Research Report 237, *An Investigation of Early-Life Air Pollution Exposure and Its Effect on the Infant Gut Microbiome and Fecal Metabolome*, by T.L. Alderete et al.

INTRODUCTION

In 2021, over 40% of the United States population was estimated to be overweight or obese.[1,2] This statistic highlights a critical public health issue because being overweight or obese is a well-known risk factor for multiple diseases, such as cardiovascular disease and type 2 diabetes.[3] Understanding the factors that might contribute to the risks of having overweight and obesity is therefore important, particularly in children, where combined rates of these health statuses have increased linearly since 1990.[2]

Some studies have demonstrated associations between ambient and traffic-related air pollution and body mass index (BMI) in children and adolescents.[4,5] However, the biological mechanisms underlying these associations are not well understood. Recent research has suggested that ambient air pollution exposures might contribute to obesity and other adverse health outcomes through alterations in the gut microbiome (microorganisms, including bacteria, fungi, viruses, and their genes, within the gastrointestinal tract) and associated bacteria-derived metabolites in the fecal metabolome (the collection of small molecules produced by gut bacteria and found in feces).[6–9]

To evaluate the potential effects of early-life exposures to ambient and traffic-related air pollution on the developing gut microbiome and fecal metabolome, Dr. Tanya L. Alderete of Johns Hopkins University submitted an application to HEI titled "Air Pollutants and the Gut Microbiota and Metabolome During Early Life: Implications for Childhood Obesity" in response to HEI's *Request for Applications 18-2:* Walter A. Rosenblith New Investigator Award. This award was established to support an outstanding new investigator at the assistant professor level in conducting research on air pollution and health; it is unrestricted with respect to the specific research topic. Dr. Alderete proposed to examine whether prenatal and postnatal exposures to ambient air pollution, including traffic-related air pollution, affect the infant gut microbiota and fecal metabolome, potentially altering infant growth trajectories in the first 2 years of life. HEI's Research Committee recommended funding Dr. Alderete's application because the study had the potential to provide new insights into the mechanisms through which air pollution might contribute to obesity, with potential implications for precision prevention and treatment. The study began in 2020.

This Commentary provides the HEI Review Committee's independent evaluation of the study. It is intended to aid the sponsors of HEI and the public by highlighting both the strengths and limitations of the study and by placing the results presented in the Investigators' Report into a broader scientific and regulatory context.

SCIENTIFIC AND REGULATORY BACKGROUND

Several studies have shown that exposure to ambient air pollutants emitted by traffic and other sources — such as particulate matter ≤2.5 µm in aerodynamic diameter ($PM_{2.5}$), particulate matter ≤10 µm in aerodynamic diameter (PM_{10}), nitrogen dioxide (NO_2), and nitrogen oxides (NO_x) — is associated with higher BMI and increased risk of obesity in children.[4,5,10] However, other studies have demonstrated no association between ambient or traffic-related air pollution exposures and childhood obesity,[11,12] and the overall evidence for this relationship remains mixed.[13,14] Studies have also shown that ambient air pollution is associated with low birth weight,[15,16] and infant birth weight is closely linked to the composition of the infant gut microbiome.[17] This connection between weight and the gut microbiome might help explain the mixed evidence regarding the relationship between early-life air pollution exposures and obesity.

The mechanisms and risk factors linking air pollution exposures and obesity — both generally and specifically in children — are not well understood. Potential mechanisms include the effects of air pollution on changes in gene expression that occur without altering DNA sequences (i.e., epigenetic modulation), oxidative stress and inflammation, and disruption of neuroendocrine pathways, which can alter metabolic processes and appetite regulation.[13,18] Additionally, recent work has suggested that ambient air pollution exposures might contribute to obesity by affecting metabolic health through changes in the gut microbiome and fecal metabolome (**Box 1**), due to alterations in gut bacteria composition and function.[7,9] For example, a study in adolescents demonstrated correlations between higher exposures to traffic-related air pollution and the abundances of gut bacteria previously

Dr. Tanya L. Aldrete's 3-year study, "Air Pollutants and the Gut Microbiota and Metabolome During Early Life: Implications for Childhood Obesity," began in May 2020. Total expenditures were $500,000. The draft Investigators' Report was received for review in October 2024. A revised report, received in March 2025, was accepted for publication in April 2025. During the review process, the HEI Review Committee and the investigators had the opportunity to exchange comments and clarify issues in the Investigators' Report and its Commentary. Review Committee member Kiros Berhane did not partake in the review of the report due to a conflict of interest.

*A list of abbreviations and other terms appears at the end of this report.

linked to changes in metabolism and obesity.[6] Other research has shown associations between ambient air pollution and changes in gut bacteria during early life with childhood BMI and obesity risk.[19,20] Nonetheless, at the time Dr. Alderete's study began, few studies had examined mechanisms linking prenatal and postnatal ambient air pollution exposures with the gut microbiome and microbiome-derived metabolites among infants in the context of providing potential insights for childhood obesity.

