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Near-roadway Air Pollution Exposure and Altered Fatty Acid Oxidation Among Adolescents and Young Adults – the Interplay with Obesity

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Abstract

Background: Air pollution exposure has been shown to increase the risk of obesity and metabolic dysfunction in animal models and human studies. However, the metabolic pathways altered by air pollution exposure are unclear, especially in adolescents and young adults who are at a critical period in the development of cardio-metabolic diseases.

Objectives: The aim of this study was to examine the associations between air pollution exposure and indices of fatty acid and amino acid metabolism.

Methods: A total of 173 young adults (18–23 years) from eight Children's Health Study (CHS) Southern California communities were examined from 2014–2018. Near-roadway air pollution (NRAP) exposure (freeway and non-freeway) and regional air pollution exposure (nitrogen dioxide, ozone and particulate matter) during one year before the study visit were estimated based on participants' residential addresses. Serum concentrations of 64 targeted metabolites including amino acids, acylcarnitines, non-esterified fatty acid (NEFA) and glycerol were measured in

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fasting serum samples. Principal component analysis of metabolites was performed to identify metabolite clusters that represent key metabolic pathways. Mixed effects models were used to analyze the associations of air pollution exposure with metabolomic principal component (PC) scores and individual metabolite concentrations adjusting for potential confounders.

Results: Higher lagged one-year averaged non-freeway NRAP exposure was associated with higher concentrations of NEFA oxidation byproducts and higher NEFA-related PC score (all p's≤0.038). The effect sizes were larger among obese individuals (interaction p=0.047). Among females, higher freeway NRAP exposure was also associated with a higher NEFA-related PC score (p=0.042). Among all participants, higher freeway NRAP exposure was associated with a lower PC score for lower concentrations of short- and median-chain acylcarnitines (p=0.044).

Conclusions: Results of this study indicate that NRAP exposure is associated with altered fatty acid metabolism, which could contribute to the metabolic perturbation in obese youth.

Keywords

traffic; air pollution; obesity; metabolic diseases; metabolomics

INTRODUCTION

Childhood obesity rates have steadily risen over the past few decades, contributing to the increased epidemic of cardio-metabolic diseases in children and adults.[1–5] Unhealthy diet, inadequate exercise, and genetics are well-known risk factors in the burgeoning obesity epidemic, but recent work suggests an important role for environmental exposures, including air pollution. A growing body of evidence indicates that early-life near-roadway air pollution (NRAP) and regional air pollution exposures are associated with childhood obesity, higher growth trajectory of body mass index (BMI) and higher attained BMI at age 18 years.[6,7] In addition, air pollution exposure has been associated with obesity-related metabolic dysfunction, including glucose intolerance and insulin resistance.[8–12] However, the mechanism linking air pollution exposure and metabolic dysfunction remains unclear.

Air pollution exposure may increase the risk of obesity and metabolic dysfunction by altering key metabolic pathways. Application of metabolomics technology allows measurement of a broad array of metabolites, including amino acids, fatty acids, and acylcarnitines in biological fluids such as serum.[13] A series of metabolomics studies have linked alterations in amino acid, fatty acid, glucose, bile acid, and choline metabolism with increased adiposity,[14–18] including visceral and liver fat,[19–25] glucose intolerance,[26–30] and insulin resistance.[31–35] Furthermore, mounting evidence suggests that increased NRAP and regional particulate matter with aerodynamic diameter <2.5 μm ($PM_{2.5}$) exposures are associated with dysregulated metabolism of fatty acids, amino acids and leukotrienes,[36–39] which may contribute to the onset and progression of cardiovascular diseases and asthma. To our knowledge, no human study has used metabolomics to investigate links of NRAP and regional air pollution exposure to obesity and metabolic dysfunction, especially among adolescents and young adults.

In this study, we leveraged an existing cohort of adolescents and young adults from the Southern California Children's Health Study (CHS)[6] to examine the influence of NRAP and regional air pollution exposures on serum concentrations of amino acids, ketones, non-esterified free fatty acids, glycerol and acylcarnitine intermediates that report on mitochondrial metabolism, reflecting key metabolic pathways for three main classes of nutrients – carbohydrates, lipids and protein. We also investigated the relationship between metabolomic profiles and the outcome of obesity and BMI. Finally, increasing evidence suggests that there are potential sex and ethnicity disparities in the metabolic effect of air pollution exposure, possibly due to pollution inequity and physiology differences.[40–43] Furthermore, metabolomic profiles can also be sex-, ethnicity- and obesity-dependent.[19,44–46] Therefore, we explored whether the associations between air pollution and metabolomic profiles differed by obesity status, ethnicities and sex.

MATERIALS AND METHODS

Participants and Health Measures:

A total of 173 participants from eight Southern California communities were selected from the original CHS cohort[6] for targeted metabolomics analysis. Because the main aim of this study was to examine the influence of air pollution exposures on metabolic dysfunction in adolescents, we first identified 1,154 participants who were overweight or obese (age- and sex-specific BMI of ≥85th percentile compared to the CDC 2000 BMI growth curves[47]) at their last in-school visit of the CHS follow-up (October 2011-June 2012). Among these participants who are at a higher risk for metabolic dysfunction, we further used a probability weighted sampling approach to enroll 137 participants with the selection probability as a logistic function of the quadratic form of percentiles of individual exposures to NRAP within each community during the last visit in the CHS. Therefore, participants with extremely high and low NRAP exposure within each community have the highest probability to be selected. To increase the generalizability to a normal weight population, a similar exposure-weighted sampling approach was used to additionally enroll 36 participants from a total of 1,957 CHS participants who were normal weight at their last CHS visit. Participants were excluded if they had type 1 or type 2 diabetes or if they were using a medication or diagnosed with a condition known to influence insulin and/or glucose metabolism or body composition.

In this project, participants completed the study visit at Diabetes and Obesity Research Institute (DORI) or Clinical Trials Unit (CTU) at the University of Southern California between 2014 and 2018. The 7-hour clinical visit included several questionnaires as well as clinical measures. Questionnaires detailing demographic, health and occupational history, parental health information, updated residential history and smoking history were administered. Individual height and weight were measured by a trained technician where height was measured to the nearest centimeter and weight to the nearest 0.1 kilogram without shoes. These objective measures of height and weight were used to calculate BMI (kg/m^2), as the primary obesity outcome. BMI was further categorized into normal weight, overweight and obese groups based on CDC criteria[48,49] as a secondary outcome. In a subset of participants who were overweight or obese at their last in-school CHS visit (N=132), self-

reported physical activity status was assessed by the questions of “Have you taken any exercise classes, lessons, or special programs (e.g., dance, martial arts, aerobics, gymnastics or tumbling and swimming) during the past 12 months?” and “Please place yourself on the scale (0–100) to rate your usual physical activity”. The self-evaluation of physical activity scale was further categorized into three categories of low- (0–40), moderate- (50–60) and high- (70–100) activity levels. Additionally, two non-consecutive 24-hour diet recalls[50] including serving sizes of 168 food items were collected among the subset of 132 participants to estimate total calorie and macronutrient intake, as well as glycemic index. The 24-hour recalls were analyzed with the Nutrition Data System for Research (NDSR) software (Version 2014),[51] which is based on Nutrition Coordinating Center (NCC) Food and Nutrient Database. Informed written consent was obtained from all participants for this study. The USC Institutional Review Board (IRB) approved this study.

