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Authors

Li, Zhenjiang

Pan, Shudi

Baumert, Brittney O

et al.

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Beyond the Surface: Mapping DDE's Metabolic Footprint on Adolescent Obesity

Zhenjiang Li^{1,*}, Shudi Pan¹, Brittney O. Baumert¹, Jiawen Carmen Chen¹, Jesse A. Goodrich¹, Hongxu Wang¹, Sarah Rock¹, Justin Ryder², Damaskini Valvi³, Todd Jenkins⁴, Stephanie Sisley⁵, Xiangping Lin⁶, Scott M. Bartell⁷, Thomas H. Inge², Stavra Xanthakos⁸, Brooklynn McNeil⁹, Anna R. Robuck¹⁰, Catherine E. Mullins¹¹, Sandrah P. Eckel¹, Rob S. McConnell¹, Michele A. La Merrill¹², Douglas I. Walker¹¹, David V. Conti¹, Lida Chatzi¹

Affiliations:

1. Department of Population and Public Health Sciences, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA
2. Department of Surgery, Northwestern University Feinberg School of Medicine and Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago, IL, USA
3. Department of Environmental Medicine and Climate Science, Icahn School of Medicine at Mount Sinai, New York, NY, USA
4. Division of Biostatistics & Epidemiology, Cincinnati Children's Hospital Medical Center, Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, OH, USA
5. USDA/ARS Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine, Houston, TX, USA
6. Department of Genetics, Stanford University School of Medicine, Stanford, CA, USA
7. Department of Environmental and Occupational Health, Department of Epidemiology and Biostatistics, and Department of Statistics, University of California, Irvine, CA, USA
8. Division of Gastroenterology, Hepatology, Nutrition, Cincinnati Children's Hospital Medical Center, Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, OH, USA
9. Irving Institute for Clinical and Translational Research, Columbia University, New York, NY, USA
10. Graduate School of Oceanography, University of Rhode Island, Narragansett, RI, USA

11. Gangarosa Department of Environmental Health, Emory University, Atlanta, GA, USA

12. Department of Environmental Toxicology, University of California, Davis, CA, USA

CONTACT INFO

Corresponding author: Dr. Zhenjiang Li, Department of Population and Public Health Sciences, Keck School of Medicine, University of Southern California, 2001 N. Soto Street, Los Angeles, CA 90032, USA. Email: lizhenji@usc.edu

Conflicts of interest

Dr. Chatzi has been financially compensated for her services as an expert consultant for plaintiffs in litigation related to PFAS-contaminated drinking water. Dr. Ryder receives support from Boehringer Ingelheim Pharmaceuticals in the form of drug/placebo and serves on an advisory board for Calorify. Dr. Inge has received consulting fees from Standard Bariatrics, Teleflex, Medtronic, Mediflix, Independent Medical Expert Consulting Services, and royalties from Wolters Kluwer (UpToDate) all unrelated to this project.

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Abstract

Background: Bariatric surgery is an intervention for severe obesity, leading to significant weight loss and metabolic improvements. However, the release of lipophilic chemicals accumulated in adipose tissue during weight loss presents a unique clinical challenge and research opportunity. Dichlorodiphenyldichloroethylene (DDE) is a persistent organic pollutant increasingly recognized as obesogen, while the biological mechanisms through which DDE influences body mass index (BMI) and waist circumference remain underexplored.

Objectives: We aimed to identify metabolic signatures mediating the association between DDE exposure and weight loss by plasma and adipose tissue metabolomics.

Methods: We conducted a longitudinal study involving 60 adolescents with severe obesity undergoing bariatric surgery. We quantified *p,p'*-DDE concentrations in visceral adipose tissue collected during surgery and analyzed metabolic profiles from both adipose tissues collected at surgery and plasma samples collected at surgery, 6 months, 1 year, and 3 years postoperatively, using gas/liquid chromatography and high-resolution mass spectrometry. We used meet-in-the-middle approach coupled with a series of metabolome-wide association analyses to identify overlapping metabolite associated with both DDE exposure and weight loss outcomes.

Results: Our findings revealed that DDE concentrations in visceral adipose tissue are associated with significant alterations in metabolites locally in adipose tissue pre-surgery and systemically in plasma post-surgery. Eight plasma metabolites were significantly associated with DDE exposure over time, while 27 were associated with BMI. Notably, methylbutyryl-carnitine was identified as a potential mediator, showing a positive association with both exposure and outcome. Eight adipose tissue metabolites were associated with both DDE and longitudinal changes in plasma metabolites, suggesting an indirect pathway linking DDE and weight changes.

Discussion: These findings enhance our understanding of DDE's obesogenic effect. The involved metabolic changes could attenuate the efficacy of bariatric surgery in achieving sustained weight loss, underscoring the importance of integrating environmental health considerations into clinical practice.

