Chemical exposures assessed via silicone wristbands and endogenous plasma metabolomics during pregnancy

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Abstract

\textbf{Background:} Metabolomics is a promising method to investigate physiological effects of chemical exposures during pregnancy, with the potential to clarify toxicological mechanisms, suggest sensitive endpoints, and identify novel biomarkers of exposures.

\textbf{Objective:} Investigate the influence of chemical exposures on the maternal plasma metabolome during pregnancy.

\textbf{Methods:} Data were obtained from participants (n=177) in the New Hampshire Birth Cohort Study, a prospective pregnancy cohort. Chemical exposures were assessed via silicone wristbands worn for one week at \textasciitilde13 gestational weeks. Metabolomic features were assessed in plasma samples obtained at \textasciitilde24-28 gestational weeks via the Biocrates Absolute\textit{IDQ® p}180 kit and...
nuclear magnetic resonance (NMR) spectroscopy. Associations between chemical exposures and plasma metabolomics were investigated using multivariate modeling.

**Results:** Chemical exposures predicted 11 (of 226) and 23 (of 125) metabolomic features in Biocrates and NMR, respectively. The joint chemical exposures did not significantly predict pathway enrichment, though some individual chemicals were associated with certain amino acids and related metabolic pathways. For example, N,N-diethyl-m-toluamide was associated with the amino acids glycine, L-glutamic acid, L-asparagine, and L-aspartic acid and enrichment of the ammonia recycling pathway.

**Significance:** This study contributes evidence to the potential effects of chemical exposures during pregnancy upon the endogenous maternal plasma metabolome.

**Keywords**
Exposome; metabolome; silicone wristband; pregnancy; exposure; multipollutant

**Introduction**

Human development during the prenatal period is an intricately ordered and tightly regulated process (1–3), and perturbation of maternal physiology during pregnancy by exposure to environmental chemicals can adversely affect maternal health and fetal development (4–6). Of particular concern are cumulative or joint effects of chemical exposures, as multiple chemicals can act upon common physiological processes in additive or synergistic fashions (7–9). Metabolomics, the study of the totality of small molecules within an organism or biological media (10–12), is an emerging and promising method to investigate the influence of chemical exposures (13–16), including their joint impacts on maternal health during pregnancy and subsequent development. Epidemiologic studies of metabolites or metabolic pathways affected by chemical exposures may elucidate toxicological mechanisms, indicate potential downstream effects of exposure, identify novel biomarkers of exposure, and lead to potential targets for intervention.

A growing number of epidemiologic studies have employed metabolomic methods to investigate physiological effects of chemical exposures during pregnancy (17–21), and some of these studies have investigated the impacts of chemical co-exposures upon the pregnancy metabolome (19–21). In one study, Maitre et al. investigated a diverse set of 35 biomarkers of chemical exposures measured in multiple biological media in relation to the urine metabolome in a cohort of pregnant women in Spain (n = 750) (19). The investigators reported relations between urinary arsenic and urinary metabolites related to gut microbial methylamine metabolism and fish consumption, urinary cotinine and urinary metabolites related to coffee metabolism, and serum PCB-153 and urinary metabolites related to ketogenosis. In a second study, Zhou et al. investigated the impact of phthalate exposure, including sums of phthalate metabolites, upon the plasma and urine metabolome in a prospective cohort of pregnant women in California (n = 115) (21). The investigators observed that these exposures were related to lipid, steroid, and nucleic acid metabolism, and also inflammatory pathways. Recently, Yang et al. investigated maternal blood levels of 37 pesticides during pregnancy in relation to birth weight and gestational duration and
mediation by maternal blood metabolomic features in a pregnancy cohort (n = 102) in China (20). The investigators reported that a mixture of the most frequently detected pesticides was associated with decreased birthweight and that β-hexachlorocyclohexane was the primary driver of this association. Subsequent metabolomic analyses indicated that disrupted thyroid hormone synthesis might mediate this relation. These studies demonstrate the potential for metabolomics to support research of physiological effects of chemical co-exposures during pregnancy, though additional work is needed to advance our understandings of such relations.

Therefore, we investigated chemical exposures in relation to plasma metabolomics during pregnancy in a prospective cohort of pregnant women in New Hampshire. Specifically, we used a silicone wristband analyzed with a targeted quantitative method to assess a broad array of chemical exposures (described in Doherty et al. (22)) and we used two metabolomics platforms (one targeted, one untargeted) to assess the pregnancy plasma metabolome. Our primary objectives included the investigation of individual chemicals and chemical exposures in relation to individual metabolites and metabolic pathways using targeted and untargeted approaches.

Materials and Methods

Study population

Data for this analysis were collected as part of the New Hampshire Birth Cohort Study (NHBCS), an ongoing prospective pregnancy and birth cohort of mother-child pairs receiving prenatal care in central New Hampshire (23–25). Eligible women were identified at participating prenatal care clinics and invited to participate if they satisfied the following criteria: 18 to 45 years of age, carrying a singleton pregnancy, able to communicate in English, having a private well as the source of water at their residence, and having resided at the same address since their last menstrual period and intending to remain at that residence throughout the pregnancy. A total of 200 NHBCS participants enrolled between March 2017 and February 2019 provided data on both chemical exposures and plasma metabolomics during pregnancy, and this analysis includes a subset of 177 of these women who had complete and valid data on the timing of the chemical exposure and plasma metabolomic assessments (detailed below). The protocols for NHBCS were approved by the Committee for the Protection of Human Subjects at Dartmouth College and all participants provided written informed consent.