Air Pollution Exposures:

NRAP exposure was estimated through detailed residential history obtained at the study visit. Residential addresses were standardized at street level and geocoded using the Texas A&M Geocoder (http://geoservices.tamu.edu/Services/Geocode/). Latitude and longitude of residences were given to Sonoma Technology Inc., who then provided monthly NRAP exposure data for up to 12 months prior to each visit for each participant. NRAP exposure from freeway and non-freeway roads was estimated using modelled nitrogen oxides (NOx) at residential addresses by applying the California line-source dispersion (CALINE4) model. CALINE4 dispersion model is an air quality model designed to estimate the incremental ambient concentration contributed by vehicle emissions on local roadways.[52] The modeled annual concentration estimates (parts per billion, ppb) were based on roadway geometry, traffic counts, traffic volumes, vehicle NO_x emission rates, and meteorological conditions. Traffic counts and road geometry were obtained from Caltrans and TeleAtlas/GDT. Annual average daily traffic volumes were assigned based on calendar year. All road emissions from freeway or non-freeway sources were calculated within 5-kilometer buffer of the residence. It is noted that the modeled NRAP exposures reflect the mixture of multiple pollutants from nearby traffic, and the high correlation of pollutants in the mixture precludes identifying the effect of any specific pollutant in the mixture, as described in previous publications.[53,54]

Regional air pollution exposure levels of each participant are estimated based on residential histories for three regional air pollutants: nitrogen dioxides (NO_2), $PM_{2.5}$ and ozone (O_3).[55–57] Hourly air quality data from ambient monitoring stations were downloaded from the U.S. Environmental Protection Agency’s Air Quality System (AQS, http://www.epa.gov/ttn/airs/airsaqs) from year 2011–2018 and averaged to daily level. In California, air monitor stations are spaced 20–30 km apart and provide a monitoring network with good characterization of the pollution gradients across Los Angeles. The gaseous pollutants were measured using Federal Reference Method (FRM) continuous monitors, whereas $PM_{2.5}$ data was restricted to FRM or Federal Equivalent Method (FEM) monitors. Monthly averages were calculated from the daily data using a 75% completeness criterion, and monthly exposure values were spatially interpolated from the air quality monitoring station’s locations within 50 km of each residential address using an inverse distance-squared weighting (IDW2) algorithm, as previously described.[58] Data from up to four monitors were

used to estimate exposure for each location. Prior work by our group[59] has shown that the IDW2 method in California was robust to a leave one out validation for monthly monitoring AQS site data and performs as well as more sophisticated models that are limited by shorter spatial-temporal coverage.

Based on monthly air pollution exposure data, we further calculated lagged annual average exposures (as representatives of long-term exposures) to freeway, non-freeway and total (combined freeway and non-freeway) NRAP, as well as regional air pollutants (NO_2, $PM_{2.5}$ and O_3) as the cumulative averages of monthly exposure concentrations over one year prior to the DORI/CTU study visit and were weighted by time spent at each different address since some subjects began attending college and no longer lived at home. In these instances, one-year average air pollution exposure prior to the study visit accounts for both college and home air pollution exposures. Additionally, short-term air pollution exposures were represented by one-month exposure level prior to the clinical visit.

Metabolomic Signatures:

Concentrations of 64 endogenous metabolites were assayed in fasting serum samples as previously described.[31,38,60–62] These 64 targeted metabolites have been successfully used in many studies of obesity and cardiometabolic diseases,[13,61,63,64] and have been linked to increased adiposity,[31,32] insulin resistance,[61,64] as well as air pollution exposures.[38] In order to assure the stability of analyzed serum metabolites,[65] all unthawed serum samples were stored at −80 °C and were transferred to the laboratory at Duke University on dry ice. Targeted analyses included three analytic modules: 1) 15 amino acids, 2) 45 acylcarnitines, and 3) three conventional metabolites: non-esterified free fatty acids (NEFA), lactate, beta-hydroxybutyrate were assayed across three batches with samples block randomized by sex on each assay plate. Later, we measured glycerol using the leftover samples that had been freeze-thawed once in a separate batch as an additional measurement of lipolysis. Lipolysis is a process of degrading triglycerides into fatty acids and glycerol,[66] therefore, serum NEFA and glycerol are expected to increase with enhanced adipocyte lipolysis.

Proteins were first removed by precipitation with methanol. Aliquoted supernatants were dried, and then esterified with hot acidic methanol (acylcarnitines) or n-butanol (amino acids). Acylcarnitines and amino acids were analyzed by flow injection-tandem mass spectrometry (MS/MS).[31,67,68] Quantitative and reproducible measurement of metabolites was achieved by inclusion of stable isotope-labeled internal standards for the amino acid and acylcarnitine modules,[31,38,60–62] with data expressed in molarities rather than relative units. A Beckman Unicel DxC 600 autoanalyzer was used for analysis of conventional metabolites.[31]

Based on the concentration data of all metabolites, principal components analysis (PCA) with varimax orthogonal rotation was used for dimension reduction and to classify correlated metabolites into clusters of fewer uncorrelated factors, principal components (PC).[69,70] To eliminate the influence of variations across batches and freeze-thaw cycles of serum samples in the final PC classifications, glycerol was not included in the PCA. There were two acylcarnitines (C6 and C7-DC) with >20% zero-value due to their concentrations below the lower limits of quantification for that assay (Supplemental Table 1). To further

assess the influence of zero-value data, we applied a Markov chain Monte Carlo-based multiple imputation (MI)[71,72] for C6, C7-DC, and calculated averaged concentrations from 1000 imputation samples before the PCA to verify the consistency of PC classifications. The scree plot was used to determine the number of prioritized PCs for the main association analyses. Metabolites with a factor load of an absolute value ≥ 0.4 were reported as the main components of a given PC. Scoring coefficients were used to calculate metabolomic PC scores for each individual.

Statistical Methods:

Details of model forms of the regression models are presented in the supplemental material. Briefly, a generalized additive model with a cubic smoothing spline of the exposure variable was used to assess nonlinear relationships between air pollution exposures and metabolomic factor scores, as well as relationships of exposure variables of metabolomic factor scores with BMI as the outcome. If no nonlinear relationships were found, linear regression models were applied in subsequent analyses. Generally, mixed effects models were used to examine the associations of long-term and short-term NRAP and regional air pollution exposures with the outcome of each metabolomic PC score and individual metabolite concentrations after adjusting for potential confounders including age, sex, race/ethnicity, parental education (proxy for social economic status), BMI, whether or not the participant smoked cigarettes during the last week, ever/never smoked electronic cigarettes, season of the study visit and random intercepts for CHS communities and metabolomics analytical batches. Beyond the analysis of individual pollutants, multi-pollutant models with exposures to multiple air pollutants as independent variables were used to assess the independent effects of each air pollutant on metabolomic outcomes adjusting for exposures to other air pollutants.

For the analyses of individual metabolite concentrations, we focused on the metabolites that were representative of significant PCs (a factor loading of an absolute value ≥ 0.4, Supplemental Table 2) that had significant associations with air pollution exposures. The false discovery rate (FDR) method[73] was used to adjust for multiple testing that could inflate type 1 error. Statistical tests were considered significant with an FDR-adjusted p-value less than 0.1.

Next, the mixed effects model was used to assess the joint associations between all prioritized metabolomic PC scores and the primary obesity outcome of BMI as a continuous variable after adjusting for potential confounders including age, sex, ethnicity, parental education levels as a proxy for socioeconomic status, whether or not cigarettes were smoked during the last week, ever/never smoked electronic cigarettes, and random intercepts for CHS communities and metabolomics analytical batches. In addition, BMI categories [normal weight (BMI<25 kg/m^2), overweight (25⩽BMI<30 kg/m^2) and obese (BMI⩾30 kg/m^2)] were used as a secondary outcome for obesity. Multinomial logistic regression with random intercepts for CHS communities and metabolomics analytical batches was used to analyze the associations between metabolomic PC scores and BMI categories.

Additionally, in a subset of 132 participants, we assessed the influence of physical activity (whether or not exercise classes were taken outside of the school in the previous year and

self-rated physical activity scale) and diet (total calorie intake, percent calorie intake from fat and protein, and glycemic index estimated from the 24-hr recalls) in the association of metabolomics factor scores with air pollution exposures or the obesity outcomes of BMI as a continuous variable or BMI categories.