In the United States, regulatory efforts have sought to moderate the health effects of $PM_{2.5}$ and NO_2. The National Ambient Air Quality Standards, established by the United States Environmental Protection Agency (US EPA), limit the 3-year annual average $PM_{2.5}$ concentration to 9 $\mu g/m^3$ and the annual average NO_2 concentration to 53 parts per billion.[21,22] In the most recent integrated science assessments for both particulate matter and oxides of nitrogen, obesity was considered a risk factor for air pollution-related health outcomes.[23,24] In those assessments, the US EPA concluded that existing evidence suggests an increased risk for $PM_{2.5}$-related health effects among individuals with obesity compared with non-obese individuals, and that evidence remains inadequate to determine NO_2-related health effects.

STUDY OBJECTIVES

The overall objective of Dr. Alderete's study was to evaluate whether prenatal or postnatal exposures to ambient air pollution, including traffic-related air pollution, affect the infant gut microbiome and fecal metabolome during the first 2 years of life. Using stool samples collected longitudinally from infants aged 1, 6, 12, 18, and 24 months, the team sought to explore two specific aims:

Aim 1. Determine whether prenatal or postnatal exposure to air pollution is associated with a) lower gut bacterial diversity and altered abundances of gut bacteria and b) levels of fecal metabolites, at each timepoint (cross-sectional analyses).

Aim 2. Determine whether prenatal or postnatal exposure to air pollution is associated with a) the developmental trajectory of the infant gut microbiota (i.e., lower average bacterial diversity or altered average relative abundances of gut bacteria) and b) changes in average fecal metabolite levels over time during the first 2 years of life (longitudinal analyses).

For ease of comprehension, various terms used throughout this Commentary that refer to the outcomes and exposures assessed in the study are defined in **Box 2**.

Box 1: An Introduction to the Infant Gut Microbiome and Fecal Metabolome

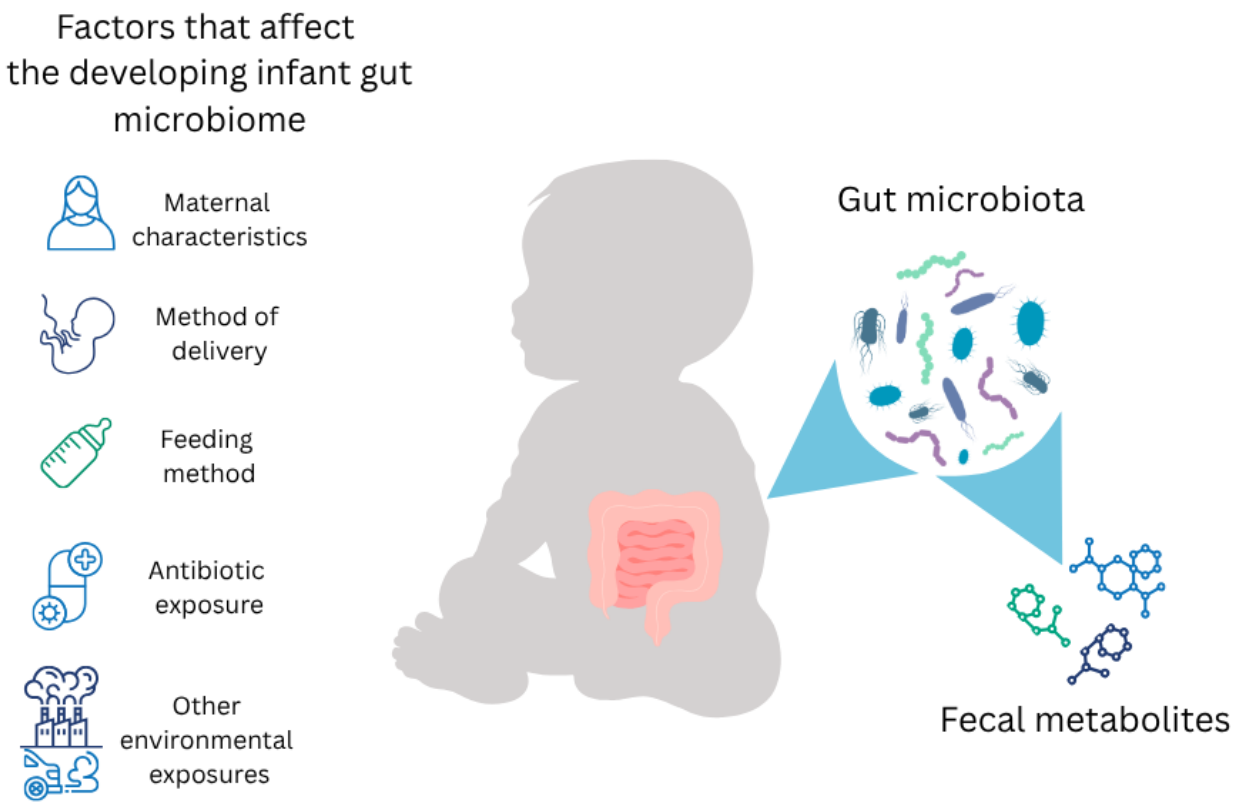
The infant gut microbiome consists of the microbiota, including bacteria, fungi, and viruses, found in the infant gastrointestinal tract. The infant fecal metabolome refers to the collection of metabolites, or small molecules, that reflect diet and metabolism, as well as metabolites produced by gut bacteria and fungi, or influenced by viral activity, as part of metabolic processes. It thus can partly provide a functional readout of the infant gut microbiome[25] (**Commentary Figure 1**).