Chemical exposures

To assess chemical exposures during pregnancy, NHBCS used silicone wristbands provided and analyzed by MyExposome, Inc. (Philadelphia, PA) (26–28). In brief, participants wore silicone wristbands for ~7 days, during which environmental chemicals are absorbed by the silicone; these chemicals can then be extracted from the wristbands and their concentrations can be measured to provide an estimate of exposure during the period of wear. The wristbands primarily capture inhalation and dermal exposures. The application of the silicone wristbands as passive samplers in NHBCS has been previously detailed (22). Prior to deployment, the silicone wristbands (https://24hourwristbands.com, Houston, TX)
were prepared using previously described methods (27). During a prenatal care visit at ~13 gestational weeks, participants were provided a silicone wristband enclosed in an airtight polytetrafluoroethylene (PTFE) bag (Welch Fluorocarbon, Dover, NH). Participants were instructed to wear the wristband for seven days and to perform their normal activities and to avoid directly spreading lotion onto the wristband. A period of 7 days was selected to capture a week’s cycle of chemical exposures, and chemical concentrations were standardized to the duration of wear by dividing the chemical concentrations by the reported number of days worn. Participants were instructed to return the wristbands to NHBCS study coordinators by mail in a sealed PTFE bag. Upon receipt, wristbands were stored at room temperature until they were shipped overnight in the PFTE bags to MyExposome for analysis. MyExposome screened for concentrations of 1530 chemicals in the wristbands using gas chromatography coupled with mass spectrometry (GC-MS) with previously published methods (28). These 1530 chemicals include a diverse set of organic chemicals, including chemicals in commerce, chemicals in personal care products, chemicals in consumer products, pesticides, flame retardants, polycyclic aromatic hydrocarbons (PAHs), and pharmaceuticals; the full analyte list is available online (http://www.myexposome.com/fullscreen). The quality of the measurements in the silicone wristbands are maintained using several quality control (QC) samples and techniques, which have been previously detailed (22, 28). The methods for calculating the limits of quantitation (LOQ) for the chemicals in our study, as well as the values of the LOQs, are reported by Bergmann et al. (28)

**Plasma metabolomics**

At a study visit at ~24 gestational weeks, participants were provided blood collection kits and instructed to bring the kits to their routine antenatal care visit at ~24-28 gestational weeks (range: 20, 38). Blood samples were obtained using K2 EDTA tubes. The samples were couriered at 4°C and received by the blood processing laboratory within 24 hours of collection. Samples were centrifuged for 15 minutes at 2500g, and separated plasma was collected and stored at −80°C until shipment. Samples were shipped overnight on dry ice in insulated shipping containers to the CHEAR Metabolomics and Exposome Laboratory at the University of North Carolina at Chapel Hill Nutrition Research Institute for metabolomics analyses. Plasma metabolomic features were measured via two platforms: the Biocrates Absolute**IDQ®** p180 kit and broad spectrum nuclear magnetic resonance (NMR) spectroscopy.

**Targeted Metabolomics**

The targeted mass spectrometry (MS) based quantitative to semi-quantitative assay Biocrates Absolute**IDQ®** p180 kit (Biocrates Life Sciences AG, Innsbruck, Austria; hereafter, “Biocrates”) was used for the measurement of metabolite concentrations. This kit is comprised of two parts that are analyzed by multiple reaction monitoring (MRM) tandem MS analysis (MS/MS). The first part is a high-performance liquid chromatography (HPLC) based method that quantifies 42 metabolites (21 amino acids and 21 biogenic amines), and the second part is a flow injection analysis (FIA) that quantifies 146 metabolites (40 acylcarnitines, 38 acyl/acyl side chain phosphatidylcholines, 38 acyl/alkyl side chain phosphatidylcholines, 14 lysophosphatidylcholines, and 15 sphingolipids in the positive polarity mode, and the total concentration hexoses in the negative polarity mode). In
addition to the 188 metabolites, the kit calculates 44 derivatives of the metabolites (e.g., sums, ratios; Supplemental Table 1), producing a total of 232 metabolomic features. The Biocrates kit was chosen as a scalable high-throughput targeted platform which provides information on endogenous metabolites and metabolic pathways that influence a wide variety of physiological processes.

Ten μL of the stable labeled internal standard mixture and 10 μL of blood plasma were pipetted onto the filter paper in each of the sample wells. After drying under high purity N\(_2\) gas, the sample metabolites were derivatized with phenol isothiocyanate, extracted, split into two parts, and then diluted in preparation for analysis. MS analyses were performed on a 4000 Q-Trap® ESI-LC-MS/MS System (Sciex, Framingham, MA) equipped with an Agilent 1200 Series HPLC (Agilent Technologies, Palo Alto, CA) using an Agilent Zorbax® Eclipse XDB-C\(_{18}\) (3.5 μm) 3.0 x 100 mm column. The system was controlled by a workstation installed with Analyst® 1.6.2 software (Sciex LP, Ontario, Canada). A unique MRM ion pair (precursor MS1 and product MS2 “transition” ions) that is unique to each analyte was used to measure the analytes and their stable labeled internal standards. The internal standard was used to determine absolute and/or relative quantification. All raw data were processed using a combination of Analyst® 1.6.2 (Sciex LP, Ontario, Canada) instrument control and data processing software and MetIDQ Carbon 6.4.8-DB105-2809 Laboratory Information Management System software (Biocrates Life Sciences AG, Innsbruck, Austria).

Assay performance was assessed by the evaluation of QC samples. Three kit-provided QCs at three known levels of concentration for each metabolite were analyzed in each plate. Additionally, four replicates of the mid-level QC and 6 CHEAR plasma reference materials were included in each plate. QC assessments within an analytical run and pre- and post-analysis system suitability checks were performed. The QC procedures involved reviewing the quality of each plate. The metrics included the signal stability of the internal standards, retention time drifts of the standards and its effects on peak integration, and accuracy of points on the standard curves of each analyte in the LC based analysis, as well as the regression model used and the graph weighting to generate the curves.

**Broad Spectrum NMR Metabolomics**

Aliquots of maternal plasma were received and stored at −80°C after being logged in for metabolomics analysis. The samples were randomized into NMR batches for processing and data acquisition. The plasma samples were thawed at 4°C overnight. An aliquot (350 μL) of thawed plasma was transferred into labeled 2.0 mL LoBind Eppendorf tubes. Low volume samples (below 350 μL) were made up to 350 μL with water. A volume of 1050 μL of Methanol was added to each sample, vortexed for 2 min on multi-tube vortexer at speed 5, and centrifuged at 16,000 rcf for 20 min. A 1000 μL aliquot of clean supernatant was transferred into new pre-labeled LoBind Eppendorf tubes and dried on the SpeedVac (ThermoFisher, Waltham, MA). The dried extracts of samples were reconstituted in 700 μL of D\(_2\)O phosphate buffer NMR Master Mix (containing 0.5 mM DSS-d\(_6\) and 0.2% NaN\(_3\) (w/v) in D\(_2\)O, pH = 7.4). Sample tubes were vortexed for 2 min on multi-tube vortexer at speed 5, centrifuged at 12,000 rcf for 4 min, and a 600 μL aliquot of the supernatant
was transferred into numbered 5 mm NMR tubes. Aliquots (350 μL) of CHEAR plasma reference samples were included in each NMR batch, processed identically to the study samples described above, and used for QC purposes.

