae (below v. above median)	0.77 (0.58 to 1.02)	0.92 (0.66 to 1.29)
Genus *Clostridium* (below v. above median)	0.94 (0.71 to 1.26)	1.06 (0.76 to 1.48)
Genus *Veillonella* (below v. above median)	0.71 (0.53 to 0.95)	0.79 (0.57 to 1.09)
Enterobacteriaceae (below v. above median)	0.73 (0.55 to 0.97)	0.62 (0.45 to 0.87)
Genus *uncl. Enterobacteriaceae* (below v. above median)	0.72 (0.54 to 0.95)	0.60 (0.43 to 0.83)
Genus *Haemophilus* (below v. above median)	1.14 (0.86 to 1.52)	0.84 (0.60 to 1.18)

Note: AOR = adjusted odds ratio, CI = confidence interval, OR = odds ratio.
*Adjusted for mode of delivery, breastfeeding status, direct and indirect exposure to antibiotics, household disinfectant and detergent in first 3 months, maternal allergy during pregnancy, maternal overweight and fecal sampling age.

significant average causal mediation effect on the association of top 30th centile disinfectant use with age 3 BMI *z* score ($p = 0.02$) and with overweight status ($p = 0.04$) (Figure 4, Appendix 1r).

After adjustment for fecal abundance of *Enterobacteriaceae* and other covariates, the association of use of eco-friendly products greater than the median with BMI *z* score at age 3 was no longer significant (difference in *z* score −0.12, 95% CI −0.24 to 0.002), but the association with child overweight remained significant (AOR 0.44, 95% CI 0.22 to 0.86) (Table 4). Fecal *Enterobacteriaceae* did not significantly mediate associations of greater use of eco-friendly products with BMI *z* score ($p = 0.8$) or with child overweight ($p = 0.2$; Appendix 1r). However, among vaginally born infants with a lower abundance of *Enterobacteriaceae* microbes at age 3–4 months, greater use of eco-friendly products

Table 4: Crude and adjusted odds ratios for overweight or obesity at 3 years of age with frequent household use of disinfectant and eco-friendly products

Disinfectant products	**Crude model (*n* = 675) Difference in BMI *z* score (95% CI)**	**Adjusted model* (*n* = 655) Difference in BMI *z* score (95% CI)**
BMI *z* score coefficient		
Disinfectant top 30th centile	0.17 (0.01 to 0.33)	0.11 (−0.02 to 0.25)
Lachnospiraceae (high v. low)	0.28 (0.14 to 0.43)	0.12 (−0.01 to 0.24)
BMI *z* score 1 year	0.48 (0.42 to 0.53)	0.45 (0.39 to 0.50)
Disinfectant Top 40th centile	0.14 (−0.01 to 0.29)	0.08 (−0.05 to 0.21)
Lachnospiraceae (high v. low)	–	0.12 (−0.01 to 0.24)
BMI *z* score 1 year	–	0.45 (0.39 to 0.50)
Disinfectant top 50th centile	0.12 (−0.03 to 0.26)	0.07 (−0.05 to 0.20)
Lachnospiraceae (high v. low)	–	0.12 (−0.01 to 0.24)
BMI *z* score 1 year	–	0.45 (0.39 to 0.50)
Overweight or obesity	**OR (95% CI)**	**AOR (95% CI)**
Disinfectant top 30th centile	1.32 (0.78 to 2.25)	1.32 (0.68 to 2.57)
Lachnospiraceae (high v. low)	1.79 (1.06 to 3.03)	1.18 (0.63 to 2.21)
BMI *z* score 1 year	3.19 (2.27 to 4.30)	3.01 (2.18 to 4.14)
Disinfectant top 40th centile	1.58 (0.95 to 2.64)	1.55 (0.81 to 2.96)
Lachnospiraceae (high v. low)	–	1.14 (0.60 to 2.14)
BMI *z* score 1 year	–	3.04 (2.20 to 4.20)
Disinfectant top 50th centile	1.66 (0.98 to 2.80)	1.55 (0.80 to 3.03)
Lachnospiraceae (high v. low)	–	1.13 (0.60 to 2.13)
BMI *z* score 1 year	–	3.00 (2.18 to 4.13)
Eco-friendly products	**Crude model (n = 675) Difference in BMI *z* score (95% CI)**	**Adjusted model† (*n* = 655) Difference in BMI z score (95% CI)**
BMI *z* score coefficient		
Eco-friendly products 50th centile	−0.25 (−0.40 to −0.11)	−0.12 (−0.24 to 0.002)
Enterobacteriaceae (high v. low)	−0.001 (−0.15 to 0.14)	−0.014 (−0.14 to 0.11)
BMI *z* score 1 year	0.48 (0.42 to 0.53)	0.45 (0.40 to 0.50)
Overweight or obesity	**OR (95% CI)**	**AOR (95% CI)**
Eco-friendly products 50th centile	0.36 (0.20 to 0.63)	0.44 (0.22 to 0.86)
Enterobacteriaceae (high v. low)	0.66 (0.39 to 1.10)	0.51 (0.27 to 0.96)
BMI *z* score 1 year	3.19 (2.37 to 4.30)	3.01 (2.19 to 4.14)