Studies of the infant gut microbiome and fecal metabolome are important because the first 1,000 days after birth represent a critical period for the growth and development of the gut microbiome, with broad effects on the infant's immune system, metabolism, and neurodevelopment.[26,27] An array of factors influences this development, including maternal characteristics (such as diet, weight, and age) and, more importantly, the type of birth (vaginal delivery or cesarean section), the feeding method (human milk or formula), antibiotic exposure, and infant diet after the introduction of solid foods.[26–28] Additionally, other early-life exposures, such as exposures to air pollution, pets, metals, and chemicals, have been linked to alterations in the infant gut microbiota.[29–31]

Metagenomics and metabolomics are common methods used to study the gut microbiome and fecal metabolome, respectively. Generally, both approaches rely on stool samples, which are processed and analyzed using sequencing and mass spectrometry techniques, respectively.[32]

Metagenomics provides an overview of the composition, diversity, and function of the entire genomes of bacterial, fungal, and viral members of the microbiome by randomly sequencing DNA fragments within a sample. Metagenomic sequencing can provide phylogenetic information about the gut microbiota in a sample, including the abundance and diversity of various microbes,[25] and information related to the functional potential of the microbiome based on the presence of genes with specific known functions.[33] Abundance refers to the amount of a given type of microbe within a sample, whereas diversity refers to both the number of species (richness) and their distribution or relative abundance (evenness). Microbial diversity can be measured using alpha-diversity and beta-diversity metrics. Alpha-diversity focuses on microbial richness and evenness within a given sample.[34] Examples of alpha-diversity measures used in this study include the Shannon and Simpson indices. Beta-diversity focuses on differences in microbial composition between samples and was measured in this study using the Bray–Curtis dissimilarity metric.[35]

Fecal metabolomics provides insight into the metabolic processes occurring in the distal gut, which are partly driven by microbial metabolism. This approach involves identifying and quantifying metabolites within a sample using mass spectrometry. Fecal metabolic intensity reflects the relative levels of metabolites in a stool sample. The identification and quantification of metabolites can be either targeted (focusing on specific classes of compounds such as amino acids or fatty acids) or untargeted (aiming to identify as many metabolites as possible).[25]



Commentary Figure 1. Schematic of the infant gut microbiome and fecal metabolome.

Box 2: Defining Terms Used Throughout This Commentary

- Gut bacterial abundance: The amount of a specific gut bacterium within a sample.
- Gut bacterial diversity: A measure of the richness (number of unique bacterial types) or evenness of their distribution within a sample.
- Identity and levels of fecal metabolites: The type and quantity of small molecules detected in fecal samples, potentially produced by gut bacteria as byproducts of metabolism (referred to as "fecal metabolite intensity" in the Investigators' Report).
- Prenatal air pollution exposure: The average of monthly air pollutant concentrations for the 9 months before an infant's birth.
- Postnatal air pollution exposures: This study assessed three measures:
  - Short-term air pollution exposure: The average air pollutant concentration in the month before an infant's clinical visit.
  - Cumulative air pollution exposure: The average of monthly air pollutant concentrations from birth to a clinical visit.
  - Fluctuation from long-term air pollution exposure: The difference between the average of short-term air pollutant concentrations across all clinical visits and the monthly average concentration in the month before a clinical visit.

To address the study aims, Alderete and colleagues used a cohort of more than 200 Hispanic mother–infant pairs participating in the Southern California Mother's Milk Study. Pairs were enrolled at 1 month postpartum and attended subsequent clinical visits at various timepoints up to 24 months postpartum. Participants were all located in Southern California, and most resided in the Los Angeles area. Detailed information was available regarding both the mother and infant (such as age, sex, and socioeconomic status), as well as infant feeding practices. At each clinical visit, an infant stool sample was collected. All data were processed using metagenomic and metabolomic analysis techniques.