1H NMR spectra of plasma samples were acquired on a Bruker Avance 700 MHz NMR spectrometer (Bruker, Billerica, MA) using a 5 mm cryogenically cooled ATMA inverse probe and ambient temperature of 25 °C. A 1D NOESY pre-saturation pulse sequence (29) (noesypppr1d, [recycle delay (RD)-90°-t₁-90°-tₘ-90°-acquire free induction decay (FID) was used for data acquisition. For each sample 64 transients were collected into 64k data points using a spectral width of 12ppm, 2 s relaxation delay, 10 ms mixing time, and an acquisition time of 3.9 s per FID. The water resonance was suppressed using resonance irradiation during the relaxation delay and mixing time. NMR spectra were processed using TopSpin 3.5 software (Bruker-Biospin, Rheinstetten, Germany). Spectra were zero filled, and Fourier transformed after exponential multiplication with line broadening factor of 0.5 Hz. Phase and baseline of the spectra were manually corrected for each spectrum. Spectra were referenced internally to the DSS-d₆ signal (δ 0 ppm). The quality of each NMR spectrum was assessed for the level of noise and alignment of identified markers. Spectra were assessed for missing data and underwent quality checks. NMR bins (0.50-8.50 ppm) were made after excluding water (4.70 – 4.90 ppm), EDTA (2.53 – 2.62 ppm, 2.68 – 2.72 ppm, 3.57 - 3.66 ppm) and methanol (3.03 - 3.37 ppm) regions, using intelligent bucket Integration with a 0.04 ppm bucket width and 50% looseness using ACD Spectrus Processor 2019 (ACD Labs Inc., Toronto, Canada). Integrals of each of the bins were normalized to total integral of each of the spectrum. A total of 125 bins were available for analysis.

**Supporting covariate data**

Participants completed questionnaires that solicited information on demographic characteristics, anthropometrics, and lifestyle factors. Trained NHBCS staff extracted relevant supporting information from participants’ prenatal medical records. Potential confounding factors of the chemical-metabolomic associations and predictors of the metabolomic features were selected for inclusion as covariates in subsequent analyses, and these included maternal age at enrollment (linear), Body Mass Index (BMI; <24.9, 24.9 to 29.9, >29.9), maternal education (less than college graduate, college graduate, more than college graduate), parity (0, ≥1), and season of wear for the wristband (Spring, Summer, Fall, Winter).

**Data Pre-Processing**

The wristbands detected 171 unique chemicals, and 27 chemicals detected in >25% of the wristbands were used in subsequent analyses. The ComBat function in the R package sva was used to reduce batch-related differences (30, 31). Because the statistical method used to investigate relations between chemical exposures and metabolomic features (partial least squares regression, PLS) is poorly suited to adjust for confounding variables and accommodate missing data on covariates, before analysis with PLS the chemical exposure data were adjusted for covariates using multiple imputed covariates data. Specifically, missing covariate data was imputed with multiple imputation by chained equations via the R package mice (32) to generate 25 datasets with complete data on chemical exposures.
and covariates; then, each chemical concentration was regressed on covariates within each complete dataset and the residuals of the models were averaged across the datasets. The averaged residuals of the chemical concentrations were used in subsequent analyses. Lastly, the residuals of the chemical concentrations were mean-centered and unit-scaled.

The Biocrates data were filtered by removing spermine, spermidine, and related derivatives at the suggestion of the manufacturer (4 features) and also compounds with <1% non-zero values (2 features, PEA and Carnosine). The filtered dataset included 226 metabolites and metabolite derivatives that included a small amount of missing data (0.2% of the data points). These missing Biocrates data were imputed using multiple imputation by chained equations to generate 25 datasets with complete data on Biocrates and covariates, where only covariates were used to impute missing data; the imputed Biocrates data were then averaged across the datasets to obtain a single complete Biocrates dataset. Then, the ComBat function in the R package sva was used to reduce batch-related differences. Then, covariate adjustment was performed in a similar manner as in the chemical exposure data. Lastly, the residuals of the Biocrates metabolites were mean-centered and unit-scaled.

The NMR binned data were first batch corrected and then covariate adjusted as previously described, and then the resulting residuals were mean centered and Pareto scaled.

**Statistical analysis**

Descriptive statistics, including univariate statistics (e.g., counts and percentiles) and Spearman correlations, were calculated to characterize the study sample, chemical exposures, and plasma metabolomic features.

To investigate the relationship between chemical exposures and plasma metabolites (or features), PLS regression was used and implemented via the mixOmics package version 6.12.2 (33). A two component PLS model was generated, where the chemical exposures were used to predict the plasma metabolomic features (Biocrates and NMR in separate models). The proportion of variance in the chemical exposures and in the plasma metabolomics that was explained by the model was calculated, as well as the proportion of covariance between the chemical exposures and plasma metabolomics that was explained by the model. To assess the ability of the chemical exposures to predict plasma metabolomic features, Q^2 statistics were calculated for the metabolomic features via 100 repetitions of 10-fold cross-validation of the PLS model; if a metabolomic feature has Q^2 > 0, then the model has predictive accuracy superior to a random model for that metabolomic feature. Therefore, metabolomic features with Q^2 > 0 on both PLS components were considered to be predicted by the chemical exposures. To assess the strength of relationship between individual chemical exposures and individual plasma metabolomic features, pairwise similarity scores were calculated, which were proposed by Gonzalez et al. (34) as a measure of the strength of a pairwise relation between two variables in a multivariate model; a pairwise similarity score ranges from [−1, 1] and may be interpreted similarly to a correlation coefficient. To identify individual chemical exposures that most strongly contributed to the prediction of a given predicted metabolomic feature, chemical exposures whose pairwise similarity score magnitude for that metabolomic feature exceeded the 75th percentile of all pairwise similarity scores magnitudes (for either Biocrates or NMR) were selected. In summary, a set
of metabolomic features that were meaningfully predicted by the model (i.e., metabolomic features with \( Q^2 > 0 \) on both PLS components) and a set of chemical exposures that were most strongly related to each of those metabolomic features (i.e., chemical exposures with pairwise similarity scores for a metabolomic feature whose magnitude exceeded the 75th percentile for all pairwise similarity score magnitudes) were obtained. We additionally report Variable Importance in Projection (VIP) statistics for the chemical exposures in both the Biocrates and NMR models, to indicate which chemicals were most strongly related to the full set of metabolomic features of the respective platform. All statistical analyses were performed using R version 4.03 (35).