Lastly, we investigated whether sex, race/ethnicity and BMI categories could modify the effects of air pollution exposure and metabolomic PC scores by including multiplicative interaction terms in the model. All independent statistical tests were considered significant with a two-sided p-value less than 0.05. SAS version 9.4 (SAS Institute Inc., Cary, North Carolina) was used for data analysis.

RESULTS

This study included 173 participants who had complete metabolomic data, weight and height measures, and at least exposure data for one air pollutant. Sociodemographic characteristics are described in Table 1. The age range of our participants at the study enrollment was 18–23 years old with a mean age (standard deviation, SD) of 19.8 (1.1) years. The mean BMI (SD) was 28.2 (5.2) kg/m^2, and 73.4% participants were overweight or obese.

Means (SDs) of air pollution exposure concentrations are presented in Table 2. Correlations among long-term and short-term air pollution exposures are presented in Supplemental Table 3. Generally, lagged 1-month (short-term) NRAP and regional air pollution exposures were highly correlated with lagged 1-year averages (long-term) of exposures (all $R^2 \geq 0.5$), except O_3. However, the correlations between NRAP exposures and regional air pollution exposures were relatively small (all $R^2 < 0.3$).

Classifications of Metabolomic PCs

Means (SDs) of serum concentrations of all metabolites analyzed from 173 samples are presented in Supplemental Table 1. Similar PC patterns were identified comparing raw metabolomic data and the MI-imputed data (Supplemental Table 2). Therefore, we used PCs classified from the raw metabolomic data for all the following analyses. Overall, principal component analysis identified 14 orthogonal factors with eigenvalues ≥1.0. In our main analysis, we focused on the top five PCs, which explained a total of 52.6% variance of serum concentrations of 63 metabolites (Figure 1, Supplemental Table 2 and Supplemental Figure 1). Among these five PCs, PC1 mainly represents a group of short- and median-chain acylcarnitines, PC2 is characterized by NEFA and its oxidation by-products, PC3 represents metabolites involved in branched-chain amino-acid (BCAA) catabolism, PC4 represents amino acids involved in general amino acid catabolism and the urea cycle, and PC5 represents metabolites related to glycine metabolism.

NRAP exposure was associated with lower concentrations of short- and median-chain acylcarnitines and higher concentrations of NEFA oxidation by-products

No significant departure from a linear relationship was observed between short-term or long-term NRAP and regional air pollution exposures with the outcome variables of metabolomic factor PC scores (all p's for cubic spline terms>0.10). Therefore, results described below are based on the linear relationships between air pollution exposure and PC scores.

Table 3 presents the associations between NRAP exposure and metabolomic PC1-PC5 scores. After adjusting for potential confounders, higher long-term (lagged one-year average) freeway NRAP and total NRAP (a combination of freeway and non-freeway NRAP) exposure was associated with lower PC1 score, which represents lower concentrations of various short- and median-chain acylcarnitines (p=0.044 and 0.049, respectively). Meanwhile, higher exposure to long-term non-freeway NRAP was associated with higher NEFA-related PC2 score (p=0.038). Similar associations were found for short-term (lagged one-month) exposures to freeway, non-freeway and total NRAP with metabolomic PC scores (Supplemental Table 4). However, due to the relatively small sample size, none of the associations were statistically significant after multiple testing adjustment for five PC scores (all FDR's >0.19).

Findings from single pollutant models were further supported by the multi-pollutant regression analyses, which included both freeway and non-freeway NRAP exposure in one model for each PC (Supplemental Table 5). Among all study participants, long-term freeway NRAP exposure was negatively associated with PC1 score and non-freeway NRAP exposure was positively associated with PC2 score (p=0.05 and 0.025, respectively). No significant associations were found between NRAP exposure and other PC scores.

Next, we explored long-term NRAP exposure association with concentrations of individual metabolites among 32 metabolites that have loadings>0.4 in either PC1 or PC2 (Supplemental Table 6). Glycerol was also included in the single metabolite analyses as an additional measurement of lipolysis.[66] Higher long-term and short-term non-freeway NRAP exposures were significantly associated with increased circulating glycerol and higher concentrations of NEFA-oxidation products: two long-chain acylcarnitines (C18:1-DC and C20-OH/C18-DC), 3-Hydroxybutyrylcarnitine (C4-OH) (FDR-adjusted p≤0.1). No other NRAP exposures were found to be significantly associated with concentrations of individual metabolites after adjusting for multiple testing (data not shown).

Furthermore, multi-pollutant analysis including exposures to three regional and near-roadway air pollutants in one model for each PC showed that higher lagged one-month exposure to regional $PM_{2.5}$ was associated with lower PC1 score, while higher lagged one-month exposure to regional NO_2 was associated with higher PC1 score (Supplemental Table 7, p=0.041 and 0.002, respectively). We further confirmed that the previously observed associations between freeway NRAP exposure with PC1 score were not significantly influenced by adjustment for regional air pollution exposures. However, adjustment for regional air pollution exposure attenuates the association between nonfreeway NRAP exposure and metabolomic PC2 score. No other significant associations were observed for long-term or short-term exposures to regional air pollutants with metabolomic PCs in this study. It was not possible to disentangle the relative importance of short- *versus* long- term exposures due to the high collinearity between these two exposure metrics (Supplemental Table 3).

Lastly, in a subset of 132 participants who had data from 24-hr diet recalls and self-reported physical activity status in the previous year (detailed characteristics are presented in Supplemental Table 8), we confirmed that the associations between nonfreeway NRAP

exposure and PC2 score remained significant after additionally adjusting for total calorie intake, percent calorie intake from fat and protein, glycemic index in the daily diet, whether or not participating in exercise classes in the previous year and self-rated physical activity scale (Supplemental Table 9). The associations between freeway and total NRAP exposures with PC1 score were still negative but not statistically significant among this subset of participants. It is noted that sociodemographic characteristics among this subset of participants were not significantly different from the entire study sample, though there was a 10% higher frequency of overweight and obese participants in the subset of participants compared to the entire study sample.

Associations between NRAP exposure and metabolomic PCs are differentiated by BMI categories and sex

The associations of long-term non-freeway NRAP exposure with NEFA oxidation-related PC2 score were different across BMI categories (interaction p=0.047) (Table 3). Non-freeway NRAP exposure had the strongest positive association with PC2 score among overweight and obese participants. Two standard deviations (2.02 ppb) increase in lagged one-year average of non-freeway NRAP exposure was associated with 0.5 and 0.7 unit increase in PC2 score among overweight and obese participants [β (p) for overweight: 0.51(0.039) and obese 0.68 (0.007)]. While associations between non-freeway NRAP exposure and PC2 score had smaller effect size and was not statistically significant among normal-weight participants (β=−0.21, p=0.51). Similar effect modifications by BMI categories were also found for the associations of short-term non-freeway NRAP exposure with PC2 score (Supplemental Table 4). In addition, although there was no significant association between NRAP exposure and the BCAA-related PC3 score, higher freeway and total NRAP exposure was associated with lower BCAA-related PC3 score in obese participants (p=0.006 and 0.005, respectively).

Associations of long-term freeway NRAP exposure with NEFA-related PC2 score were more evident in females than in males (Table 4, interaction p=0.049). Two standard deviations (12.4 ppb) increase in lagged one-year average of freeway NRAP exposure was associated with 0.5 unit increase in PC2 score among females (β=0.52, p=0.042), but there was no significant association between freeway NRAP exposure and PC2 score among males (β=−0.13, p=0.55). Similar effect modification was found for short-term freeway NRAP exposure and PC2 score [β (p) for females: 0.71 (0.008) and for males: −0.15 (0.51)]. Along with these results, the mean concentration of NEFA was 0.39 mmol/L higher in females than males (p=0.011). However, there was no significant difference in mean BMI between males and females [mean BMI (SD) for males: 28.1 (5.2) kg/m^2 and for females: 28.5 (5.3) kg/m^2]. Lastly, no significant difference was found for associations between NRAP exposure and metabolomic PC2 score between non-Hispanic white and Hispanic white participants.