ACCEPTED MANUSCRIPT

Introduction

Childhood obesity in the United States (U.S.) remains an epidemic, with its prevalence tripling over the past three decades.¹ As of 2024, approximately 1 in 5 U.S. youths aged 2-19 years were affected by obesity,² and nearly half of adolescents aged 12-19 are projected to have obesity by 2030.³ Childhood obesity has been associated with a variety of physical and mental health problems during both childhood and adulthood, such as diabetes and cardiovascular disease.⁴ Researchers have developed an expanding array of intervention strategies to treat obesity and then prevent the onset of complications.⁵ Bariatric surgery is an effective and well tolerated intervention approach for adolescents with severe obesity.⁶ It encompasses a wide variety of surgical procedures, with three most commonly performed being laparoscopic adjustable gastric banding, Roux-en-Y gastric by-pass (RYGB), and vertical sleeve gastrectomy (VSG).⁶ These procedures reduce food intake and/or induce malabsorption, leading to significant weight loss and improvements in metabolic health compared to conventional non-surgical treatments.^{7, 8} However, the weight loss would result in a release of lipophilic chemicals accumulated in adipose tissue into circulation, which poses a unique clinical challenge.⁹ The redistribution of lipophilic chemicals might explain the variation in bariatric surgery effects among adolescents and provides a research opportunity to study the effects of lipophilic chemicals on weight trajectory.^{9, 10}

Dichloro-diphenyl-trichloroethane (DDT) emerged in the 1940s as one of the first synthetic insecticides and was extensively used in agriculture and vector-borne disease control worldwide for about three decades.¹¹ Although the use of DDT has been largely phased out,^{12, 13} DDT persists in the environment for long periods and is found worldwide due to its high resistance to environmental degradation and lipophilicity.^{11, 12} DDT is primarily transformed to dichloro-diphenyldichloro-ethylene (DDE) with no further conversion to a water-soluble and excretable form.¹⁴ Even though current use of DDT has drastically decreased, DDE can be detected in water,¹⁵ marine organisms,¹⁶ agricultural products,^{17, 18, 19} and human tissues worldwide,

including human milk,²⁰ blood,²¹ and adipose tissue.²² Moreover, DDE has a long half-life in human body (6.2-8.6 years),²³ and, therefore, people who had been exposed to these chemicals still had high concentrations detectable in their bodies long after the exposure ended.^{24, 25} The lipophilic nature of DDE also makes adipose tissue a major reservoir and suitable matrix for biomonitoring long-term cumulative internal exposures.²²

Over the past decade, there has been a growing research interest in the obesogenic effect of DDT/DDE among children and adolescents.^{26, 27} Stratakis et al. performed a systematic review and identified 24 epidemiological studies investigating the association between prenatal exposure to DDE and childhood obesity outcomes of interest.²⁷ Eight studies, primarily conducted in Europe, reported a significantly positive association of prenatal exposure to DDE with obesity outcomes [e.g., body mass index (BMI) and waist circumference] among individuals aged 2-19, while another seven reported null associations.²⁷ The body of evidence was considered to provide moderate support for a causal relationship between DDE exposure and increased adiposity in human, while relying primarily on plasma DDE concentrations.²⁶ In our previous work, we found that higher DDE exposure in visceral adipose tissue was associated with less improvement in BMI and waist circumference among adolescents with obesity after bariatric surgery, and the analysis showed a dose-response relationship, with the low exposure group experiencing a greater reduction in BMI during the first year post-surgery as well as less weight regain in subsequent years.⁹ However, the mechanisms underlying DDE obesogenic activity are poorly understood. Understanding the biological mechanisms underlying DDT/DDE effects may provide a model that will help identify other environmental obesogens and reveal potential therapeutic targets which might mitigate their adverse impacts, complementing traditional interventions focusing on diet and physical activity.

To fill the knowledge gap, we have completed an integrative meet-in-the-middle approach via adipose tissue and plasma metabolomics. The integrative approach evaluated both the local and systemic effects of DDE exposure among an adolescent population with severe obesity

who had undergone the bariatric surgery (Figure 1). We measured *p,p'*-DDE and metabolic profiles in visceral adipose tissue collected at the surgery, while plasma metabolic profiles and weight loss outcomes were measured repeatedly before and after bariatric surgery. Depending on the structure of the metabolic profiles, we performed a series of metabolomics-wide association studies (MWAS) cross-sectionally and longitudinally to examine the associations among DDE exposure, adipose tissue metabolic profile, and plasma metabolic profile. The integrative approach linked metabolic changes in adipose tissue, which is the major accumulation site of DDE,²² to longitudinal post-surgery alterations observed in the body's overall metabolic profile. The primary aim was to identify metabolic signatures mediating the association between DDE exposure and weight loss.

Materials and Methods

Study population

The Teen-Longitudinal Assessment of Bariatric Surgery (Teen-LABS) consortium is a prospective, multi-center, observational cohort, which evaluates the efficacy and safety of bariatric surgery among adolescents in the USA has been described previously.¹⁰ Briefly, the study enrolled 242 adolescents aged 13 to 19 years between 2007 and 2012, who underwent bariatric surgery at five clinical centers in major metropolitan areas across the United States. Four centers were free-standing pediatric hospitals with dedicated adolescent-only weight loss surgery (WLS) programs, while one was an adult tertiary facility with an integrated adolescent program.²⁸ The eligibility criteria for surgery included severe obesity (BMI ≥ 40 kg/m² with comorbidity or ≥ 35 kg/m² with serious comorbidity) and comprehensive preoperative evaluation.²⁸ Participants were interviewed by trained medical personnel at enrollment (≤ 30 days pre-surgery, we also refer to this timepoint as “at surgery” or “baseline” throughout the paper) and followed up at 0.5 years, 1 year, and 3 years after surgery, following a standardized protocol. Fasting plasma samples were collected at surgery and at each follow-up visit. Visceral adipose tissue was collected in a subset of patients during surgery. We analyzed data from 60 participants with both visceral adipose tissues and repeated plasma available from two medical centers (Cincinnati Children’s Hospital Medical Center and Baylor College of Medicine). Two primary procedures, RYGB (N=42) and VSG (N=18), were performed among these included participants. Ethics approval for this study was provided by the University of Southern California Institutional Review Board (IRB protocols HS-19-00057). Prior to participation, written informed assent/consent were obtained from participants and their guardians.