NMR bins that were predicted by the chemical exposures were annotated by library matching in Chenomx NMR Suite 8.6 Professional software, a library of NMR spectral signatures for 338 endogenous metabolites (Supplemental Table 2).

Enrichment analyses were performed for the combined chemical mixture and for each chemical that contributed to the prediction of at least one metabolomic feature. For the enrichment analyses of the combined chemical mixture, all metabolomic features with \( Q^2 > 0 \) on both PLS components were included. For the enrichment analyses of the individual chemicals, the set of metabolomic features that were predicted with \( Q^2 > 0 \) on both PLS components was first considered and then features related to each chemical with pairwise similarity score above the 75th percentile of all pairwise similarity scores were selected. In both instances, the set of metabolites influenced by the exposures was entered into MetaboAnalyst version 4.0 to determine metabolic pathway perturbations. Specifically, the following parameters were used: Enrichment Analyses, the Small Molecule Pathway Database (SMPDB; 99 human metabolic pathways), metabolite sets containing at least 2 compounds, and a reference metabolome that included all metabolites capable of measurement in Biocrates and annotation in Chenomx; the reference metabolome included a total of 630 molecules. Due to the exploratory nature of the study, pathways enriched at a nominal p-value < 0.01 or false discovery rate (FDR, via Benjamini-Hochberg procedure (36)) p-value < 0.2 were considered to be of interest; these p-values were used for the prioritization of results for discussion and were not used to perform tests of causality.

Results

Study sample

Of the 200 participants who wore the silicone wristbands at ~13 gestational weeks and who provided plasma for metabolomics analyses at ~24-28 gestational weeks, 177 participants had complete and valid information on the timing of the wristband and plasma sampling. Participants were omitted for the following reasons: missing plasma sampling date (n=12), missing wristband end date (n=2), and plasma samples collected before they returned the wristbands (n=9). The analysis sample of 177 women had median age 31 years (IQR: 28, 35) and median BMI 25 (IQR: 22, 30), and most participants were Non-Hispanic White (91%), married (82%), college graduates (73%), and reported no first- or second-hand exposure to tobacco smoke during pregnancy (89%) (Table 1). Participants excluded from the analysis sample due to missing or invalid timing of exposure assessment and/or
metabolomics assessment (n=23) were generally similar to participants in the analysis sample (Table 1).

Chemical exposures

Silicone wristbands were worn by study participants for a median of 7.0 days (range: 4, 25) at a median of 13 gestational weeks (IQR: 12, 14). The median length of time between the end of the wristband sampling period and plasma sampling was 14 weeks (IQR: 12, 15). Of 1530 chemicals assayed, 171 chemicals were detected in at least one wristband (Supplemental Table 3). A median of 23 chemicals (IQR: 20, 26) was detected in each wristband, and chemicals in commerce, personal care products, and pesticides were most frequently detected chemical classes (Supplemental Table 4). A subset of 27 chemicals was detected in >25% of wristbands (Table 2), which were included in subsequent analyses. The concentrations of these chemicals were weakly correlated with one another (Supplemental Table 5), with a median Spearman Correlation of 0.07 (IQR: –0.01, 0.16) and ranging from –0.41 (butylated hydroxytoluene and 2,4-di-tert-butylphenol) to 0.58 (lilial and galaxolide).

Chemical exposures in relation to plasma metabolomics

In the PLS model of the chemical exposures predicting metabolites identified using the Biocrates p180 kit (Supplemental Table 6), the first and second model components explained 7% and 6% of the variance in the chemical exposure data, 18% and 20% of the variance in the metabolites, and 1.2% and 0.9% of covariance between these two sets of variables, respectively (Supplemental Table 7). In cross-validation, the 228 metabolites had a median Q^2 of –1.5x10^{-2} and –9.5x10^{-3} on the two components (Supplemental Table 8), and a total of 11 metabolites had Q^2 > 0 on both components (Table 3, Supplemental Table 8). Pairwise similarity scores between the chemical exposures and Biocrates metabolites ranged from –0.19 to 0.19 and the absolute value of the scores had a median value of 0.04 (IQR: 0.02, 0.07) (Supplemental Table 9). VIP statistics indicated that the chemicals butyl benzyl phthalate, N,N-diethyl-m-toluamide, benzyl benzoate, di-n-nonyl phthalate, 2,4-di-tert-butylphenol, and diethyl phthalate were most important in the prediction of the Biocrates metabolites (i.e., VIP statistic > 1 on both model components) (Supplemental Table 10).