Lower concentrations of short- and median-chain acylcarnitines and higher concentrations of BCAA-related metabolites were associated with higher BMI and an increased odd of obesity.

We found that lower metabolomic PC1 score (i.e., lower concentrations of short- and median-chain acylcarnitines), higher PC3 score (i.e., higher concentrations of BCAA and related metabolites), higher PC4 score (i.e., higher concentrations of intermediates involved in catabolism of multiple amino acids), and lower PC5 scores (i.e., lower concentrations of glycine and other related amino acids) were all significantly associated with higher BMI after adjusting for potential confounders (Table 5, all p's≤0.001). A one-unit increases in the PC1 score was associated with 44% and 57% lower odds for an individual to be overweight and obese rather than normal weight [Figure 2, odds ratios (ORs) and 95% confidence intervals (CIs)=0.56 (0.34, 0.94) and 0.43 (0.23, 0.84), respectively]. Meanwhile, individuals with one-unit increases in the PC3 score were 2–4 fold more likely to be overweight and obese compared to normal weight [Figure 2, ORs (95% CIs) = 2.09 (1.12, 3.89) and 3.86 (1.82, 8.15), respectively].

We further explored the associations between individual metabolite concentrations and odds of being overweight and obese (Figure 3). After adjusting for multiple testing across 33 metabolites that had the loadings in PC1, PC3, PC4 and PC5 greater than 0.4, as well as glycerol, we found that increased concentrations of eight acylcarnitines had significant associations with reduced odds of being overweight or obese (all FDR-adjusted p's<0.1). Conversely, increased concentrations of six amino acids (including valine, leucine/isoleucine) and byproducts of BCAA metabolism, C3 and C5 acylcarnitines, were associated with increased odds of being overweight or obese (all FDR-adjusted p's <0.1).

In the subset of 132 participants who had complete dietary and physical activity data collected, we verified that the association of lower PC1 and PC5 scores, as well as higher PC3 and PC4 scores with higher BMI and increased odds of obesity remained statistically significant after adjustment for total calorie intake, percent calorie intake from fat and protein, glycemic index in the daily diet, whether or not participating exercise classes in the previous year and self-rated physical activity scale (Supplemental Table 10 and Supplemental Figure 2).

CONCLUSIONS

A main finding of this study is that higher NRAP exposure, especially non-freeway NRAP, was associated with higher concentrations of glycerol and metabolites related to NEFA oxidation. This finding provides parallel evidence for our previous observations from the longitudinal CHS cohort that increased NRAP exposure during early life and childhood periods was associated with increased risk of obesity in later life.[6,54,74] It is well-known that plasma levels of NEFA and its oxidation byproducts such as C4-OH are associated with increased adiposity and insulin resistance.[75–78] Expanding fat mass releases correlate with increases in circulating NEFA and glycerol, which serve to induce insulin resistance and inflammation.[75–77] Accordingly, we found that higher NRAP exposure was associated with an increased PC score related to increased concentrations of a mixture of metabolites including NEFA and its oxidation byproducts (C2, C4-OH and several long-chain

acylcarnitines). Higher NRAP exposure was also associated with increased serum glycerol. These results suggest that NRAP exposure could contribute to increased demand for mitochondrial NEFA oxidation. However, the association between air pollution exposure and serum NEFA concentration was not statistically significant in this study, which suggests that adolescents and young adults may still have adequate mitochondrial capacity to compensate for environmental stressors such as air pollution exposures.

Consistent with the foregoing constructs, we also found that the positive association between NRAP exposure and NEFA-related PC was strongest among obese participants. The effect modification by obesity status indicates that increased NRAP exposure could exacerbate obesity-induced inflammation and metabolic dysfunction. NRAP exposure can stimulate lung and systemic inflammation through the activation of immunomodulatory pattern-recognition receptors such as Toll-like receptors (TLRs).[79–82] Meanwhile, NEFA plays an important role in obesity-induced inflammation.[75–78] One pathway for NEFA to activate systemic inflammation is through its activation of proinflammatory nuclear factor (NF)-κB pathway, which was partially mediated by the TLR-4.[83–85] Therefore, NRAP exposure could share similar inflammatory pathways as NEFA and have far-reaching impact on systemic inflammation and insulin resistance in obese individuals.

Another interesting finding about the NEFA oxidation-related PC is that even though freeway NRAP exposure was not significantly associated with NEFA-related PC score in the entire sample, there was a statistically significant positive association between freeway NRAP exposure and NEFA-related PC score among females. Previous studies showed that sex differences in metabolomic patterns were observed in childhood and may persist in adolescents and adults.[86,87] In this study, we also observed that female participants had higher mean serum NEFA concentrations than males. Although the overall BMI was not significantly different between males and females, differences in body fat distribution[19,88,89] and sex steroids[90] may contribute to differential impacts of NRAP exposure on NEFA-related metabolomic profiles.

Meanwhile, we also found that lower concentrations of a variety of short- and medium-chain acylcarnitines were associated with an increased risk of obesity. The lower levels of acylcarnitines in obese subjects observed here are consistent with observations from another metabolomics study.[91] One possible interpretation of these findings is that with increased demand for mitochondrial NEFA oxidation, early oxidative intermediates (embodied by the long-chain acylcarnitines) begin to accumulate, while levels of downstream metabolites decrease, possibly suggesting saturation of early steps in the β-oxidative pathway. This signature in adolescence may eventually give way to a more complete dysregulation of fatty acid metabolism as has been observed in adults, involving accumulation of essentially all lipid-derived acylcarnitine intermediates, suggested to be a signal of mitochondrial substrate overload.[92]

Our study also confirms the association of BCAAs and metabolites generated by their oxidation, C3, C5 acylcarnitines, with BMI. Similar associations between dysregulated metabolism of BCAAs, obesity and insulin resistance have been widely observed in adult animal[31,64] and human studies.[13–16,31–35,93] We also found freeway NRAP exposure was

associated with lower BCAA and related metabolites only among obese participants, suggesting that NRAP exposure could interact with obesity in the BCAA profile. The inverse relationship between NRAP and BCAA may be confounded by diet and other uncontrolled socio-behavior factors.[94–97] Therefore, more studies are needed to explore the associations between NRAP exposure and BCAA-related metabolism.

Based on the above findings, we summarized the potential pathways for NRAP exposure to influence lipid and amino acid metabolism in Figure 4. NRAP exposure might increase adipose lipolysis and release of NEFA and glycerol, contributing to activation of oxidative stress and inflammation pathways.[98–102] Increased circulating NEFA increases demand for fatty acid oxidation. Even though adolescents and young adults have generally normal mitochondrial function to compensate for the increased demand of NEFA oxidation, this early adaptive metabolic plasticity is very likely to be diminished over time from youth to adulthood, especially for obese individuals. Finally, it is expected that persistent NRAP exposure and obesity from childhood to adulthood will eventually cause mitochondrial dysfunction and the development of metabolic diseases.