Assessment of p,p' -DDE exposure

Visceral adipose tissues were frozen in liquid nitrogen and stored at -80°C until chemical analysis. As p,p' -DDT was the main component of DDT and primarily converted to p,p' -DDE in

human body,^{11, 14} we quantified its concentration using gas spectrometry coupled with ultra-high-resolution mass spectrometry (GC-UHRMS) that allowed quantification using gold-standard isotope dilution methods. The limit of detection (LOD) for *p,p'*-DDE was established as 0.10 ng/g. None of the samples included in this study had *p,p'*-DDE concentrations below the LOD. We also measured other DDT isomers, including *o,p'*-DDE and *o,p'*-DDT, but they were excluded as their levels were below the LOD in all adipose tissue samples. Throughout the Methods and Results sections, the term “DDE” specifically refers to *p,p'*-DDE. No *o,p'*-DDE was analyzed in this study. Furthermore, we categorized the continuous exposure variable by tertiles (high vs. moderate vs. low exposure groups) based on its distribution in our samples and treated the categorized exposure variable as a three-level continuous variable in all models. It is important to note that the DDE concentrations observed in our study are relatively low compared to some previous studies, likely due to the ban on its use in the 1970s as well as the younger age of our study population.¹³ The “high exposure” group defined here is not truly high when compared to concentrations reported in earlier studies;²⁹ rather we used the term “high” for ease of interpretation and relative comparison within our cohort. A detailed description of the quantification method can be found elsewhere.⁹ Briefly, approximately 0.15g of tissue was weighed, placed in plastic vial containing 10 glass beads and treated with 3.5mL of liquid chromatography-mass spectrometry (LC-MS) grade water. Each sample was spiked with 12.5 μ L of 2-propanol solution containing 100 ng/mL ¹³C-labelled internal standards and then homogenized using six cycles of 15-sec on/15-sec off in a BioSpec Mini-Beadbeater-24 maintained at 4°C. The homogenate was sonicated for 10 min in a sonicating bath and then transferred to a 50 mL conical tube containing 10mL acetonitrile, 4000mg MgSO₄ and 1000mg NaCl, and vortexed briefly immediately after. The original homogenate tube was rinsed with another 3.5 mL of water, vortexed and added to 50mL vial with extraction salts. The mixture was vortexed for 5 min and then centrifuged for 5 mins at 3,900 rpm. The resulting supernatant of 8 mL was transferred to a clean, 15 mL tube, and stored overnight

at -20°C to precipitate remaining lipids. Following overnight storage, the tubes were centrifuged for 5 min at 4°C and a 4.5mL aliquot of supernatant was then transferred to a 15mL tube containing 300 mg primary and secondary amine exchange material (PSA), 300 mg C18 and 900 mg MgSO₄. The extract and sorbents were vortex-mixed for 5 min and centrifuged for 5 min. Following cleanup, 2.5ml of supernatant was evaporated and solvent exchanged to toluene under a gentle stream of N₂ to 150μL. The extract was then transferred to a GC vial containing a low volume insert and stored at -20°C until analysis. Tissue extracts were analyzed using a Thermo Scientific 1310 GC connected to a Q Exactive GC Orbitrap GC-MS/MS UHRMS and Triplus RSH autosampler. A 2 μL aliquot of extract was injected into an inlet maintained at 250°C in pulsed split-less mode. The analytes were separated on an Agilent DB-5MSUI capillary column (30m length × 0.25mm inner diameter × 0.25μm film thickness) using high purity helium (99.999% purity) as the carrier gas at a constant flow rate of 1 mL/min. The oven temperature program consisted of an initial temperature of 100°C for 1 min, increased to 180°C at 25°C/min; followed by a temperature ramp to 215°C at 5°C/min, and finally increased to 300°C at 25°C/min and held for 10 min, resulting in a total run time of 26.6 min. Mass spectral data were obtained using an electron ionization source, operated at a source temperature of 250°C, electron impact energy of -70 eV and transfer line temperature maintained at 280°C. The UHRMS was operated in full-scan mode over mass-to-charge (m/z) range 85-850 and 60,000 resolution. DDE concentrations were determined using an isotope dilution approach in combination with a five-point calibration curve. Only peaks within the analyte retention time +/- 0.2 minutes were considered for quantification. The method detection limit (MDL) was determined based on the average concentration detected in process blanks or the lowest curve point with a signal to noise ratio exceeding 10; the higher value was selected for the MDL.