In the PLS model of chemical exposures predicting the NMR bins, the first and second model components explained 6% and 6% of the variance in the chemical exposure data, 31% and 34% of the variance in NMR bins, and 1.1% and 0.8% of covariance between these two sets of variables, respectively (Supplemental Table 7). In cross-validation, the 125 NMR bins had a median Q^2 of –4.1x10^{-3} and –2.7x10^{-4} on the two components (Supplemental Table 11), and a total of 22 bins, corresponding to 23 unique annotated metabolites, had Q^2 > 0 on both components (Table 3, Supplemental Table 11). Pairwise similarity scores between the chemical exposures and NMR bins ranged from –0.32 to 0.31 and the absolute values of the scores had a median value of 0.04 (IQR: 0.02, 0.07) (Supplemental Table 12). VIP statistics indicated that the chemicals triphenyl phosphate, N,N-diethyl-m-toluamide, 2,4-di-tert-butylphenol, and benzothiazole were most important in the prediction of the binned NMR data (Supplemental Table 13).
In enrichment analyses of metabolites predicted by the combined chemical mixture, no pathways were enriched at the level of statistical significance considered for our analysis (i.e., nominal p-value < 0.01, or FDR p-value < 0.2, Supplemental Table 14). The pathways most strongly associated with the combined chemical mixture were ammonia recycling (p-value = 0.02, FDR = 1.0) and methylhistidine metabolism (p-value = 0.03, FDR = 1.0).

In enrichment analyses for individual chemical exposures (Table 4, Supplemental Table 14), there were a total of 14 combinations of chemical exposures and enriched pathways, including 6 unique chemical exposures and 9 unique pathways. The chemical N,N-diethyl-m-toluamide was most strongly associated with enrichment of ammonia recycling (p-value = 0.0001, FDR = 0.01), driven by associations with the metabolites glycine (↑), L-glutamic acid (↑), L-asparagine (↑), and L-aspartic acid (↑). N,N-diethyl-m-toluamide was also associated with enrichment of malate-aspartate Shuttle (p-value = 0.004, FDR = 0.2), aspartate metabolism (p-value = 0.006, FDR = 0.2), and glutamate metabolism (p-value = 0.007, FDR = 0.2). The chemical tonalide was associated with enrichment of alanine metabolism (p-value = 0.002, FDR = 0.1), glutathione metabolism (p-value = 0.002, FDR = 0.1), ammonia recycling (p-value = 0.006, FDR = 0.2), and glutamate metabolism (p-value = 0.009, FDR = 0.2), which were each driven by negative associations with the metabolites glycine (↓) and L-glutamic acid (↓). Three chemicals, lilial, benzyl salicylate, and amyl cinnamal, were associated with enrichment of phenylalanine and tyrosine metabolism. Other associations included galaxolide and methionine metabolism, galaxolide and glycine and serine metabolism, benzyl salicylate and ammonia recycling, and permethrin II and thyroid hormone synthesis.

Discussion

Using a novel method to assess chemical exposures and both targeted and untargeted metabolomic assays in a prospective pregnancy cohort, we observed that a diverse set of chemical exposures measured in silicone wristbands in early pregnancy were associated with endogenous metabolomic features in plasma and metabolic pathways in mid pregnancy. While we did not observe that the combined mixture of chemical exposures was significantly related to metabolic pathway enrichment, we observed that certain individual chemicals, such as N,N-diethyl-m-toluamide, tonalide, galaxolide, and benzyl salicylate, were associated with certain amino acids and potential perturbations in various metabolic pathways, including ammonia recycling, glutamate metabolism, and phenylalanine and tyrosine metabolism.

We observed associations between individual chemical exposures and a set of amino acids and their derivatives (i.e., glycine, L-glutamic acid, L-asparagine, L-aspartic acid, L-tyrosine, L-phenylalanine, L-methionine, methionine sulfoxide). Amino acids, including those we observed to be most strongly associated with the chemical exposures in our study, are essential to a wide range of physiological processes (37, 38) and function as precursors for many essential compounds (e.g., proteins, neurotransmitters) and regulators of gene expression, cellular signaling, nutrient transport, and endocrine function. Consequently, amino acids play critical roles in physiological processes during pregnancy and fetal development (39–41), and perturbations to the availability and action of these essential compounds during pregnancy could adversely impact maternal health and fetal development.
For example, as the elementary units of protein synthesis, a sufficient and balanced supply of amino acids from the mother is essential to support normal fetal growth and neurodevelopment (37, 38, 42).

We observed that N,N-diethyl-m-toluamide (also known as DEET, a common insect repellent) was positively associated with associated with the amino acids glycine, L-glutamic acid, L-asparagine, L-aspartic acid and enrichment of the ammonia recycling pathway. Previous in vitro (43) and in vivo (44) experimental studies have reported associations between N,N-diethyl-m-toluamide and ammonia recycling; to our knowledge, our study is the first to observe this relation in human populations. N,N-diethyl-m-toluamide was also associated with enrichment of the malate-aspartate shuttle pathway, aspartate metabolism, and glutamate metabolism through positive associations with subsets of the aforementioned metabolites. Because these amino acids serve several critical roles in the proper functioning of the nervous system, our results suggest that neurodevelopmental endpoints may be susceptible to influence by prenatal exposure to N,N-diethyl-m-toluamide.

We observed that other chemical exposures were positively and negatively associated with certain amino acids and enrichment of related metabolic pathways. Other epidemiologic studies have reported associations between chemical exposures and amino acids and related pathways, which are summarized in a recent review by Dai et al. (45) These findings suggest that amino acids may be sensitive to prevalent chemical exposures with implications for maternal health and fetal development, though such findings would be strengthened by replication in other populations and/or observation of perturbations of related physiological outcomes (e.g., related proteomic signatures, downstream endpoints). Ultimately, our exploratory work demonstrates that the combination of exposomic sampler technology with metabolomics may indicate perturbations of metabolic pathways by prevalent environmental chemicals, which may suggest hypotheses for future work.