To our knowledge, this is the first targeted-metabolomics study in adolescents and young adults that investigates the role of metabolomic profiles in the impact of air pollution exposure on metabolic dysfunction. Several advantages of this study include 1) robust measures of targeted metabolites that participate in metabolic pathways of the three major classes of macronutrients—lipids, carbohydrates, and protein, 2) unique study population of adolescents and young adults who are at the initial stage in developing metabolic diseases, and 3) state-of-the-art air pollution exposure assessment and detailed sociodemographic and health behavior covariates. Overall, results of this study support the link between air pollution exposure and dysregulated lipid metabolism, which was observed in several other non-targeted and targeted metabolomics studies.[36–39] More importantly, results of this study further provide evidence that dysregulated fatty acid metabolism could be an essential pathway in obese youth for NRAP exposure to influence metabolism and trigger the development of cardio-metabolic diseases in later life.

We also acknowledge that the current study has several limitations. First, the cross-sectional and observational design of our study precludes us from conclusions inferring any causal relationship between air pollution exposures, metabolomic changes, and the development of metabolic dysfunction. Directionality cannot be discerned - i.e., whether the metabolites are leading to obesity or obesity is modifying metabolism cannot be distinguished from this study. Future longitudinal studies and animal models are needed to verify the mediation role of metabolomic changes in the relationship between air pollution exposure and the development of obesity and metabolic diseases. Also, the sample size of this study is relatively small. Findings of this study will need to be verified by other large replication cohorts. Second, we were not able to discriminate the impact of short-term and long-term air pollution exposures on metabolomic outcomes because the short-term and long-term air pollution exposures were highly correlated in this study. Furthermore, future longitudinal studies with dynamic assessment of life-time air pollution exposure and health outcomes are warranted to identify critical exposure window during life time for the adverse effect of air pollution exposures on metabolomic dysfunction in children and youth. Third, there could

be residual confounders not included in the analysis such as geospatial clustering and early-life socio-behavioral factors, which may explain our observation of the opposing effect of NO_2 on the PC1 score of short- and median-chain acylcarnitines compared to the effect of $PM_{2.5}$ and freeway NRAP exposures. Finally, most of our significant findings of NRAP associations with altering fatty acid metabolism were related to non-freeway NRAP exposure. It has been shown that chemical composition of NARP exposure from freeway versus non-freeway roads are different due to differences in vehicle types and vehicle volume.[103] Non-freeway NRAP exposure also contains more non-exhaust particles (e.g. brake wear and tire wear). It is also possible that non-freeway NRAP exposures capture other neighborhood characteristics (e.g. housing and built environment) that introduce some residual confounding. Future studies are needed to identify which specific components or chemicals of NRAP are detrimental to metabolic dysfunction.

In conclusion, our study suggests that NRAP exposure contributes to lipolysis and the altered fatty acid metabolism, manifest by increased circulating glycerol and NEFA oxidation byproducts. This pollution-triggered alteration in fatty acid metabolism may contribute to greater susceptibility of pollutant-exposed adolescents to metabolic diseases associated with obesity. However, these findings warrant future larger cohorts to replicate and explore these associations. Also, future studies are needed to account for sex and racial/ethnic differences in studying the impact of air pollution exposure on metabolic perturbations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Z.C. conducted the analyses and wrote the article. Z.C., F.D.G., E.R.H., C.B.N. and C.B. contributed to study design. Z.C., F.D.G., J.K., T.L.A., O.I., E.A., F.L. and M.M. contributed to the data collection. Z.C., E.R.H., D.C.T. and K.B. contributed to the development of statistical methods. E.R.H., D.C.T., K.B., J.K., T.L.A., C.B.N., C.B., L.C., T.M.B., E.A., F.L., R.M., and F.D.G. edited the article and contributed to discussion. All authors reviewed the article. Z.C. is the guarantor of this work, and as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors declare that they have no conflict of interest.

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Abbreviations:

NRAP	near-roadway air pollution
BMI	body mass index
$PM_{2.5}$	particulate matter with aerodynamic diameter <2.5 μm

CHS	Children's Health Study
DORI	Diabetes and Obesity Research Institute
CTU	Clinical Trials Unit
CDC	Centers for Disease Control and Prevention
NDSR	Nutrition Data System for Research
NCC	Nutrition Coordinating Center
IRB	Institutional Review Board
NOx	nitrogen oxides
CALINE4	California line-source dispersion
NO_2	nitrogen dioxides
O_3	ozone
FRM	Federal Reference Method
FEM	Federal Equivalent Method
IDW2	inverse distance-squared weighting
NEFA	non-esterified free fatty acids
PCA	principal components analysis
PC	principal components
MI	multiple imputation
FDR	false discovery rate
SD	standard deviation
BCAA	branched-chain amino-acid

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Highlights:

- Near-roadway air pollution exposure is associated with altered fatty acid metabolism
- Near-roadway air pollution exposure interacts with obesity and sex in the association with fatty acid metabolism.
- Air pollution-triggered alteration in fatty acid metabolism may contribute to greater susceptibility of pollutant-exposed adolescents to metabolic diseases associated with obesity

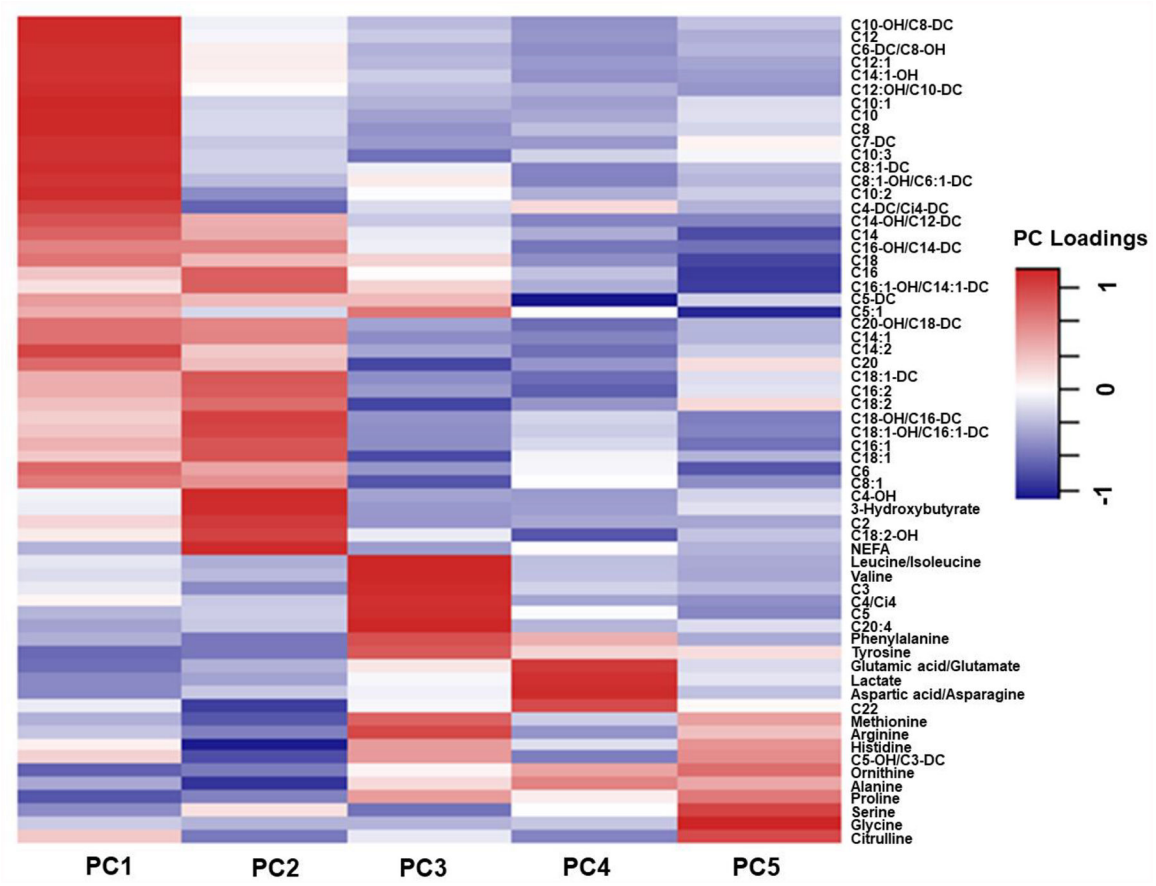

Figure1.