Assessment of plasma metabolome

We measured untargeted metabolite profiles in fasting plasma samples collected at enrollment, 0.5 years, 1 year, and 3 years after surgery from the 60 included participants. Due to incomplete plasma availability, we analyzed a total of 197 samples. No significant differences were observed between participants with complete plasma sample availability and those with partially missing samples in terms of key demographic variables (age, sex, race, and parental income) or baseline BMI distribution (Table S1), suggesting minimal risk of selection bias. The sample preparation and feature extraction followed established protocols.³⁰ Briefly, biological samples were stored at -80°C. They were then randomized into 10 batches, and in each batch, we added up to 12 pooled human plasma samples and the National Institute of Standards and Technology (NIST) 1950 standard reference samples for quality control. Each batch was analyzed on the same day to minimize technical variation. The thawed plasma underwent a series of sample preparation, performed as described by Goodrich et al.³⁰ Then, we analyzed the samples by liquid chromatography and high-resolution mass spectrometry (LC-HRMS, Thermo Vanquish Duo with Q-Exactive HFX) with dual column and dual polarity approaches. To maximize detection of metabolites, we employed four analytical configurations for each sample, including: reverse-phase C18 liquid chromatography with negative electrospray ionization (ESI-), C18 with ESI+, hydrophilic interaction liquid chromatography (HILIC) with ESI-, HILIC with ESI+. The detected metabolite profiles were extracted using *apLCMS* with modifications by *xMSanalyzer*.^{31, 32} The extracted metabolites were defined by mass-to-charge ratio (m/z), retention time as identifiers and ion intensity. Prior to downstream analysis, we adjusted for potential batch variation among metabolites.³³ We further excluded features with >80% of missing values, imputed remaining missing values as the half of feature-wise minimum, and removed features where QC samples have >30% coefficient of variance. The final metabolomic data contained 4,195; 5,141; 7,894; and 7,145 metabolite features under C18 with ESI-, C18 with ESI+, HILIC with ESI-, and HILIC with ESI+, respectively. Details of the analytical process

can be found elsewhere.³⁰ Confirmed metabolites were determined by matching accurate m/z and retention time to an in-house metabolite database, resulting in 569 confirmed metabolites. Specifically, the in-house database was created by analyzing authentic chemical references under the same experimental conditions. For the significant metabolite features of interest that were not included in the in-house database, we searched the online Human Metabolome Database (HMDB) Mass Spectrum database by their m/z and reported annotation matches with the corresponding adduct form.³⁴ Annotated metabolites were considered to be confidence level 4.³⁵

Assessment of adipose tissue metabolome

Adipose tissue samples were prepared and analyzed in one batch with repeated QC samples, following a similar workflow to plasma metabolome. Prior to analysis, adipose tissues were thawed at 4°C, and up to 30mg of tissue was treated with 5 μ L acetonitrile containing ^{13}C labeled internal standards (ISs) per mg tissue and homogenized in a bead beater. Homogenized samples were vortexed for 2 minutes, equilibrated at 4°C for 30 min, and then centrifuged for 45 min at 3,220 $\times$ g, at 4°C. Two aliquots of supernatant (30 μ L) were then transferred to vials containing 60 μ L water (for the C18 column) or 60 μ L 1:1 acetonitrile/water (for the HILIC column). These were vortexed 2 minutes and placed in a refrigerated autosampler until analysis. We accomplished untargeted analysis using two separate systems configured for C18 or HILIC analysis that included a Vanquish Duo Ultra Performance Liquid Chromatography (Thermo Fisher Scientific, Rockford, IL, USA) coupled to an Exploris120 HRMS system (Thermo Fisher Scientific, Rockford, IL, USA). Liquid chromatography column temperatures were set to 40°C (HILIC) and 30°C (C18), and the automatic sampler temperature set at 5°C. We analyzed samples using four analytical configurations for each sample: C18 with ESI-, C18 with ESI+, HILIC with ESI-, HILIC with ESI+. The metabolic profiles were extracted in the same way as plasma metabolic profiles. Prior to downstream analysis, we adjusted for potential within-batch

variation by dividing the samples into two roughly equal portions.³³ We further excluded features with >80% of missing values, imputed remaining missing values as the half of feature-wise minimum, and removed features where QC samples have >30% coefficient of variance. The final metabolomic data contained 3,619; 4,824; 7,615; and 5,292 metabolite features under C18 with ESI-, C18 with ESI+, HILIC with ESI-, and HILIC with ESI+, respectively. Metabolites were identified by comparison of m/z, retention time, and ion dissociation patterns to authentic chemical reference standards analyzed using the identical method and instrument parameters via tandem mass spectrometry (MS/MS). For metabolites without available reference standards, putative annotation was conducted by matching accurate mass against publicly available spectral libraries.³⁴ We obtained 284 confirmed metabolites.

Assessment of adipose tissue transcriptome

Total RNA was extracted from adipose tissues using the RNeasy Lipid Tissue Mini Kit (Cat. No. 74804, Qiagen, Germantown, MD) according to the manufacturer's instructions. The detailed process of RNA sequencing could be found elsewhere.³⁶ Briefly, gene expression profiling was carried out using a 3'-Tag-RNA-Seq protocol. Barcoded sequencing libraries were prepared from total RNA of each sample using the QuantSeq FWD kit (Lexogen, Vienna, Austria) for multiplexed sequencing according to the recommendations of the manufacturer using the UMI Second-Strand Synthesis modules (Lexogen). The profiles were preprocessed using HTStream (version 1.3.3) and aligned to GRCh38 reference genome using STAR (version 2.7.9a).³⁷ Unique molecular identifier (UMI) deduplication was performed with UMI-tools (version 1.0.1).³⁸ Gene-level read counts were obtained using featureCounts (Subread version 1.6.3).³⁹ After filtering out minimally expressed genes [those with count-per-million (CPM) < 1 among more than 80% of the study population] and genes on Chromosome X and Y, we retained 13,625 probes. We further performed the trimmed mean of M-values normalization method to eliminate composition biases.⁴⁰ We further performed the trimmed mean of M-values normalization

method to eliminate composition biases. The preprocessing of count data was performed via the R package edgeR (version 4.0.16).⁴¹

Assessment of clinical outcomes

We used body mass index (BMI, kg/m²) and waist circumference (cm) as outcome measures to evaluate adiposity change following bariatric surgery. Data collection occurred during the enrollment and three follow-up visits (0.5, 1, and 3 years) after surgery. Body weight (kg) and height (m) data were collected using a calibrated stadiometer and an electronic scale (TBF-310; Tanita, Tokyo, Japan), and BMI was calculated by dividing the weight in kilograms by the squared height in meters. Waist circumference was measured at the midpoint between the lower rib margin and the iliac crest. BMI and waist circumference were treated as continuous variables in the present study.