Our study has strengths and limitations. First, our analytic sample size was modest, potentially impeding detection of subtle metabolomic impacts of the chemical exposures we studied; however, our sample size was comparable to other existing chemical exposure and metabolomics studies, which have ranged from 102 to 750 participants (19–21). Second, the plasma samples used for our metabolomic assessments were collected approximately 14 weeks after the chemical exposure assessments; therefore, for the chemical exposures to cause the observed metabolic perturbations, it is necessary to assume that the effects of the chemicals upon the metabolome are observable several weeks after exposure, or that chemical exposures are consistent over this time period. Per the former assumption, there is little evidence pertaining to the duration of impacts of chemical exposures upon the metabolome. Per the latter assumption, in a pilot study within NHBCS of the consistency of chemical concentrations measured in wristbands worn by participants once at ~13 gestational weeks and again at ~24-28 gestational weeks, we observed that some of the chemicals with which we observed associations demonstrated good reproducibility (e.g., lilial, tonalide, galaxolide), while others were less reproducible (e.g., N,N-diethyl-m-toluamide, benzyl salicylate) (further detailed in Doherty et al. (22)). Therefore, the chemical-metabolomic associations we observed are temporally plausible, though our conclusions would be strengthened by additional evidence of the timing and persistence.
of chemical-metabolome relations. As a related point, the interval between exposure and outcome assessment was a median of 14 weeks and varied considerably (e.g., 10th and 90th percentiles of 3 and 17 weeks, respectively). Such variation complicates interpretation of our results, and we may have been limited in our ability to observe short-lived effects of the chemicals upon the metabolome. The analysis and interpretation of future studies may benefit from the collection of chemical exposure and metabolomic data in a shorter and tighter interval. Nonetheless, the prospective study design supports causal interpretations of our results, as the exposure assessment preceded the metabolomic assessments. Third, our study sample is fairly homogenous with respect to certain demographic and lifestyle features (e.g., race/ethnicity, socioeconomic factors, smoke exposure), which limited our ability to control for potential bias due to such factors (e.g., covariate adjustment) and may limit the generalizability of our results to other populations. Such homogeneity may also be a strength, however, as it may limit potential bias due to confounding by these factors, and NHBCS participants reflect the source population in rural NH. Fourth, our observational design is vulnerable to potential confounding and selection biases; however, we adjusted our models for several potentially important confounding variables. However, it is possible that other confounding factors, such as diet, could have biased our results. Additionally, it is possible that infrequently detected chemicals that were omitted from our analysis (and other chemicals not measured by the wristbands) may have confounded our observed relations. Speculation as to the nature of any potential uncontrolled confounding is difficult, given the diversity of the chemical exposures and metabolomic features considered in our analyses. Fifth, we used silicone wristbands to assess chemical exposures, which may not capture important exposures through ingestion. However, these silicone samplers enabled measurement of a diverse set of chemicals in a non-invasive and minimally burdensome manner. Importantly, this broad exposure assessment supported the investigation of chemical exposures, which better approximate real world exposures and potential additive or synergistic effects (7–9). Relatedly, although most correlations observed among the chemicals surveyed were weak to moderate, stronger correlations ($\rho > 0.40$) were observed among phthalates, lilial, tonalide, and other chemicals commonly found in fragrances. Phthalates are commonly used as scent retainers in products containing fragrance (46, 47), and benzyl salicylate, ethylene brassylate, galaxolide, lilial, and tonalide are synthetic musk and scent chemicals (48, 49). Taken together, this may suggest that a common source of exposure could be personal care products or other home products containing fragrance. Lastly, the metabolomics assays we used covered a broad space of endogenous metabolites, which supported the investigation of a wide range of potential metabolomic effects of chemical exposures during pregnancy. However, it is possible that metabolomic features not covered by these assays could be sensitive to chemical exposures and are also important for maternal health and fetal development. It will therefore be necessary to investigate relations between chemical exposures and additional metabolomic features to determine which metabolomic features and pathways are most sensitive to chemical exposures during pregnancy; these findings will be further strengthened if they are observed in multiple populations.

In conclusion, we observed that chemical exposures may influence the plasma metabolome during pregnancy. This work contributes evidence to the potential adverse impacts of
chemical exposures upon maternal health during pregnancy and fetal development. Going forward, evidence of metabolomic effects of chemical exposures may be strengthened by replication in other populations. It will also be important to investigate such relations during multiple periods of pregnancy to identify windows of vulnerability (1–3). Lastly, such work may be further validated by investigating maternal health or developmental endpoints related to the metabolic pathways affected by the chemical exposures.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

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Table 1.
Characteristics of participants included in the analysis sample (n = 177) and participants excluded from the analysis sample due to missing or invalid timing of exposure assessment or metabolomics assessment (n = 23).

| Characteristic                                      | Analysis sample (n = 177) | Excluded participants (n = 23) |
|-----------------------------------------------------|---------------------------|-------------------------------|
| Age                                                 | Median (IQR)              | 31 (28, 35)                   | 32 (29, 36) |
|                                                     | Missing                   | 0                             | 0           |
| BMI                                                 | Median (IQR)              | 25 (22, 30)                   | 25 (23, 31) |
|                                                     | Missing                   | 18                            | 7           |
| Race and ethnicity                                   |                           |                               |             |
| Non-Hispanic White                                  | 148 (91)                  | 17 (100)                      |
| Other                                               | 15 (9)                    | 0 (0)                         |
| Missing                                             | 14                        | 6                             |
| Relationship status                                 |                           |                               |             |
| Married                                             | 118 (82)                  | 13 (76)                       |
| Single                                              | 26 (18)                   | 4 (24)                        |
| Missing                                             | 33                        | 6                             |
| Education level                                     |                           |                               |             |
| Less than college                                   | 39 (27)                   | 3 (19)                        |
| College graduate                                    | 60 (42)                   | 8 (50)                        |
| More than college graduate                          | 43 (30)                   | 5 (31)                        |
| Missing                                             | 35                        | 7                             |
| Parity                                              |                           |                               |             |
| 0                                                   | 68 (46)                   | 5 (29)                        |
| ≥1                                                  | 81 (54)                   | 12 (71)                       |
| Missing                                             | 28                        | 6                             |
| Tobacco smoke exposure                              |                           |                               |             |
| None                                                | 133 (89)                  | 17 (94)                       |
| Any first- or second-hand                           | 17 (11)                   | 1 (6)                         |
| Missing                                             | 27                        | 5                             |
| Wristband - gestational age at start (weeks)        | Median (IQR)              | 13 (12, 14)                   | 19 (12, 29) |
|                                                     | Missing                   | 0                             | 1           |
| Wristband - gestational age at end (weeks)          | Median (IQR)              | 14 (13, 16)                   | 16 (13, 30) |
|                                                     | Missing                   | 0                             | 2           |
| Wristband - duration worn (days)                    | Median (IQR)              | 7.0 (7.0, 7.0)                | 7.0 (7.0, 7.0) |
|                                                     | Missing                   | 0                             | 2           |
| Wristband - season worn                             |                           |                               |             |
| Spring                                              | 40 (23)                   | 6 (27)                        |
| Summer                                              | 35 (20)                   | 2 (9)                         |
| Fall                                                | 64 (36)                   | 6 (27)                        |
| Winter                                              | 38 (21)                   | 8 (36)                        |
| Missing                                             | 0                         | 1                             |
| Plasma metabolomics - gestational age at blood draw (weeks) | Median (IQR) | 28 (27, 28) | 28 (28, 30) |
|                                                     | Missing                   | 0                             | 12          |
| Difference between gestational age of wristband end and blood draw (weeks) | Median (IQR) | 14 (12, 15) | −1.3 (−1.6, −1.1) |
|                                                     | Missing                   | 0                             | 14          |

Values indicate n (%) unless otherwise indicated.