Heatmap of loadings for the first five principal components identified from targeted metabolite concentrations among 173 adolescents and young adults enrolled in the Children's Health Study.

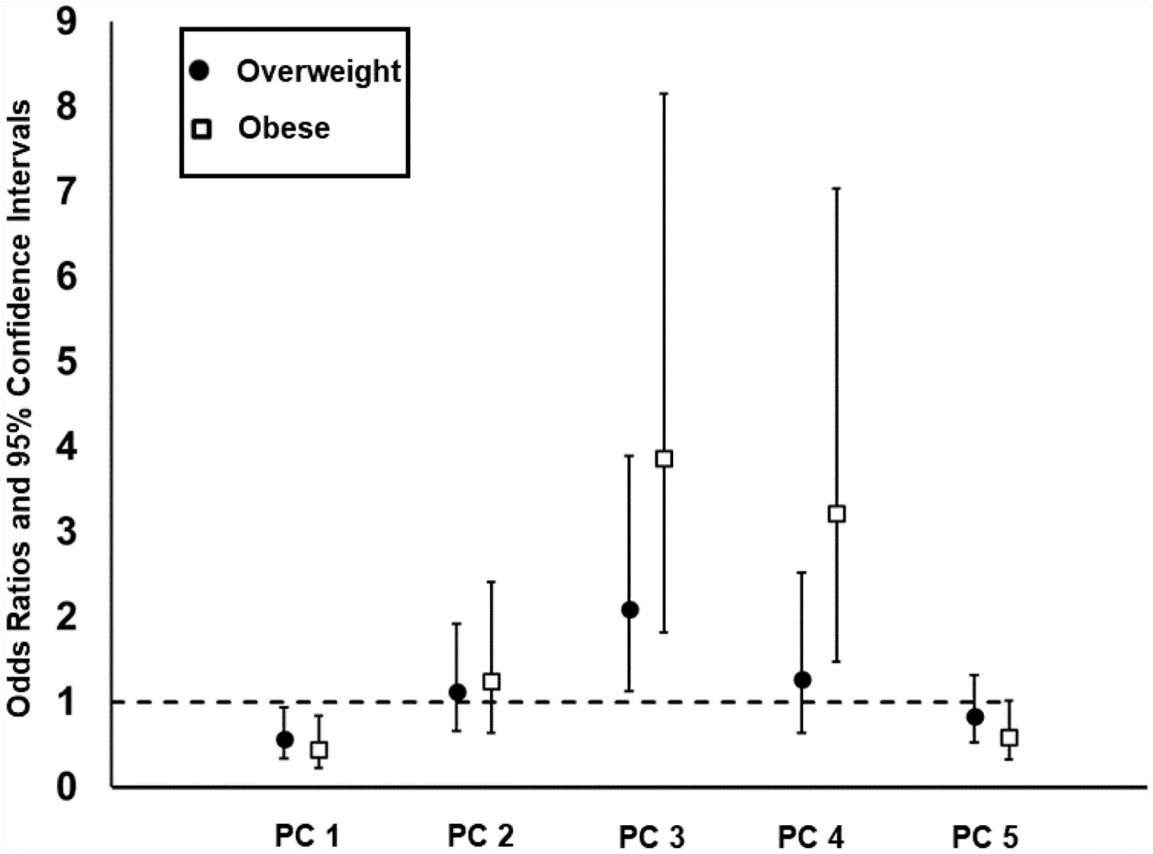

Figure 2.

Associations between metabolomic principal component (PC) scores and relative odds to be overweight or obese compared to normal weight. Odds ratios and 95% confidence intervals are presented for comparing odds to be overweight and obese compared to normal weight with one unit increase in metabolomic PC scores (PC1-PC5) among 173 adolescents and young adults.

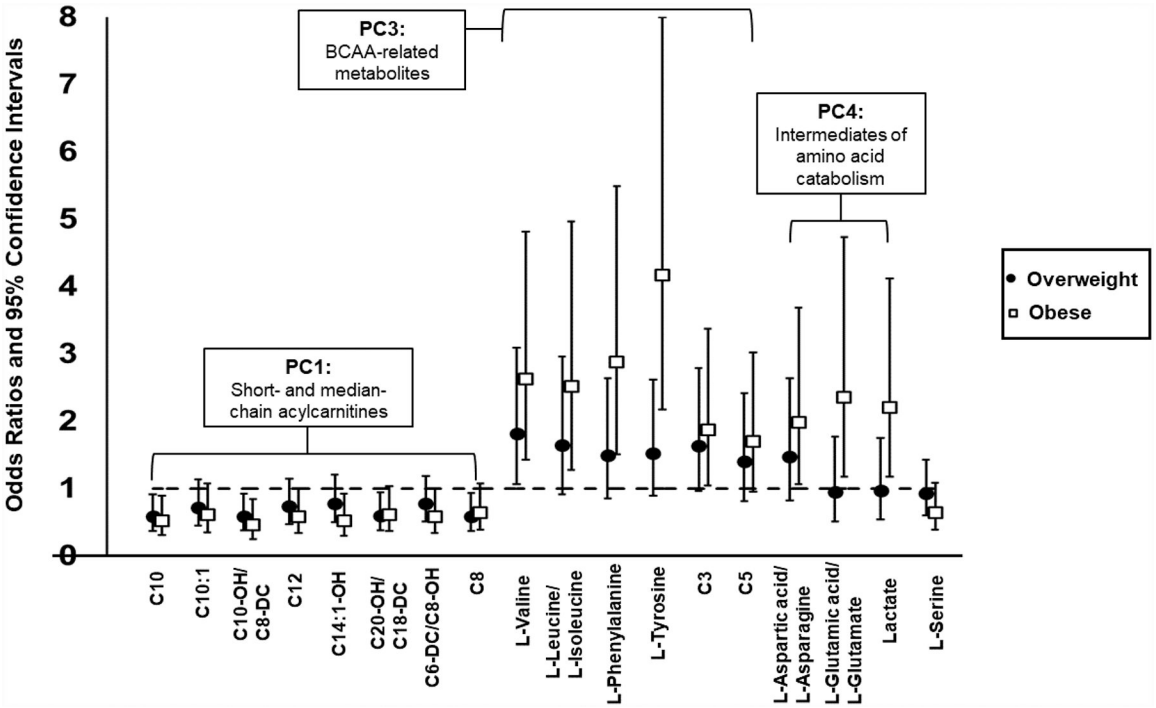

Figure 3.

Significant associations of concentrations of individual metabolites that represent principal components (PC) - PC1, PC3 and PC5 with relative odds of being overweight or obese compared to normal weight. Odds ratios and 95% confidence intervals are presented for comparing odds to be overweight and obese compared to normal weight with one standard deviation increase in each metabolite among 173 adolescents and young adults. Significant associations are selected based on the criterion of FDR-adjusted p-values<0.1.