Assessment of covariates

We selected the covariates based on previous literature and constructed a directed acyclic graph to visualize paths for adjustment of confounding (Figure S1). We derived participant characteristics from the Teen-LABS study, including sex (male vs. female), race (White vs. others), age at baseline (years), parents' annual income level (less than \$25,000; \$25,000 to \$74,999; \$75,000 or more). Additional methodological details are available online.⁴² Race was self-reported, with participants instructed to mark all that apply. Response options included: White or Caucasian, Black or African American, Asian, American Indian or Alaska Native, Native Hawaiian or Other Pacific Islander, Other, and Unknown. Given the limited sample size for non-White participants, we re-categorized race as White vs. Others for statistical modeling. Race was included as a confounder based on its potential association with both exposure (DDE levels) and outcome (weight change), as supported by Figure S1. We considered parents' annual income level as a proxy for socioeconomic status of the participants. Time since surgery

was treated as a continuous variable in years (i.e., 0, 0.5, 1, and 3 years after surgery). Medical center (Cincinnati Children's Hospital Medical Center vs. Baylor College of Medicine) was included as a binary variable to control for potential selection and information bias. Total energy intake in kcal/day was estimated by the Minnesota Nutrition Data System at the baseline, 0.5 years, and 1 year after surgery, and was treated as a surrogate for the dietary intake.⁴³ The variable was only used for a sensitivity analysis to assess the potential impact of dietary intake on the associations of interest. Though total energy intake information was only available for a subset of 20 participants out of the study participants, we compared demographic characteristics between participants with and without dietary data to evaluate the potential risk of bias.

Statistical analysis

To identify the local and systemic mechanisms underlying the association between DDE exposure and weight loss, we leveraged plasma metabolomics, adipose tissue metabolomics, and adipose tissue transcriptomics through a series of analyses (Figure 1): 1) the longitudinal metabolome-wide association study (MWAS) of DDE exposure with plasma metabolomics; 2) the longitudinal MWAS of plasma metabolomics with BMI and waist circumference; 3) the cross-sectional MWAS of DDE exposure with adipose tissue metabolomics; 4) the longitudinal MWAS of selected adipose tissue metabolites (independent) with plasma metabolomics (dependent); and 5) the target 249 gene set analysis for DDE exposure cross-sectionally based on adipose tissue RNA sequencing data. We further adopted a framework of the meet-in-the-middle approach by comparing the results of the Analysis 1) and 2),⁴⁴ in order to identify the overlapping metabolites (i.e., systemic metabolic signatures) that were significantly associated with both DDE exposure and weight loss outcomes. The purpose of the meet-in-the-middle approach was to empirically discover potential mediators between DDE exposure and weight change. Then, analysis 3) and 4) were combined via the same method to identify the local

metabolic signatures potentially mediating the association between DDE exposure and systemic metabolic signatures. The targeted genes set analysis served as a compliment to provide a more comprehensive picture of biological pathways involved in the local impact of DDE exposure.

For Analysis 1), we developed a linear mixed-effect model with a random intercept by participants to evaluate the association of DDE exposure with longitudinal metabolic measures after bariatric surgery [Eq. (1)]. We treated the DDE exposure as a 3-level continuous variable defined by tertiles in the model. The temporal trend of metabolite features was allowed to vary by DDE exposure through the inclusion of an interaction term between p,p' -DDE tertile and time since surgery. Because we observed a steep decline in BMI up to 1 year after bariatric surgery, followed by a small increase in subsequent years (Figure S2), the model also included a piecewise linear spline on time since surgery with a single knot at the 1-year timepoint. This pattern might be explained by lipid turnover at the 1-year timepoint, affecting the temporal trend of metabolite features and requiring a nonlinear model to obtain a more accurate profile of metabolic signature associated with DDE exposure. This modeling approach allows for different slopes before and after the one-year mark, effectively capturing the effect of DDE during both pre- and post-knot periods. Accordingly, we reported the associations between DDE exposure and the metabolic signature for the 1st year (β_3) and 1st to 3rd years (β_5) after surgery, respectively.

$$\log(Z_{ij}) = \beta_0 + \mu_i + \beta_1 DDE_i + (\beta_2 + \beta_3 DDE_i) \times time_j + (\beta_4 + \beta_5 DDE_i) \times (time_j - 1) + \beta_6 age_i + \beta_7 sex_i + \beta_8 race_i + \beta_9 income_i + \beta_{10} site_i + \epsilon_{ij} \quad \text{Eq. (1)}$$

where $\log(Z_{ij})$ refers to the log-transformed relative intensity in plasma of the metabolite feature for individual i at time j since surgery; β_1 is the effect estimates for DDE exposure at baseline; $time_j$ is the time since surgery; β_3 and β_5 are the effect estimates which quantifies the moderated temporal change in metabolite features by DDE levels. We tested the significance of

the association between DDE exposure and changes in plasma metabolite features over time with two strategies: 1) a partial F test for all interaction terms; 2) significance of each coefficient for the two interaction terms in Eq. (1). We used Benjamini-Hochberg procedure to adjust for multiple comparison correction due to the high dimensionality of metabolomics data.⁴⁵ The significance threshold was set at q -value < 0.05 (i.e., adjusted p -values).