Note: AOR = adjusted odds ratio, BMI = body mass index, CI = confidence interval, OR = odds ratio.
*Adjusted for exposure to household disinfectant, household detergent and household smoke, maternal overweight, BMI *z* score at 1 year and *Lachnospiraceae.*
†Adjusted for exposure to household disinfectant, household eco-friendly products and household smoking, maternal overweight, BMI *z* score at 1 year and *Enterobacteriaceae.*

was associated with decreased odds of overweight or obesity at age 3 (AOR 0.24, 95% CI 0.06–0.86); no associations were found in infants delivered by cesarean (Appendix 1s).

Interpretation

In a subsample of 757 Canadian infants, derived from a population-based birth cohort, household cleaning product use affected gut microbiota at 3–4 months of age, independently of infants' exposure to antibiotics, birth mode, breastfeeding and other microbe-altering covariates. Associations with altered microbiota were most compelling for frequent use of household disinfectants, which showed reduced abundance of genus *Haemophilus* and of genus *Clostridium*. These changes are compatible with the bacterial-killing actions of disinfectants containing bleach and hydrogen peroxide.[29,30] At the same time, *Lachnospiraceae* were 1.3 times more likely to be overrepresented in infant gut microbiota after frequent cleaning with disinfectants. This enrichment with *Lachnospiraceae* at 3–4 months of infant age strongly predicted a higher BMI *z* score at age 1 which, in turn, was a strong determinant of BMI *z* score at age 3. Moreover, we found evidence of statistical mediation by fecal *Lachnospiraceae* of the association between weekly to daily cleaning with disinfectant during infancy and an increase in BMI *z* score at age 3.

Infant fecal abundance of *Lachnospiraceae* rose with frequency of disinfectant cleaning in a dose-dependent manner, with 2-fold higher odds of higher microbial abundance in the highest usage category. Commonly found in infants with detected *Lachnospiraceae*,[31] genus *Ruminococcus* became 1.6 times more likely to have higher abundance with frequent use of disinfectants in our study infants, in conjunction with lowered abundance of genus *Haemophilus* and *Clostridium*. This same compositional profile is typical of eczema in children.[32] Elevated fecal abundance of *Lachnospiraceae* (specifically *Blautia*) concurrent with lowered *Haemophilus* is also a signature of diabetes, as shown in a study on 11-year-old children.[33] Blooms in *Lachnospiraceae* have been observed with subtherapeutic doses of antibiotic treatment in a murine model of obesity[34] and in newborn piglets after environmental aerosolization with a disinfectant.[9] Greater prominence of the *Lachnospiraceae* or individual species in gut microbiota has been associated with higher visceral white adipose tissue mass and insulin resistance in mice,[35] and with higher body fat and insulin resistance in human adults.[36]