Based on the mothers' residential address histories, the team assigned estimates of prenatal and postnatal exposures to PM_{10}, $PM_{2.5}$, NO_2, ozone (O_3), and NO_x (used as a proxy for traffic-related air pollution) for each mother–infant pair. A cumulative 9-month average of air pollutant concentrations before birth was used to estimate prenatal exposure. Postnatal exposures in cross-sectional analyses included both

short-term (i.e., prior-month) and cumulative estimates of air pollution concentrations, whereas postnatal exposures in longitudinal analyses were based on fluctuations from long-term air pollution concentrations during the first 2 years of life. All exposure estimates were calculated using monthly concentrations of ambient air pollutants (PM_{10}, $PM_{2.5}$, NO_2, O_3) derived from US Environmental Protection Agency (US EPA) monitoring data and were spatially interpolated between central monitors. Monthly concentrations of traffic-related air pollutants (NO_x) were estimated using an air quality dispersion model.

Alderete and colleagues used a combination of negative binomial models and linear mixed-effects models to evaluate associations between air pollution exposure estimates and several outcomes of interest, including the abundances and diversity of gut bacteria in the infant gut microbiome and the identities and levels of fecal metabolites in the infant fecal metabolome.

The study also originally included a third aim to use mediation analysis to determine whether infant gut microbiota or fecal metabolites associated with higher estimated ambient or traffic-related air pollution exposures mediated changes in infant growth trajectories over time. However, this analysis could not be conducted due to substantial delays in the study related to the COVID-19 pandemic.

SUMMARY OF METHODS AND STUDY DESIGN

MOTHER'S MILK STUDY POPULATION

The study cohort was drawn from the Southern California Mother's Milk Study, a prospective cohort study of 219 Hispanic mother–infant pairs designed to examine the effects of human milk feeding on early-life growth and development. Eligible individuals were self-identifying Hispanic mothers who were at least 18 years old at the time of delivery; had a healthy, term (≥37 weeks) singleton birth; and intended to breastfeed for at least 6 months. Mother–infant pairs were enrolled at 1 month postpartum and attended follow-up visits at 6, 12, 18, and 24 months postpartum. Each visit included infant stool sample collection and completion of questionnaires on infant feeding practices (e.g., frequency of human milk feeding and age at which solid foods were introduced). Overall, the study sample included 207 infants with gut microbiome data; 127 of these infants were included in the high-resolution fecal metabolomics analysis.

The outcomes of interest for Dr. Alderete's study were 1) abundances of gut bacteria, 2) diversity of gut bacteria, and 3) identities and levels of fecal metabolites.

EXPOSURE ASSESSMENT

The investigators estimated monthly concentrations of PM_{10} (μg/m^3), $PM_{2.5}$ (μg/m^3), NO_2 (ppb), and O_3 (ppb) through spatial interpolation of monitoring data from the US EPA Air Quality System,[36] based on participants' residential address histories. NO_x was used as a proxy for traffic-related air pollution, and monthly concentrations of NO_x within 5 km of participants' homes were estimated using the California Line Source Dispersion Model (CALINE4),[37] an air quality model that integrates information regarding traffic emissions, traffic volume, roadway geometry, and meteorology to estimate pollutant concentrations near roadways.

Alderete and colleagues computed several measures of air pollutant exposure for each mother–infant pair, broadly categorized into prenatal and postnatal exposures, as previously defined in Textbox 1.

STOOL SAMPLE ANALYSIS AND DATA PROCESSING

For the gut microbiome analysis, the investigators performed DNA extraction and sequencing on infant stool samples. DNA sequence reads were taxonomically classified using the RefSeq database of bacterial, viral, plasmid, human, and vector sequences[38]; these reads were used to identify the relative abundances of gut bacterial taxa (categories used to classify bacteria based on shared biological characteristics) at the species level. The average number of reads per sample was about 17 million, which is considered relatively low in the context of fecal metagenomics. Shallow sequencing, in which a smaller amount of DNA is sequenced, provides a less detailed analysis of the microbiome compared with deep sequencing methods but is often more cost-effective. Gut bacterial diversity was assessed by calculating "alpha-diversity measures" to quantify species diversity within each sample (Shannon index, species richness, species evenness, and Simpson index) and "beta-diversity measures" to quantify differences in microbiome composition between samples (Bray–Curtis dissimilarity).

For the fecal metabolome analysis, stool samples were analyzed using liquid chromatography–high-resolution mass spectrometry (LC-HRMS). Fecal metabolites were profiled and analyzed using an untargeted approach (i.e., as many metabolites as possible were identified and quantified without prior knowledge of their identity or profile).

MAIN STATISTICAL ANALYSES

Alderete and colleagues conducted multiple statistical analyses to address their study aims. For brevity, see **Commentary Table 1** for a summary of the study population, exposure assessment, and statistical methods used.