For Analysis 2), we developed a similar linear mixed-effect model [Eq. (2)] with metabolite features as the independent variable. Eq. (2) was performed for the outcomes (Y) of BMI and waist circumference, separately. We adjusted for the following covariates in Eq. (2): age at baseline, sex assigned at birth, race, parental income, and site of medical center. We adjusted for DDE exposure and its interaction with time since surgery in Eq. (2) additionally to block the potential direct effect of DDE exposure on the outcomes (i.e., BMI and waist circumference).

The forms of the models were:

$$Y_{ij} = \theta_0 + \psi_i + \theta_1 \log(Z_{ij}) + \theta_2 time_j + \theta_3 (time_j - 1) + \theta_4 DDE_i + \theta_5 DDE_i \times time_j + \theta_6 DDE_i \times (time_j - 1) + \theta_7 age_i + \theta_8 sex_i + \theta_9 race_i + \theta_{10} income_i + \theta_{11} site_i + \omega_{ij} \quad \text{Eq. (2)}$$

where Y_{ij} refers to BMI or waist circumference for individual i at time j since surgery; θ_1 is the effect estimate associated with a one-unit increase in $\log(Z_{ij})$. The significant metabolite features associated with BMI or waist circumference were determined at a q -value of 0.05.

For Analysis 3), we performed a cross-sectional analysis via multiple linear regression as shown in Eq. (3). We included log-transformed intensity of metabolites extracted from the adipose tissue as dependent variable and adjusted for age at baseline, sex assigned at birth, race, parental income, and site of medical center.

$$\log(Z_i^{(adipose)}) = \delta_0 + \delta_1 DDE_i + \delta_2 age_i + \delta_3 sex_i + \delta_4 race_i + \delta_5 income_i + \delta_6 site_i + \epsilon_i \quad \text{Eq. (3)}$$

For Analysis 4), we created a similar linear mixed-effect model as Analysis 1) with the selected metabolites in adipose tissue as independent variables and the plasma metabolite features as dependent variables. The metabolites in adipose tissue were selected based on the

significance of their associations with DDE exposure. We focused on the 569 confirmed metabolites in plasma as dependent variables. Similarly, we reported the associations between adipose tissue metabolites and plasma metabolites for the 1st year and 1st to 3rd years after surgery, respectively. We tested the significance of the association between adipose tissue metabolites and plasma metabolites over time with the same strategy as Analysis 2). The forms of the models were:

$$\log(Z_{ij}) = \gamma_0 + \varphi_i + \gamma_1 \log(Z_i^{(adipose)}) + [\gamma_2 + \gamma_3 \log(Z_i^{(adipose)})] \times time_j + [\beta_4 + \gamma_5 \log(Z_i^{(adipose)})] \times (time_j - 1) + \beta_6 age_i + \beta_7 sex_i + \beta_8 race_i + \beta_9 income_i + \gamma_{10} site_i + \epsilon_{ij} \quad \text{Eq. (4)}$$

For Analysis 5), we evaluated the impact of DDE exposure on a predefined set of genes and applied a multiple linear model to test for differentially expressed genes via the R package *limma*.⁴⁶ The predefined list of genes for transcriptomic analysis was selected based on the results of the adipose tissue metabolome and plasma metabolome analyses performed so far. We first transformed the read counts of RNA sequencing data into log-transformed CPM while taking into account the mean-variance relationship by *voom*.⁴⁷ The *voom* function in R assesses the mean-variance relationship of log-transformed counts, assigns a precision weight to each observation, and integrates these weights into the *limma* empirical Bayes analysis.⁴⁷ We fitted a linear model for each gene as the dependent variable and adjusted for the same covariates as Analysis 3). Then, we applied the empirical Bayes shrinkage on the gene-wise variances, which estimated the moderated t-statistics and the associated *p*-values. The gene set was selected based on the aforementioned MWAS results with reference to the KEGG pathway database.⁴⁸

Additionally, we visualized untargeted plasma MWAS results using Manhattan plots in the Supplementary Materials (Figure S3 & S4) which plotted the retention time of each metabolite feature on the x-axis against the $-\log_{10}(p\text{-values})$ on the y-axis. We conducted pathway enrichment analysis from the results from Analysis 1) and 2) using metabolite features meeting the significance criterion (*q*-value (derived from the joint significance test) < 0.05) using

mummichog (version 2.6.1) in Python (version 3.8.0), a bioinformatics platform that infers and categorizes functional biological activity directly from mass spectrometry output.⁴⁹ We conducted pathway enrichment analyses separately for four analytical configurations: C18 with ESI-, C18 with ESI+, HILIC with ESI-, and HILIC with ESI+. A p -value for each pathway was computed by applying a penalty to pathways containing fewer significant metabolite features and ascribing higher significance to pathways with a greater number of significant features. We extracted pathways with p -value < 0.05 with at least two significant features enriched to alleviate possibility of false positive findings. Similarly, we detected the biological pathways that were enriched by significant metabolite features associated with weight loss via mummichog as well.