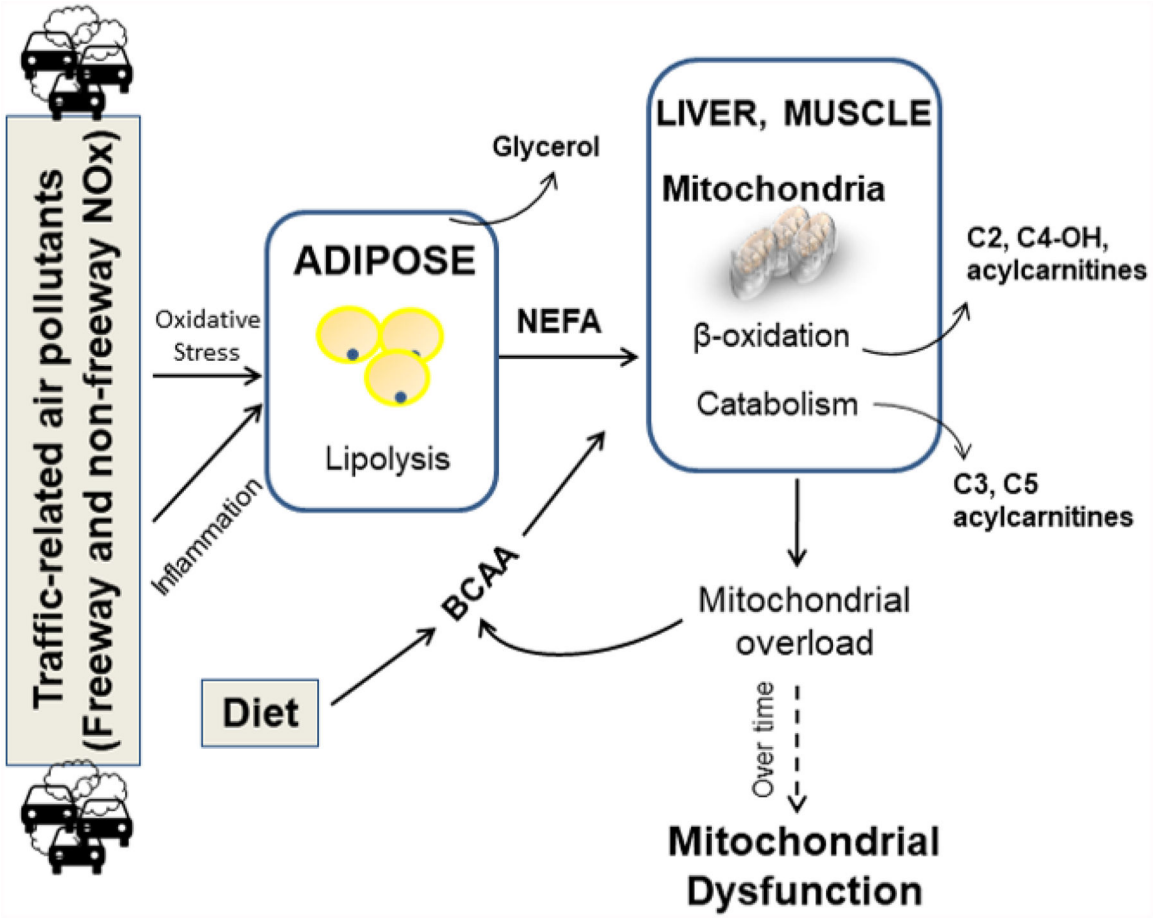

Figure 4.

The potential pathways for near-roadway air pollution (NRAP) exposure to influence lipid and amino acid metabolism. NEFA: Non-esterified fatty acids; BCAA: branched-chain amino acids. Increased NRAP exposure might increase adipose lipolysis and release of NEFA and glycerol. Increased circulating NEFA requires increased mitochondrial function for NEFA oxidation, which competes the mitochondrial capacity of amino acid metabolism. Therefore, serum concentrations of BCAAs and other amino acids can be elevated at the same time.

Table 1.

Sociodemographic characteristics of 173 Children's Health Study (CHS) adolescents and young adults enrolled during year 2014–2018.

	Entire sample (N=173)	
	N	**%**
Sex		
Female	79	45.7
Male	94	54.3
Race/Ethnicity		
Non-Hispanic White	50	28.9
Hispanic White	97	56.1
Other[1]	26	15.0
Parental education		
Less than high school	58	33.5
Completed high school	52	30.1
Some college or higher	59	34.1
Unknown	4	3.3
Ever used e-cigarette		
Yes	46	26.6
No	127	73.4
Cigarette smoke in the last week		
Yes	9	5. 2
No	164	94.8
Body mass index (BMI) categories		
Normal weight (<25 kg/m^2)	46	26.6
Overweight (≥25 and <30 kg/m^2)	77	44.5
Obese (≥30 kg/m^2)	50	28.9

1. Other race/ethnicity includes African American, Asian/Pacific Islander, mixed three or more races, other Non-White race/ethnicity and unknown race/ethnicity.

Table 2.

Means and standard deviations of short-term[1] and long-term[2] near-roadway and regional air pollution exposure levels among 173 adolescents and young adults enrolled from the Southern California Children's Health Study during year 2014–2018.

	Mean	Standard Deviation
Short-term Exposures		
Near-roadway air pollutants (NRAP)		
Freeway NRAP (ppb)[3]	4.8	6. 3
Non-freeway NRAP (ppb)[3]	1.5	1.0
Total NRAP (ppb)[3]	6.3	6.6
Regional air pollutants		
NO_2 (ppb)[3]	15.4	5. 9
O_3 (ppb)[3]	48.3	14.3
PM_{10} ($\mu g/m^3$)	29.5	10.2
$PM_{2.5}$ ($\mu g/m^3$)	12.0	4.4
Long-term Exposures		
Near-roadway air pollutants (NRAP)		
Freeway NRAP (ppb)[3]	5.1	6. 2
Non-freeway NRAP (ppb)[3]	1.5	1.0
Total NRAP (ppb)[3]	6.6	6.6
Regional air pollutants		
NO_2 (ppb)[3]	15.7	4. 0
O_3 (ppb)[3]	48.2	6.4
PM_{10} ($\mu g/m^3$)	30.2	7.3
$PM_{2.5}$ ($\mu g/m^3$)	12.0	2.6

1. Short-term air pollution exposure was estimated from the individual residential history for lagged 1-month exposure level prior to the study visit.

2. Long-term air pollution exposure was estimated from the individual residential history for lagged 1-year exposure level prior to the study visit.

3. ppb: parts per billion, as the unit of NRAP, NO_2 and O_3 exposure

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Table 3.

Associations between long-term[1] near-roadway air pollution (NRAP) exposure and metabolomic principal component (PC) scores estimated from 173 adolescents and young adults in the Children's Health Study.

	Entire sample (N=173)		Normal weight (N=46)[3]		Overweight (N=77)[3]		Obese (N=50)[3]		Interaction p[4]
	β[2]	p[3]	β[2]	p[3]	β[2]	p[3]	β[2]	p[3]	
Outcome=PC1[5]									
Freeway NRAP	**−0.34**	**0.0 44**	−0. 77	0.23	−0. 36	0.18	−0.48	0.09	0.71
Non-freeway NRAP	−0.07	0.63	−0.67	0.12	−0.37	0.16	0.45	0.08	0.10
Total NRAP	**−0.33**	**0.049**	−0.88	0.15	−0.39	0.14	−0.37	0.21	0.51
Outcome=PC2[6]									
Freeway NRAP	0.09	0.58	0.08	0.85	0.01	0.97	0.10	0.73	0.53
Non-freeway NRAP	**0.30**	**0.038**	−0.21	0.51	**0.51**	**0.039**	**0.68**	**0.007** *	**0.047**
Total NRAP	0.14	0.42	0.003	0.99	0.07	0.78	0.23	0.44	0.48
Outcome=PC3[7]									
Freeway NRAP	−0.21	0.18	0.25	0.64	−0.03	0.91	**−0.80**	**0.006** *	0.66
Non-freeway NRAP	0.03	0.81	−0.01	0.99	0.17	0.47	0.01	0.96	0.87
Total NRAP	−0.19	0.22	0.22	0.67	0.002	0.99	**−0.86**	**0.005** *	0.60
Outcome=PC4[8]									
Freeway NRAP	−0.01	0.92	−0.07	0.82	−0.10	0.66	0.16	0.57	0.49
Non-freeway NRAP	−0.03	0.80	−0.24	0.24	0.001	1.00	−0.36	0.18	0.96
Total NRAP	−0.02	0.87	−0.11	0.68	−0.09	0.69	0.09	0.76	0.50
Outcome=PC5[9]									
Freeway NRAP	0.21	0.24	−0.20	0.71	0.30	0.33	0.22	0.44	0.75
Non-freeway NRAP	0.21	0.19	0.25	0.49	0.38	0.22	−0.27	0.30	0.17
Total NRAP	0.24	0.18	−0.10	0.85	0.34	0.27	0.17	0.56	0.66

1. Long-term air pollution exposure was estimated by cumulative averages of monthly exposure concentrations over one year prior to the study visit and were weighted by time spent at each different address.