Aim 1. Associations of Air Pollution Exposures with the Infant Gut Microbiome and Fecal Metabolome at Each Timepoint (Cross-Sectional Analyses)

The investigators used negative binomial and linear models and a variance test to evaluate associations between estimated prenatal and postnatal air pollution exposures and the infant gut microbiome. In these analyses, associations of estimated prenatal, cumulative, and short-term exposures to all air pollutants with gut bacterial abundances and diversity were examined for each infant follow-up visit. For the fecal metabolome analysis, associations of estimated prenatal,

Commentary Table 1. Summary of Main Statistical Analyses Conducted in This Study

Aim 1. Cross-Sectional Analyses				
Analysis	**Study Population**	**Estimated Exposures**	**Method**	**Outcome**
Infant gut microbiome	N = 207 infants with gut microbiome data 1-month visit (N = 196) 6-month visit (N = 157) 12-month visit (N = 155) 18-month visit (N = 143) 24-month visit (N = 171)	Prenatal, cumulative, and short-term estimates of PM_{10}, $PM_{2.5}$, NO_2, O_3, and NO_x	1. Negative binomial models[a] 2. Linear models[a] 3. Permutational multivariate analysis of variance (PERMANOVA)	1. Abundances of gut bacterial taxa at each timepoint 2. Alpha-diversity of gut bacterial taxa at each timepoint 3. Beta-diversity of gut bacterial taxa at each timepoint
Infant fecal metabolome	N = 127 infants with fecal metabolite data 1-month visit (N = 124) 6-month visit (N = 116) 12-month visit (N = 120) 18-month visit (N = 123) 24-month visit (N = 126)	Prenatal, cumulative, and short-term estimates of PM_{10}, $PM_{2.5}$, NO_2, O_3, and NO_x	Linear models[a]	Identity and levels of fecal metabolites at each timepoint
Aim 2. Longitudinal Analyses				
Analysis	**Study Population**	**Estimated Exposures**	**Method**	**Outcome**
Infant gut microbiome	N = 207 infants with gut microbiome data	Prenatal estimates of PM_{10}, $PM_{2.5}$, NO_2, O_3, and NO_x	Negative binomial models[a]	Average abundances of gut bacterial taxa across timepoints
		Fluctuations from long-term early-life estimates of PM_{10}, $PM_{2.5}$, NO_2, O_3, and NO_x	1. Negative binomial models[b] 2. Linear mixed-effects models[b]	1. Average abundances of gut bacterial taxa across timepoints 2. Alpha-diversity of gut bacterial taxa across timepoints
Infant fecal metabolome	N = 127 infants with fecal metabolite data	Prenatal and fluctuations from long-term early-life estimates of PM_{10}, $PM_{2.5}$, NO_2, O_3, and NO_x	Linear mixed-effects models[b]	Average levels of fecal metabolites across timepoints

[a]All models were adjusted for infant age and sex, maternal age, maternal prepregnancy BMI, socioeconomic status, human milk feedings per day, formula feedings per day, type of delivery, whether solid foods had been introduced (6-month timepoint and after), and diet quality (12-month timepoint and after).

[b]All models were adjusted for infant age and sex, maternal age, maternal prepregnancy BMI, socioeconomic status, human milk or formula feedings per day, type of delivery, whether solid foods had been introduced, diet quality, and long-term early-life air pollution exposure.

cumulative, and short-term air pollution exposures with the identities and levels of fecal metabolites were assessed using multivariable linear models.

All models were adjusted for factors such as maternal and infant demographic characteristics, infant diet, and maternal BMI. All models also included adjustment for multiple testing using the Benjamini–Hochberg procedure to control the false discovery rate.

Aim 2. Associations of Air Pollution Exposures with the Infant Gut Microbiome and Fecal Metabolome Across All Timepoints (Longitudinal Analyses)

In longitudinal analyses of the infant gut microbiome, Alderete and colleagues used negative binomial models to evaluate associations between estimated prenatal exposure and fluctuations from long-term early-life air pollution exposures with the average abundances of gut bacterial taxa across

all timepoints. Linear mixed-effects models were used to assess associations between fluctuations from long-term early-life air pollution exposures and the average diversity of gut bacterial taxa, as well as the average level of fecal metabolites across all timepoints.

All models were adjusted for a suite of characteristics, such as maternal and infant demographic characteristics, infant diet, maternal BMI, and average long-term early-life air pollution concentrations. A random intercept was included in all models to adjust for repeated measures within infants.

SUMMARY OF KEY RESULTS

POPULATION CHARACTERISTICS AND EXPOSURE ASSESSMENT

The study included 207 infants with gut microbiome data and a subset of 127 infants with fecal metabolomics data from the Southern California Mother's Milk cohort. Sample sizes varied across timepoints because some participants missed follow-up visits (Commentary Table 1). The average prepregnancy BMI of the mothers was 28.3 kg/m^2, and the average maternal age at the 1-month follow-up visit was 29 years. About 55% of infants in the cohort were female, and about 25% had been delivered by cesarean section — both percentages are similar to rates in the overall US population.

Average estimated prenatal and postnatal exposures to air pollutant concentrations among participants across all timepoints are summarized in **Commentary Table 2**. Average estimated prenatal exposures were broadly consistent with average estimated cumulative and short-term exposures.