Genus *Haemophilus* were further depleted when disinfectants were employed on a daily basis, consistent with their exquisite sensitivity to high concentrations of hydrogen peroxide[37] and frequent use of household wipe products.[38,39] Unlike in the infant nasopharynx,[40] *Haemophilus* species are low-abundant microbiota in the gut;[41,42] they decline, but genus *Clostridium* becomes more prominent as full-term and even hospitalized preterm infants get older. Less well studied than *Clostridium difficile* colonization,[43,44] other species of *Clostridium*, such as clusters XIVa and IV, are important to gut motility, water absorption and immune tolerance.[45] Our observations of both genera are remarkably consistent with the study by Schmidt and colleagues, in which the postbirth transfer of piglets to a pathogen-free environment, aerosolized with disinfectant, caused a reduction in intestinal tissue levels of *Pasteurellaceae* and *Clostridiaceae*, and a rise in *Lachnospiraceae*.[9] Increases to *Enterobacteriaceae* were also seen in these piglets.

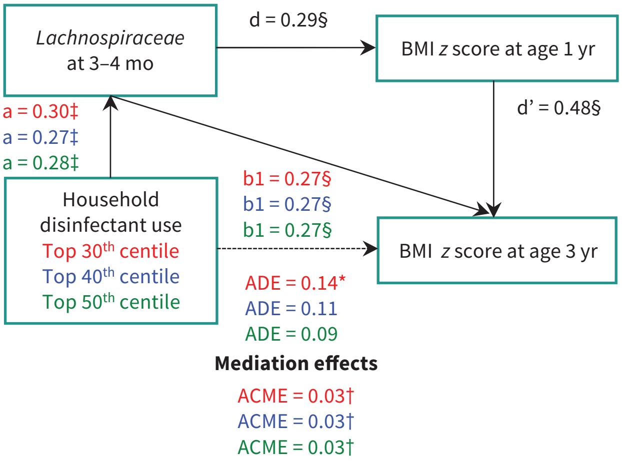

Figure 4: Hypothetical pathway for the association between exposure to household disinfectant and body mass index (BMI) *z* score at age 3 years. a, b1, ADE, ACME are taken from Appendix 1h; d is taken from Appendix 1g; d' is taken from Table 4 (a). a = association between exposure to disinfectant and *Lachnospiraceae;* b1 = association between *Lachnospiraceae* and BMI *z* score at age 3 years; d = association between *Lachnospiraceae* and BMI *z* score at age 1 year; d' = association between BMI *z* score at age 1 year and BMI *z* score at age 3 years; total effect (a total effect of X [disinfectant/eco-friendly products]) on Y (overweight/obese or BMI *z* scores 3 years) without mediator (M; *Lachnospiraceae*/*Enterobacteriaceae*). ADE (a direct effect of X [disinfectant/eco-friendly products]) on Y (overweight/obese or BMI *z* scores 3 yr) after taking into account a mediation (indirect) effect of M (*Lachnospiraceae*/*Enterobacteriaceae*). ACME (mediation effect) = total effect – ADE.*$p < 0.1$, †$p < 0.05$, ‡$p < 0.1$, §$p < 0.001$.

On the other hand, infants remained normal weight when eco-friendly products were used daily and *Enterobacteriaceae* were less abundant in their gut. Eco-friendly products have efficacy against *Escherichia coli*;[12] when these microbes are fewer in number, adiposity is less likely in toddlers.[46] However, we found no statistical evidence that *Enterobacteriaceae* mediated the strong association between postnatal use of eco-friendly products, and BMI *z* score or child overweight. This led us to speculate about lower rates of transmission for *Enterobacteriaceae* during vaginal birth when mothers used eco-friendly products or led healthy lifestyles.[47] In support of this thesis, we identified an inverse association between high use of eco-friendly products and child overweight only among infants with low levels of *Enterobacteriaceae* and who had been born vaginally.