We also performed a series of sensitivity analyses. First, as shown in Figure S1A, baseline BMI might serve as a potential confounding factor, because adipose tissue is considered an accumulation site of lipophilic toxicants, such as DDE, and previous studies reported an explanatory potential of BMI for the adipose tissue concentrations of organochlorine pollutants.^{50, 51} However, DDE was demonstrated to have an obesogenic effect by a variety of human and animal studies.²⁶ Thus, to assess the robustness of the Eq. (1) results against the baseline BMI, we included it as an additional covariate in the linear mixed-effect model and conducted the same significance tests. Second, the dietary intake could impact both metabolic profiles and body weight,⁵² which potentially confounded the association between metabolite features and weight loss (Figure S1B). To assess the impact of dietary intake, we included the total energy intake in Eq. (2). As the data on the total energy intake was available at the baseline, 0.5 years and 1 year after surgery for a subset of 20 participants, we performed Eq. (2) with and without the total energy intake on the subset and compared the significant metabolites. To offer insights into the generalizability of this sensitivity analysis, we compared the demographics between the entire study population and the subset. Third, to ensure the reliability of treating the categorical 3-level variable as continuous, we performed a sensitivity analysis using Eq. (1) with a categorical 3-level DDE variable as the exposure and BMI as the

outcome. Fourth, since three participants provided plasma samples at only a single timepoint, we conducted a sensitivity analysis to ensure that results were not driven by these samples not contributing any longitudinal information by excluding them from Eq. (1). Fifth, to further assess the potential selection bias due to the availability of biospecimens, we compared the demographics of the 60 participants included in the present analysis to the 160 participants not included from the Teen-LABS cohort. Sixth, to assess the robustness of the results against potential confounding effects of surgical procedure, we compared demographic characteristics and BMI between participants undergoing RYGB and VSG.

We performed all analyses in R (version 4.3.0), except for the pathway enrichment analysis using Python (version 3.8.0).

Results

The present study included 60 participants from the Teen-LABS cohort with available visceral adipose tissue metabolomics data at baseline and 197 plasma samples with metabolomics data at baseline, 0.5, 1, and 3 years post-surgery. Among the 60 participants, everyone provided plasma sample at baseline. Thirty participants provided plasma samples at all four time points, 20 provided plasma samples at three time points (2 of them missing at 0.5 years, 8 missing at 1 years, and 10 missing at 3 years post-surgery), 7 provided plasma samples at two points (6 missing at 0.5 years, 5 missing at 1 years, and 3 missing at 3 years post-surgery), and 3 provided plasma samples at baseline only. To assess potential selection bias, we compared demographic variables and baseline BMI between participants with complete plasma sample availability and those with partially missing samples. No significant differences were observed between the groups in terms of age, sex, race, parental income, or baseline BMI distribution (Table S1).

The study cohort had a mean age of 16.8 years (range: 13.2–19.4 years), with 73.3% female participants. *p,p'*-DDE concentrations ranged from 0.1 to 55.2 ng/g, with a median of 16.7 ng/g; exposure was categorized into low (0.1–13.6 ng/g), moderate (13.6–18.4 ng/g), and high (18.4–55.2 ng/g) groups based on tertiles (Table 1). The low exposure group had the highest proportion of white participants (75.0%), and 58% of participants in this group had household incomes below \$25,000. There was no significant correlation between income and DDE exposure. The high exposure group exhibited a 28.3% coefficient of variation (CV) in *p,p'*-DDE levels, while the moderate exposure group had a CV of 8.3%, and the low exposure group had a CV of 30.6%. All participants had obesity at baseline, with mean BMI ranging from 53.1 to 54.3 kg/m² and waist circumference from 141 to 145 cm. Longitudinal BMI changes are shown in Figure S2, showing large variation within each exposure groups. As a summary, the low exposure group exhibited CVs in BMI across different timepoints ranging from 16.8% to 27.8%.

The moderate exposure group showed CVs ranging from 20.4% to 26.5%, and the high exposure group showed CVs from 17.7% to 27.4%.

Adipose tissue DDE concentrations and longitudinal alterations in plasma metabolomics

Metabolome-wide association analysis

We used linear mixed-effects models with random intercepts to assess the impact of adipose tissue DDE concentrations on metabolic changes following bariatric surgery, incorporating interaction terms for DDE exposure and time since surgery. Time since surgery was divided into two periods (0-1 year and 1-3 years) using a piecewise linear spline with a knot at 1 year. Metabolite features were considered significant if they met any of the following criteria: 1) a significant partial F test for all interaction terms between DDE exposure and time since surgery; 2) significance of each coefficient for the interaction terms in Eq. (1). The significance threshold was set at $q\text{-value} < 0.05$ (i.e., adjusted $p\text{-values}$). We found 268 out of 24,375 metabolite features significantly altered by DDE exposure (Table 2), categorized by detection method (e.g., 7 from C18 ESI-, 27 from C18 ESI+, 47 from HILIC ESI-, 187 from HILIC ESI+). Full details can be found in the Excel Table S1-4. Notably, higher DDE exposure was linked to increased intensity in 108 features during the first year post-surgery and decreased intensity in 32 features across both time periods. Eight metabolites, including mevalolactone and thyroxine (inverse association) and methylbutyryl-carnitine, indole-3-methyl acetate, lactate, orotate, tryptophan, and undecylenic acid (positive association) were significantly associated with DDE levels and time since surgery (Figure 2, Excel Table 5).