PRENATAL AND POSTNATAL AIR POLLUTION EXPOSURES AND THE INFANT GUT MICROBIOME AND FECAL METABOLOME: MAIN STATISTICAL ANALYSES

Overall, Alderete and colleagues found that both estimated prenatal and postnatal exposures to PM_{10}, $PM_{2.5}$, NO_2, O_3, and NO_x demonstrated some associations with short-term and longer-term changes in the abundances of gut bacterial taxa in the infant gut microbiome. Similarly, they found that PM_{10}, $PM_{2.5}$, and NO_2 demonstrated some associations with changes in the level of specific fecal metabolites in the infant fecal metabolome. **Commentary Figure 2** provides an overview of the study's main findings.

Aim 1. Associations Between Air Pollution Exposures and the Infant Gut Microbiome and Fecal Metabolome at Each Timepoint (Cross-Sectional Analyses)

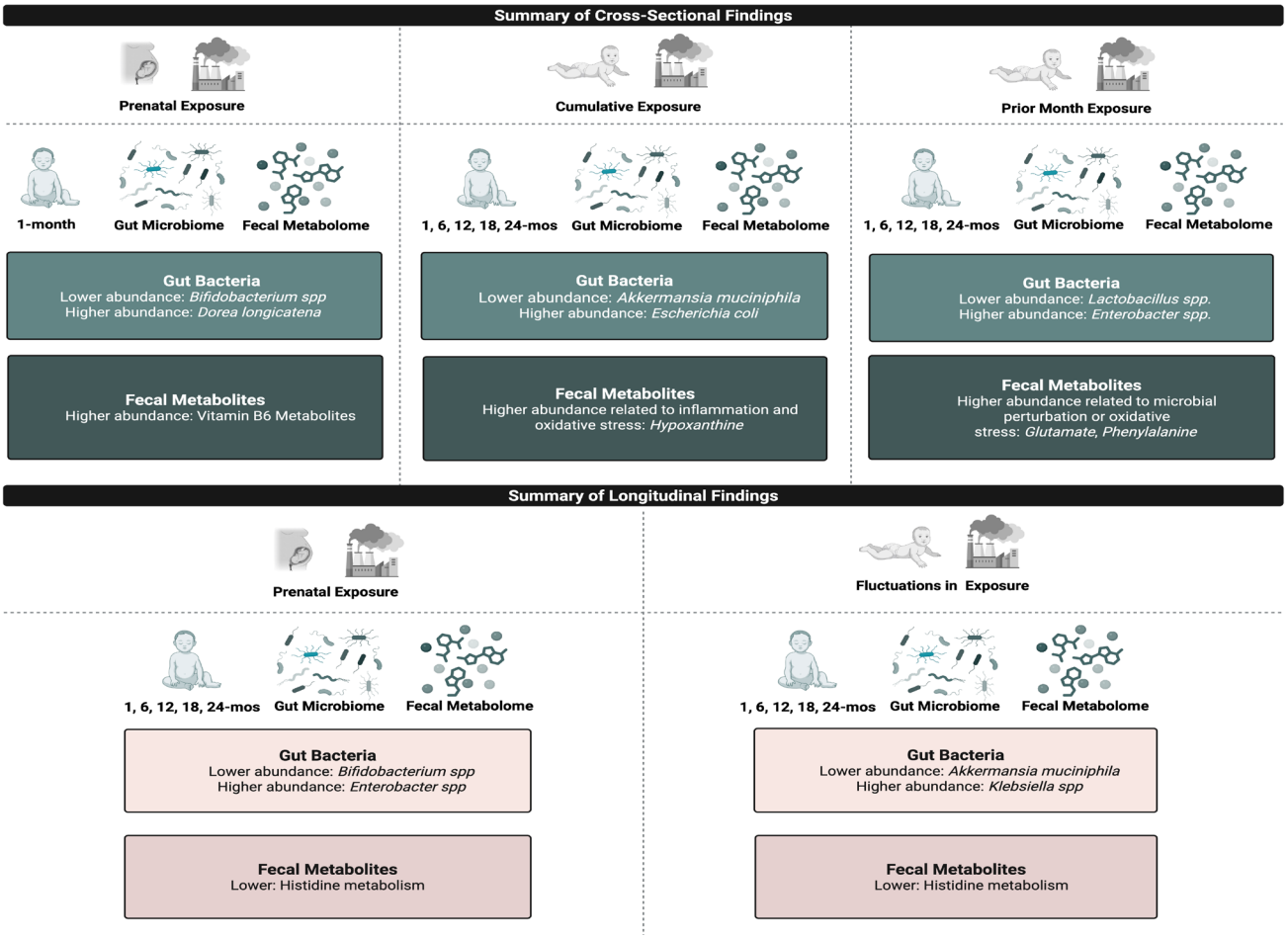
Gut Bacterial Abundance The team conducted cross-sectional analyses to evaluate associations between estimated prenatal exposures to all ambient and traffic-related air pollutants and gut bacterial abundance. They observed a mix of positive and inverse associations with the abundances of various gut bacterial taxa, potentially indicating a shift toward fewer beneficial bacteria. For example, increased PM_{10}, NO_2, and NO_x exposures were all associated with a lower abundance of *Bifidobacterium*, which is typically considered to promote gut health. Increased NO_x exposure was associated with a higher abundance of the potentially detrimental gut bacterium *Lelliottia amnigena*. Associations between estimated short-term (i.e., prior-month) and cumulative exposures to all air pollutants examined with gut bacterial abundances varied in both direction and magnitude; there were no clear patterns across timepoints, specific taxa, or pollutants.