Abbreviations: IQR, interquartile range.
Table 2.
Characteristics of chemical exposures assessed via silicone wristbands among participants in the analysis sample (n=177).

| Chemical                        | CASN    | Detection frequency (%) | Median (IQR) (ng/g silicone) | MyExposome classification          |
|--------------------------------|---------|-------------------------|------------------------------|------------------------------------|
| Di-n-butyl phthalate           | 84-74-2 | 99                      | 5570 (2910, 8790)            | Chemicals in commerce, personal care products, pesticides |
| Galaxolide                     | 1222-05-5 | 99                      | 7220 (2470, 18000)           | Chemicals in commerce, personal care products |
| Diisobutyl phthalate           | 84-69-5 | 96                      | 4980 (2600, 8310)            | Chemicals in commerce              |
| Butyl benzyl phthalate         | 85-68-7 | 93                      | 2910 (1230, 8170)            | Chemicals in commerce              |
| Benzyl salicylate              | 118-58-1 | 91                      | 5300 (1800, 14300)           | Personal care products             |
| Lilial                         | 80-54-6 | 89                      | 1080 (273, 3300)             | Personal care products             |
| Tonalide                       | 1506-02-1 | 87                      | 428 (107, 1730)              | Personal care products             |
| Benzo phenone                  | 119-61-9 | 83                      | 225 (104, 424)               | Chemicals in commerce, personal care products |
| Benzyl benzoate                | 120-51-4 | 81                      | 2550 (749, 8060)            | Pesticides                        |
| N,N-Diethyl-m-toluamide        | 134-62-3 | 81                      | 715 (197, 1710)              | Pesticides                        |
| Ethylene brassylate            | 105-95-3 | 80                      | 3970 (541, 13100)            | Personal care products             |
| Triphenyl phosphate            | 115-86-6 | 74                      | 336 (0, 819)                 | Chemicals in commerce, flame retardands |
| 2,4-Di-tert-butylphenol        | 96-76-4 | 69                      | 428 (0, 1190)                | Chemicals in commerce              |
| Di-n-nonyl phthalate           | 84-76-4 | 69                      | 817 (0, 2790)                | Chemicals in commerce              |
| Permethrin                     | 52645-53-1 | 67                      | 399 (0, 1460)               | Pesticides                        |
| Diethyl phthalate              | 84-66-2 | 65                      | 777 (0, 2220)               | Chemicals in commerce, pesticides |
| Butylated hydroxyanisole       | 25013-16-5 | 61                      | 69.6 (0, 173)               | Personal care products, pharmacologic |
| Bis(2-ethylhexyl)phthalate     | 117-81-7 | 59                      | 1070 (0, 56600)             | Chemicals in commerce, pesticides |
| Amyl cinnamal                  | 122-40-7 | 51                      | 116 (0, 679)                | Personal care products             |
| Butylated hydroxytoluene       | 128-37-0 | 50                      | 13.2 (0, 321)               | Chemicals in commerce, consumer products |
| TCPP                           | 26248-87-3 | 46                      | 0 (0, 535)                  | Chemicals in commerce, flame retardands |
| Permethrin II                  | 999046-03-6 | 43                      | 0 (0, 891)                  | Pesticides                        |
| Benzothenzole                  | 95-16-9 | 40                      | 0 (0, 127)                  | Chemicals in commerce              |
| B-Ionone                       | 79-77-6 | 38                      | 0 (0, 146)                  | Personal care products             |
| 2,4-Bis(alpha,alpha-dimethylbenzyl)phenol | 2772-45-4 | 36                      | 0 (0, 88.2)                 | Chemicals in commerce              |
| Caffeine                       | 58-08-2 | 29                      | 0 (0, 320)                  | Pesticides, pharmacologic          |
| Anthracene                     | 120-12-7 | 28                      | 0 (0, 13.9)                 | PAHs                              |

Abbreviations: IQR, interquartile range; PAHs, polycyclic aromatic hydrocarbons.
MyExposome Classifications are categories used by MyExposome to indicate potential sources of exposure, and these labels are not mutually exclusive.
Table 3.

Plasma metabolomic features predicted by chemical exposures in cross-validated PLS models.