[2] Mixed effects model was used to estimate regression associations between freeway, nonfreeway and total NRAP exposure with the outcome variables of metabolomic factor scores after adjusting for confounders including age, sex, race/ethnicity, parental education levels as a proxy for social economic status, whether or not smoked cigarettes during the last week, ever/never smoked electronic cigarettes, season of the study visit and random intercepts for CHS communities and metabolomics analytical batches. NRAP exposure was scaled by 2 standard deviations. Association estimates (β) and p-values for significance of statistical tests are presented in the table.

[3] Stratified analysis was conducted in each subgroups of body mass index (BMI) categories: normal weight (BMI<25 kg/m^2), overweight (BMI≥25 and <30 kg/m^2) and obese (BMI≥30 kg/m^2) groups.

[4] Interaction p-values<0.05 represent statistically significant differences in effect sizes for the associations between NRAP exposure and metabolomic PC scores across three BMI categories.

[5] Metabolomic principal component (PC) 1 represents a variety of short- and median-chain acylcarnitines and explains 26.3% variance of concentrations of all metabolites analyzed in the study samples. More details of the loadings of specific metabolites for each PC are described in Supplemental Table 4.

[6] PC2 represents non-esterified fatty acids (NEFA) and NEFA oxidation by-products including long-chain acylcarnitines, acetylcarnitine (C2) and 3-Hydroxybutyrylcarnitine (C4-OH), which explains 10.2% variance of concentrations of all metabolites analyzed in the study samples.

[7] PC3 represents metabolites involved in branched-chain amino-acid (BCAA) catabolism, which explains 7.2% variance of concentrations of all metabolites analyzed in the study samples.

[8] PC4 represents amino acids involved in general amino acid catabolism and the urea cycle, which explains 4.9% variance of concentrations of all metabolites analyzed in the study samples.

[9] PC5 represents amino acids involved in glycine metabolism, which explains 4.1% variance of concentrations of all metabolites analyzed in the study samples.

[*] Association tests are significant after FDR adjustment for multiple testing of five PC scores (FDR<0.1).

Table 4.

Associations between near-roadway air pollution (NRAP) exposure and NEFA-related metabolomic principal component (PC)[1] scores stratified by sex and race/ethnicity among adolescents and young adults in the Children's Health Study.

NRAP Exposure	Males (N=94)		Females (N=79)		Interaction p[3]	Non-Hispanic Whites (N=50)		Hispanic Whites (N=97)		Interaction p[4]
	β^2	p	β^2	p		β^2	p	β^2	p	
Long-term exposure[5]										
Freeway NRAP	−0.13	0.55	**0.52**	**0.0 42**	**0.049**	−0.30	0.63	0.17	0.35	0.27
Non-freeway NRAP	0.31	0.08	**0.50**	**0.032**	0.21	0.24	0.39	**0.39**	**0.044**	0.62
Total NRAP	−0.05	0.83	**0.65**	**0.008**	**0.049**	−0.05	0.93	0.23	0.21	0.29
Short-term exposure[6]										
Freeway NRAP	−0.15	0.51	**0.71**	**0.008**	**0.033**	−0.40	0.55	0.24	0.16	0.49
Non-freeway NRAP	0.32	0.09	0.45	0.06	0.44	0.31	0.30	0.32	0.11	0.75
Total NRAP	−0.07	0.76	**0.74**	**0.005**	**0.036**	−0.06	0.92	0.27	0.12	0.52

1. NEFA-related metabolomic PC (also known as PC2 in this paper) represents non-esterified fatty acids (NEFA) and NEFA oxidation by-products including long-chain acylcarnitines, acetylcarnitine (C2) and 3-Hydroxybutyrylcarnitine (C4-OH), which explains 10.2% variance of concentrations of all 63 metabolites analyzed in the study samples.

2. Mixed effects model was used to estimate regression associations between freeway, non-freeway and total NRAP exposure with the outcome variables of metabolomic factor scores after adjusting for confounders including age, sex, race/ethnicity, parental education levels as a proxy for social economic status, whether or not smoked cigarettes during the last week, ever/never smoked electronic cigarettes, season of the study visit and random intercepts for CHS communities and metabolomics analytical batches. NRAP exposure was scaled by two standard deviations. Association estimates (β) and p-values for significance of statistical tests are presented in the table.

3. Interaction p-values <0.05 represent statistically significant differences in effect sizes for the associations between NRAP exposure and PC2 score comparing males to females.

4. Interaction p-values <0.05 represent statistically significant differences in effect sizes for the associations between NRAP exposure and PC2 score comparing Non-Hispanic Whites to Hispanic Whites.

5. Long-term air pollution exposure was estimated by cumulative averages of monthly exposure concentrations over one year prior to the study visit and were weighted by time spent at each different address.

6. Short-term air pollution exposure was estimated by one-month exposure concentrations prior to the study visit.

Table 5.

Associations between metabolomic principal component (PC) scores and body mass index (BMI) among 173 adolescents and young adults.

Independent Variables	β[6]	p
Metabolomic PCs		
PC1[1]	**−1.21**	**<0.001**
PC2[2]	0.30	0.42
PC3[3]	**1.51**	**<0.001**
PC4[4]	**2.34**	**<0.001**
PC5[5]	**−1.01**	**0.001**
Race/Ethnicity		
Non-Hispanic White	*ref*	
Hispanic White	0.44	0.56
Other	0.95	0.34
Sex		
Male	*ref*	
Female	−0.42	0.55
Age	−0.01	0.96
Parental education		
Less than high school	*ref*	
Completed high school	−0.21	0.79
Some college or higher	−0.87	0.30
Unknown	−2.04	0.35
Ever used e-cigarette		
No	*ref*	
Yes	0.87	0.25
Cigarette smoke in the last week		
No	*ref*	
Yes	−1.15	0.40

[1] Metabolomic principal component (PC) 1 represents a variety of short- and median-chain acylcarnitines and explains 26.3% variance of concentrations of all metabolites analyzed in the study samples. More details of the loadings of specific metabolites for each PC are described in Supplemental Table 4.

[2] PC2 represents non-esterified fatty acids (NEFA) and NEFA oxidation by-products including long-chain acylcarnitines, acetylcarnitine (C2) and 3-Hydroxybutyrylcarnitine (C4-OH), which explains 10.2% variance of concentrations of all metabolites analyzed in the study samples.

[3] PC3 represents metabolites involved in branched-chain amino-acid (BCAA) catabolism, which explains 7.2% variance of concentrations of all metabolites analyzed in the study samples.

[4] PC4 represents amino acids involved in general amino acid catabolism and the urea cycle, which explains 4.9% variance of concentrations of all metabolites analyzed in the study samples.

[5] PC5 represents amino acids involved in glycine metabolism, which explains 4.1% variance of concentrations of all metabolites analyzed in the study samples.

[6] Mixed effects model with all five PCs in one regression model and adjustment for socio-demographic confounders was used to estimate independent association of each metabolomic PC with BMI as a continuous outcome variable. Association estimates (β) and p-values are presented for each independent variable in the analysis model.