Gut Bacterial Diversity The investigators reported statistically significant associations between estimated short-term and cumulative exposures to PM_{10}, O_3 (with and without adjustment for NO_2), and NO_x with various measures of alpha-diversity, such as the Shannon and Simpson indices. These findings indicated both greater and lesser fecal alpha-diversity, depending on the exposure metric and pollutant examined. Such associations were observed only at the 1-month and/or 6-month timepoints (see Investigators' Report Tables 2 and 6 for additional details). Only estimated short-term and cumulative NO_2 exposures were significantly associated with beta-diversity (i.e., Bray–Curtis dissimilarity), indicating differences in overall microbiome composition linked to NO_2 exposure.

Commentary Table 2. Average Estimated Exposures to Ambient Air Pollutants Across Cohort Participants for Each Exposure Measure Across the Study Period[a]

Ambient Air Pollutant	Prenatal Exposure Concentrations	Cumulative Exposure Concentrations	Short-Term (Prior Month) Exposure Concentrations	Fluctuations from Long-Term Exposure Concentrations
$PM_{2.5}$ (μg/m^3)	11.9	12.2	11.8	0
PM_{10} (μg/m^3)	29.9	30.7	28.7	−0.4
NO_2 (ppb)	18.0	17.8	16.5	−0.1
O_3 (ppb)	42.7	42.1	42.4	0
NO_x (ppb)	3.9	3.3	3.5	0

[a]Adapted from Investigators' Report Table 1.



Commentary Figure 2. Overview of main study findings. Source: Adapted from Holzhausen EA and Alderete TL (2025): *https://BioRender.com/m57g481*.

Fecal Metabolites In analyses of prenatal exposures and fecal metabolites, Alderete and colleagues found that increased exposures to PM_{10}, $PM_{2.5}$, and NO_2 were all associated with higher relative levels of fecal metabolites involved in vitamin B6 metabolism and brain function. Higher estimated prenatal NO_x exposures were associated with lower levels of metabolites linked to the breakdown of dietary sugars. No associations were observed for estimated prenatal O_3 exposures. The majority of statistically significant associations between short-term exposures (PM_{10}, $PM_{2.5}$, O_3 [adjusted for NO_2], and NO_x) or cumulative exposures (PM_{10}, NO_2, and NO_x) and levels of fecal metabolites were observed at the 6-month timepoint, although there was no apparent pattern in metabolite identity across pollutants.

Aim 2. Associations Between Air Pollution Exposures and the Infant Gut Microbiome and Fecal Metabolome Across All Timepoints (Longitudinal Analyses)

Gut Bacterial Abundance In longitudinal analyses, Alderete and colleagues evaluated estimated prenatal exposures to all ambient and traffic-related air pollutants and average gut bacterial abundances during the first 2 years of life. They observed that PM_{10}, $PM_{2.5}$, NO_2, O_3, and NO_x were associated with changes in the average abundances of gut bacterial taxa over time. Generally, associations among air pollutants and specific gut bacterial taxa varied, except for estimated prenatal $PM_{2.5}$ and NO_2 exposures, which were both associated with lower abundances of gut bacteria from the genus *Megasphaera*.

Similar to the cross-sectional analyses, some ambient air pollutants were associated with higher abundances of potentially detrimental gut bacteria. For instance, higher estimated NO_2 concentrations were associated with a higher abundance of *Klebsiella* in fecal samples. Estimated postnatal fluctuations from long-term early-life exposures to NO_2 and O_3 (with and without adjustment for NO_2) were also associated with higher abundances of certain *Klebsiella* species. In general, estimated postnatal exposures for all air pollutants showed statistically significant associations with changes in the average abundances of several gut bacterial taxa, again without an apparent trend according to pollutant or taxon. The investigators did not observe associations between estimated postnatal fluctuations from long-term early-life exposures and gut bacterial diversity (assessed using alpha-diversity measures) in longitudinal analyses.

Fecal Metabolites In longitudinal analyses of estimated prenatal exposures and average fecal metabolite levels over time, only $PM_{2.5}$ and NO_2 were associated with levels of fecal metabolites, specifically those involved in histidine and tyrosine metabolism. Regarding estimated postnatal fluctuations from long-term early-life exposures, statistically significant associations were observed for all pollutants examined except NO_x. For example, higher postnatal $PM_{2.5}$ and PM_{10} exposures were both associated with lower levels of metabolites involved in histidine metabolism. Overall, there were no obvious patterns in the associations between average levels of specific fecal metabolites and pollutant exposures.