| Metabolomic Features as Described by the Platform<sup>a</sup> | Corresponding HMDB Common Name(s)<sup>b</sup> | Biocrates | Metabolomic Features as Described by the Platform<sup>a</sup> | Corresponding HMDB Common Name(s)<sup>b</sup> | NMR |
|---------------------------------------------------------------|---------------------------------------------|-----------|---------------------------------------------------------------|---------------------------------------------|-----|
| Propionylcarnitine                                            | Propionylcarnitine                         | HMDB0000824 | Albumin                                                      |
| Methylglutarylcarnitine                                       | 3-Methylglutarylcarnitine                  | HMDB0000552 | Cholesterol                                                   |
| Aspartic Acid                                                 | L-Aspartic acid                            | HMDB000191  | Cholate                                                       |
| Glutamic Acid                                                 | L-Glutamic acid                            | HMDB000148  | Aspartate                                                     |
| Glycine                                                       | Glycine                                     | HMDB000123  | N,N-Dimethylglycine                                           |
| Phenylnalnine                                                 | L-Phenylalanine                            | HMDB000159  | Trimethylamine                                                |
| Serotonin                                                     | Serotonin                                   | HMDB000259  | Fructose                                                      |
| Lyso phosphatidylcholine a C18:1                             | LysoPC(18:1(9Z))                           | HMDB0002815 | Ascorbate                                                     |
| 2-Oleoylglycerophosphocholine                                 | HMDB0010385                                | LysoPC(18:1(11Z)) | Choline                                                 |
| 1-elaidoyl-sn-glycero-3-phosphocholine                        | HMDB0061701                                | 2-Oleoylglycerophosphocholine | Creatine                                                |
| Oleoyl-sn-glycero-3-phosphocholine                            |                                               | 1-elaidoyl-sn-glycero-3-phosphocholine | myo-Inositol                                            |
| Sum of Aromatic Amino Acids                                   | L-Phenylalanine                            | HMDB000159  | Glycerol                                                      |
| Methionine Oxidation                                          | Methionine sulfoxide                       | HMDB002005  | Methionine sulfoxide                                          |
| D-Phenylalanine                                               | D-Phenylalanine                            | HMDB000177  | Methionine sulfoxide                                          |

<sup>a</sup> Metabolomic features as described by the platform.

<sup>b</sup> Corresponding HMDB ID(s).
| Metabolomic Features as Described by the Platform<sup>a</sup> | Corresponding HMDB Common Name(s)<sup>b</sup> | Corresponding HMDB ID(s)<sup>b</sup> | Metabolomic Features as Described by the Platform<sup>a</sup> | Corresponding HMDB Common Name(s)<sup>b</sup> | Corresponding HMDB ID(s)<sup>b</sup> |
|---------------------------------------------------------------|---------------------------------|-------------------------------|---------------------------------------------------------------|---------------------------------|-------------------------------|
| L-methionine (R)-S-oxide                                      |                                 |                               | Serotonin Synthesis                                            |                                 |                               |
| D-methionine S-oxide                                           |                                 |                               | L-Tryptophan                                                   |                                 | HMDB0000929                   |
| L-Methionine                                                  |                                 | HMDB0000696                   | D-Tryptophan                                                   |                                 | HMDB0013609                   |
| D-methionine                                                  |                                 |                               | Serotonin                                                      |                                 | HMDB0000259                   |

<sup>a</sup> Metabolomic features as described by the Biocrates AbsoluteIDQ® p180 kit or NMR bin annotations (via Chenomx NMR Suite 8.6 Professional); the values in this column correspond to variables in our analysis which were predicted by the chemical exposures.

<sup>b</sup> HMDB Common Names and HMDB IDs corresponding to the metabolomic features as described by the two platforms; the manufacturers of the platforms provide materials to link the metabolomic features as described by the respective platforms to corresponding HMDB Common Names and HMDB IDs, the latter of which were used in pathway analyses.
### Table 4.

Results of enrichment analyses of chemical exposures.

| Chemical         | Pathway                        | Total | Hits | Expect | p-value | FDR    | Relevant Metabolites                              |
|------------------|--------------------------------|-------|------|--------|---------|--------|--------------------------------------------------|
| Amyl cinnamal    | Phenylalanine and Tyrosine Metabolism | 9     | 3    | 0.40   | 0.0045  | 0.44   | L-Glutamic acid ↓, L-Tyrosine ↓, L-Phenylalanine ↓ |
| Benzyl salicylate| Ammonia Recycling              | 16    | 4    | 0.79   | 0.0041  | 0.31   | Glycine ↓, L-Glutamic acid ↓, L-Asparagine ↓, L-Aspartic acid ↓ |
| Benzyl salicylate| Phenylalanine and Tyrosine Metabolism | 9     | 3    | 0.44   | 0.0063  | 0.31   | L-Glutamic acid ↓, L-Tyrosine ↓, L-Phenylalanine ↓ |
| Galaxolide       | Methionine Metabolism          | 25    | 3    | 0.41   | 0.0036  | 0.31   | Glycine ↓, L-Methionine ↓, Methionine sulfoxide ↓ |
| Galaxolide       | Glycine and Serine Metabolism  | 29    | 3    | 0.57   | 0.010   | 0.31   | Glycine ↓, L-Glutamic acid ↓, L-Methionine ↓ |
| Lilial           | Phenylalanine and Tyrosine Metabolism | 9     | 3    | 0.36   | 0.0031  | 0.30   | L-Glutamic acid ↓, L-Tyrosine ↓, L-Phenylalanine ↓ |
| N,N-Diethyl-m-toluamide | Ammonia Recycling | 16    | 4    | 0.39   | 1.3E-04 | 0.012  | Glycine ↑, L-Glutamic acid ↑, L-Asparagine ↑, L-Aspartic acid ↑ |
| N,N-Diethyl-m-toluamide | Malate-Aspartate Shuttle       | 5     | 2    | 0.12   | 0.0047  | 0.17   | L-Glutamic acid ↑, L-Aspartic acid ↑             |
| N,N-Diethyl-m-toluamide | Aspartate Metabolism          | 18    | 3    | 0.44   | 0.0053  | 0.17   | L-Glutamic acid ↑, L-Asparagine ↑, L-Aspartic acid ↑ |
| N,N-Diethyl-m-toluamide | Glutamate Metabolism           | 20    | 3    | 0.49   | 0.0073  | 0.18   | Glycine ↑, L-Glutamic acid ↑, L-Aspartic acid ↑ |
| Tonalide         | Alanine Metabolism             | 9     | 2    | 0.09   | 0.0018  | 0.11   | Glycine ↓, L-Glutamic acid ↓ |
| Tonalide         | Glutathione Metabolism         | 10    | 2    | 0.10   | 0.0022  | 0.11   | Glycine ↓, L-Glutamic acid ↓ |
| Tonalide         | Ammonia Recycling              | 16    | 2    | 0.16   | 0.0059  | 0.19   | Glycine ↓, L-Glutamic acid ↓ |
| Tonalide         | Glutamate Metabolism           | 20    | 2    | 0.20   | 0.0093  | 0.22   | Glycine ↓, L-Glutamic acid ↓ |

Abbreviations: FDR, false discovery rate.