This large, general-population birth cohort study tested associations between home cleaning products and gut microbial composition in early life, and later overweight, which were adjusted for microbe-altering covariates. We employed high-throughput genetic sequencing to profile whole fecal microbial communities. These aspects enhanced the external and internal validity of the results. Associations between cleaning product use and infant gut microbial changes were dose dependent, and showed consistency with porcine and murine model experiments, meeting 2 additional Bradford Hill criteria for causation. Finally, tests for mediation contributed evidence on the relevance of microbiota changes to the development of child overweight.

Limitations

The status of infant exposure to cleaning agents was assumed from parent report. Recall bias is thus a possibility. Nonetheless, most questions on use of household cleaning products were adapted from the Seattle–King County Healthy Homes study, which has shown the effectiveness of household interventions in reducing these exposures in children.[48] Our study did not differentiate cleaning products by brand name or the presence of specific ingredients. The latter is challenging as some ingredients are not listed on product labels, especially of eco-friendly products. Finally, infant gut microbiota were profiled at a single time point, although the fecal sample was obtained during a critical early phase of infant development.

Conclusion

Antibacterial cleaning products have the capacity to change the environmental microbiome and alter risk for child overweight. Our study provides novel information regarding the impact of these products on infant gut microbial composition and outcomes of overweight in the same population. We found *Lachnospiraceae* to be enriched in infant gut microbiota with frequent postnatal use of domestic disinfectants but not eco-friendly products; genus *Clostridium* and *Haemophilus* were reduced concurrently. Evidence of statistical mediation with *Lachnospiraceae* abundance showed a role for this disinfectant-related change to gut microbiota in causing overweight. We did not observe mediation for infant fecal *Enterobacteriaceae*, suggesting an alternate pathway for the association between postnatal eco-friendly product use and reduced child overweight. Further study is required on the mechanisms through which household cleaning products alter gut microbial composition and the subsequent role this might have on metabolic disease.