HEI REVIEW COMMITTEE'S EVALUATION

This study leveraged a unique dataset from a southern California Hispanic mother–infant cohort to evaluate potential associations between prenatal and early-life air pollution exposures and infant gut bacteria and fecal metabolites. Alderete and colleagues observed that, in both cross-sectional and longitudinal analyses, estimated prenatal and postnatal exposures to ambient and traffic-related air pollution demonstrated some associations with changes in the abundances and diversity of infant gut bacteria and the identities and levels of infant fecal metabolites, with some indication of a shift toward fewer beneficial gut bacteria.

In its independent evaluation, the HEI Review Committee concluded that this study provides a detailed set of exploratory analyses that contribute to understanding potential mechanistic links between air pollution and the gut microbiome in infants, with a possible connection to childhood obesity. The Committee also emphasized that the dataset collected for this study is highly valuable and has strong potential for use in future research. Details on the strengths and limitations of the study are discussed below.

STUDY DESIGN, DATASETS, AND ANALYTICAL APPROACHES

The Committee identified the collection of a novel dataset on the infant gut microbiome and fecal metabolome as a key strength of the study. They also appreciated the thorough initial analyses, which considered both cross-sectional and longitudinal associations.

Several limitations were highlighted by the Committee. First, the sample size for the study cohort was relatively small (207 mother–infant pairs), and only 127 infants were included in the fecal metabolomics analysis, thus limiting the statistical power of the cross-sectional analyses. Second, given the number of variables in the dataset, multiple statistical tests were conducted, which greatly increased the potential for false positives. Alderete and colleagues appropriately acknowledged these limitations and applied the Benjamini–Hochberg procedure to adjust for multiple testing.

Third, the Committee noted that the investigators conducted shallow sequencing (millions of reads per sample) rather than deep sequencing (tens to hundreds of millions of reads per sample) and therefore did not leverage the full potential of the dataset. Fourth, the outcomes selected for this study primarily focused on phylogeny, emphasizing the composition of different species or taxa in the microbiome. The Committee suggested that the inclusion of outcomes related to gut microbial function would have provided additional insights into the relationship between early-life air pollution exposures and gut health.[39] Finally, the Committee stated that future research would benefit from consideration of the viral and fungal microbiomes, rather than focusing solely on the bacterial microbiome.

FINDINGS AND INTERPRETATION

In the cross-sectional and longitudinal analyses, Alderete and colleagues observed that estimated prenatal and early-life exposures to ambient and traffic-related air pollution demonstrated some associations with lower abundances of potentially beneficial gut bacterial species and higher abundances of detrimental gut bacterial species. However, no single ecological or molecular mechanism or pattern was evident across pollutants, outcomes, or timepoints during the first 2 years of life. The investigators also found that increased prenatal or early-life exposures to PM_{10}, $PM_{2.5}$, and NO_2 were generally associated with higher levels of several fecal metabolites, some of which might indicate oxidative stress or gut inflammation (e.g., histidine). However, similar to the metagenomic findings, the fecal metabolome analyses did not reveal clear patterns across pollutants, outcomes, or timepoints.

Given the varied findings and study design limitations, the Committee determined that this work represents a comprehensive set of exploratory analyses and a valuable contribution, but it emphasized that further research is needed. The Committee appreciated that the investigators appropriately characterized this study as hypothesis-generating, with potential for further exploration in future research.

The Committee also recognized that the investigators thoughtfully outlined several future directions, including evaluation of the potential mediating effects of the gut microbiome and fecal metabolome on associations between

ambient air pollution exposure and infant growth trajectories, as well as the use of more advanced multi-omics analytical approaches to explore gut bacterial function based on genes and gene pathways. Finally, the Committee highlighted that the dataset collected for this study represents an excellent resource for other researchers to conduct additional studies.

CONCLUSIONS

In summary, Alderete and colleagues examined whether prenatal or early-life exposures to ambient and traffic-related air pollution were associated with changes in the infant gut microbiome and fecal metabolome during the first 2 years of life. They found that both prenatal and early-life air pollution exposures demonstrated some associations with alterations in the abundances of gut bacteria in the infant microbiome and in the identities and levels of fecal metabolites in the infant metabolome. Although no substantial or conclusive patterns emerged, some associations indicated lower abundances of beneficial gut bacteria, higher abundances of potentially detrimental gut bacteria, and higher levels of metabolites that might indicate oxidative stress and inflammation. Ultimately, this study represents an extensive set of exploratory analyses that can be used in future research aimed at understanding the links between air pollution and the infant gut microbiome and fecal metabolome. Moreover, future research can benefit from this study's unique dataset, which can serve as a valuable resource for additional studies in this field.

ACKNOWLEDGMENTS

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