References

1. Nazaroff WW, Weschler CJ. Cleaning products and air fresheners: exposure to primary and secondary air pollutants. *Atmos Environ* 2004;38:2841-65.
2. Matulonga B, Rava M, Siroux V, et al. Women using bleach for home cleaning are at increased risk of non-allergic asthma. *Respir Med* 2016;117:264-71.
3. Herr M, Just J, Nikasinovic L, et al. Influence of host and environmental factors on wheezing severity in infants: findings from the PARIS birth cohort. *Clin Exp Allergy* 2012;42:275-83.
4. Lankester J, Patel C, Cullen MR, et al. Urinary triclosan is associated with elevated body mass index in NHANES. *PLoS One* 2013;8:e80057.
5. Deierlein AL, Wolff MS, Pajak A, et al. Phenol concentrations during childhood and subsequent measures of adiposity among young girls. *Am J Epidemiol* 2017;186:581-92.
6. Scott E, Bloomfield SF, Barlow CG. Evaluation of disinfectants in the domestic environment under 'in use' conditions. *J Hyg (Lond)* 1984;92:193-203.
7. Josephson KL, Rubino JR, Pepper IL. Characterization and quantification of bacterial pathogens and indicator organisms in household kitchens with and without the use of a disinfectant cleaner. *J Appl Microbiol* 1997;83:737-50.
8. Medrano-Félix A, Martínez C, Castro-del Campo N, et al. Impact of prescribed cleaning and disinfectant use on microbial contamination in the home. *J Appl Microbiol* 2011;110:463-71.
9. Schmidt B, Mulder IE, Musk CC, et al. Establishment of normal gut microbiota is compromised under excessive hygiene conditions. *PLoS One* 2011;6:e28284.
10. Levy SB. Antibiotic and antiseptic resistance: impact on public health. *Pediatr Infect Dis J* 2000;19(Suppl 10):S120-S122.
11. Hooton TM, Levy SB. Antimicrobial resistance: a plan of action for community practice. *Am Fam Physician* 2001;63:1087-98.
12. Goodyear N, Brouillette N, Tenaglia K, et al. The effectiveness of three home products in cleaning and disinfection of Staphylococcus aureus and *Escherichia coli* on home environmental surfaces. *J Appl Microbiol* 2015;119:1245-52.
13. Adukwu EC, Allen SC, Phillips CA. A comparison of the sensitivity of four *Staphylococcus aureus* isolates to two chlorine-based disinfectants and an eco-friendly commercially available cleaning agent. *Int J Environ Health Res* 2015;25:115-25.
14. Rutala WA, Barbee SL, Aguiar NC, et al. Antimicrobial activity of home disinfectants and natural products against potential human pathogens. *Infect Control Hosp Epidemiol* 2000;21:33-8.
15. Stiemsma LT, Reynolds LA, Turvey SE, et al. The hygiene hypothesis: current perspectives and future therapies. *ImmunoTargets Ther* 2015;4:143-57.
16. Hesselmar B, Hicke-Roberts A, Wennergren G. Allergy in children in hand versus machine dishwashing. *Pediatrics* 2015;135:e590-7.
17. Flores G, Lin H. Factors predicting severe childhood obesity in kindergarteners. *Int J Obes (Lond)* 2013;37:31-9.
18. Leech JA, Nelson WC, Burnett RT, et al. It's about time: a comparison of Canadian and American time-activity patterns. *J Expo Anal Environ Epidemiol* 2002;12:427-32.
19. Azad MB, Bridgman SL, Becker AB, et al. Infant antibiotic exposure and the development of childhood overweight and central adiposity. *Int J Obes (Lond)* 2014;38:1290-8.
20. Penders J, Thijs C, van den Brandt PA, et al. Gut microbiota composition and development of atopic manifestations in infancy: the KOALA Birth Cohort Study. *Gut* 2007;56:661-7.
21. Kumari M, Kozyrskyj AL. Gut microbial metabolism defines host metabolism: an emerging perspective in obesity and allergic inflammation. *Obes Rev* 2017;18:18-31.
22. Ley C, Sanchez Mde L, Mathur A, et al. Stanford's Outcomes Research in Kids (STORK): a prospective study of healthy pregnant women and their babies in Northern California. *BMJ Open* 2016;6:e010810-2015-010810.
23. BMI classification. Geneva: World Health Organization; 2017. Available: www.who.int/mediacentre/factsheets/fs311/en/ (accessed 2017 Apr. 13).
24. WHO child growth standards. Geneva: World Health Organization; 2006.
25. Azad MB, Konya T, Persaud RR, et al. Impact of maternal intrapartum antibiotics, method of birth and breastfeeding on gut microbiota during the first year of life: a prospective cohort study. *BJOG* 2016;123:983-93.
26. Tingley D, Yamamoto T, Hirose K, et al. Mediation: R package for causal mediation analysis. *J Stat Softw* 2014;59:1-38.
27. Mickey RM, Greenland S. The impact of confounder selection criteria on effect estimation. *Am J Epidemiol* 1989;129:125-37.
28. McNamee R. Confounding and confounders. *Occup Environ Med* 2003;60:227-34; quiz 164, 234.
29. Edwards AN, Karim ST, Pascual RA, et al. Chemical and stress resistances of *Clostridium difficile* spores and vegetative cells. *Front Microbiol* 2016;7:1698.
30. Omidbakhsh N. Theoretical and experimental aspects of microbicidal activities of hard surface disinfectants: are their label claims based on testing under field conditions? *J AOAC Int* 2010;93:1944-51.
31. Sagheddu V, Patrone V, Miragoli F, et al. Infant early gut colonization by *Lachnospiraceae*: high frequency of *Ruminococcus gnavus*. *Front Pediatr* 2016;4:57.
32. Zheng H, Liang H, Wang Y, et al. Altered gut microbiota composition associated with eczema in infants. *PLoS One* 2016;11:e0166026.
33. Qi CJ, Zhang Q, Yu M, et al. Imbalance of fecal microbiota at newly diagnosed type 1 diabetes in Chinese children. *Chin Med J (Engl)* 2016;129:1298-304.
34. Cho I, Yamanishi S, Cox L, et al. Antibiotics in early life alter the murine colonic microbiome and adiposity. *Nature* 2012;488:621-6.
35. Poroyko VA, Carreras A, Khalyfa A, et al. Chronic sleep disruption alters gut microbiota, induces systemic and adipose tissue inflammation and insulin resistance in mice. *Sci Rep* 2016;6:35405.
36. Le Chatelier E, Nielsen T, Qin J, et al. Richness of human gut microbiome correlates with metabolic markers. *Nature* 2013;500:541-6.
37. Pericone CD, Overweg K, Hermans PW, et al. Inhibitory and bactericidal effects of hydrogen peroxide production by *Streptococcus pneumoniae* on other inhabitants of the upper respiratory tract. *Infect Immun* 2000;68:3990-7.

38. Alfa MJ, Lo E, Olson N, et al. Use of a daily disinfectant cleaner instead of a daily cleaner reduced hospital-acquired infection rates. *Am J Infect Control* 2015;43:141-6.

39. *Ready-to-use surface cleaner & intermediate level disinfectant, general virucide, bactericide, tuberculocide*. Charlotte (NC): JohnsonDiversey; 2007. Available: www.wesclean.com/catelog/productdocs/0400167.pdf (accessed 2017 Jan. 27).

40. Bosch AA, van Houten MA, Bruin JP, et al. Nasopharyngeal carriage of *Streptococcus pneumoniae* and other bacteria in the 7th year after implementation of the pneumococcal conjugate vaccine in the Netherlands. *Vaccine* 2016;34:531-9.

41. Cong X, Xu W, Janton S, et al. Gut microbiome developmental patterns in early life of preterm infants: impacts of feeding and gender. *PLoS One* 2016;11:e0152751.

42. Bäckhed F, Roswall J, Peng Y, et al. Dynamics and stabilization of the human gut microbiome during the first year of life. [published erratum in *Cell Host Microbe* 2015;17:852.] *Cell Host Microbe* 2015;17:690-703.

43. Bridgman SL, Konya T, Azad MB, et al. High fecal IgA is associated with reduced *Clostridium difficile* colonization in infants. *Microbes Infect* 2016;18:543-9.

44. Favier CF, Vaughan EE, De Vos WM, et al. Molecular monitoring of succession of bacterial communities in human neonates. *Appl Environ Microbiol* 2002;68:219-26.

45. Lopetuso LR, Scaldaferri F, Petito V, et al. *Commensal Clostridia*: leading players in the maintenance of gut homeostasis. *Gut Pathog* 2013;5:23.

46. Dogra S, Sakwinska O, Soh SE, et al. Dynamics of infant gut microbiota are influenced by delivery mode and gestational duration and are associated with subsequent adiposity. *MBio* 2015;6. pii: e02419-14. doi:10.1128/mBio.02419-14.

47. Tannock GW, Fuller R, Smith SL, et al. Plasmid profiling of members of the family *Enterobacteriaceae*, *lactobacilli*, and *bifidobacteria* to study the transmission of bacteria from mother to infant. *J Clin Microbiol* 1990;28:1225-8.

48. Krieger JW, Takaro TK, Song L, et al. The Seattle-King County Healthy Homes Project: a randomized, controlled trial of a community health worker intervention to decrease exposure to indoor asthma triggers. *Am J Public Health* 2005;95:652-9.

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