We further examined the relationship between these metabolites and outcomes such as BMI and waist circumference over 3 years. We identified 27 metabolites associated with BMI (Figure 3, Excel Table S6), 21 of which were also linked to waist circumference (Figure S5, Excel Table S7). These included amino acids, fatty acids, nucleotide derivatives, and acylcarnitines, with most showing positive associations with BMI and waist circumference. Specifically, 96, 52, 92,

and 184 metabolite features correlated with BMI, and 52, 57, 52, and 166 with waist circumference, with considerable overlap between the two outcomes (Figure 3 & Figure S5).

Pathway enrichment analysis

Pathway enrichment analysis identified five biological pathways significantly associated with DDE exposure levels, including tyrosine and tryptophan metabolism, hexose phosphorylation, and fructose and mannose metabolism (Figure 4A). Tyrosine metabolism showed the highest enrichment of metabolites linked to DDE, BMI, and waist circumference (Figure 4B & Figure S5). Notably, tyrosine metabolism was the only pathway associated with both DDE exposure and weight loss outcomes, while tryptophan metabolism, which is interconnected with tyrosine biosynthesis, was uniquely associated with DDE exposure.

Meet-in-the-middle approach

We examined the relationship between DDE exposure, confirmed metabolites, and BMI outcomes post-bariatric surgery. Among the eight metabolites associated with DDE exposure and the 27 linked to BMI, only one, methylbutyryl-carnitine, was associated with both exposure and outcome. Notably, DDE exposure was positively correlated with an increase in plasma methylbutyryl-carnitine levels over time following bariatric surgery. Furthermore, methylbutyryl-carnitine showed a positive longitudinal association with BMI. This suggests that methylbutyryl-carnitine mediated a positive indirect effect of accumulated DDE exposure on BMI changes after bariatric surgery. Additionally, an increased plasma tryptophan level was observed with higher DDE exposure, particularly during the first year post-surgery. Plasma kynurenine, another metabolite in the tryptophan-kynurenine pathway, was positively associated with BMI and waist circumference. These findings highlight the potential role of the kynurenine pathway in mediating the relationship between DDE exposure and postoperative weight changes, suggesting a mechanistic link that warrants further investigation in clinical settings.

Sensitivity analysis

In the sensitivity analysis adjusting for baseline BMI, Indole-3-methyl acetate, lactate, mevalolactone, orotate, tryptophan, and undecylenic acid remained significantly associated with DDE exposure, while methylbutyryl-carnitine lost significance (Figure S6). Despite this, its model statistics showed minimal change post-adjustment. Adjustment for total energy intake had a negligible impact on the associations of metabolites with BMI (Table S2) and waist circumference (Table S3). Demographic comparisons between participants with and without total energy intake data revealed similar distributions in age, sex, race, and parental income (Table S4), indicating that the subset results are likely representative of the broader study population. We compared the model statistics of the metabolites presented in Figure 2 between the model with categorical DDE and the continuous 3-level model. The statistics are summarized in Table S5. This sensitivity analysis shows that the high exposure group had higher absolute values of coefficients than those of the moderate exposure group for the majority of metabolites presented in Figure 2, indicating dose-response relationships. After excluding the three participants with plasma samples provided at only a single timepoint, we found that the coefficients and joint significant tests of the metabolites presented in Figure 2 remained consistent, confirming the robustness of the analysis (Table S6). Comparison of demographic variables between the 60 participants included in the present analysis and the 160 participants not included from the Teen-LABS study revealed no significant differences, suggesting that the analyzed cohort is representative of the whole Teen-LABS cohort (Table S7). We observed no significant differences in age at baseline, sex, race, and parental income between participants receiving different surgical procedures (RYGB vs. VSG). Although baseline BMI was significantly associated with the type of surgery, the percent change in BMI at 3 years post-surgery was not significantly different. These results are summarized in Table S8. After adjusting for the site of the medical center, baseline BMI was not significantly associated with

surgical procedure, indicating that the site of medical center accounted for the procedural differences.

Adipose tissue DDE concentrations and cross-sectional alterations in adipose tissue metabolomics

Meet-in-the-middle approach

We focused on the confirmed metabolites present in adipose tissue to examine the potential local impact of DDE exposure on biological processes in adipose tissue. We aimed to identify the adipose tissue metabolites that led to systemic alterations (i.e., plasma metabolic signatures detected so far) associated with weight change after bariatric surgery. In other words, we hypothesized that the metabolic alterations occurring within adipose tissue due to DDE exposure may influence systemic metabolism, which would be reflected in the plasma metabolome, as illustrated in Figure 1. To test the hypothesis, we set up a meet-in-the-middle approach with two intermediates. The exposure is DDE, the outcome is weight loss, and the two intermediates are adipose tissue metabolites and plasma metabolites, sequentially. Among the 284 confirmed metabolites in adipose tissue, 19 were associated with DDE exposure levels at a significance threshold of raw $p < 0.05$, although these associations did not remain significant after adjusting for multiple comparisons (Table S9). We further investigated whether these 19 adipose tissue metabolites were also associated with longitudinal alterations in plasma metabolites that were associated with DDE exposure using Eq. (4). Specifically, Eq. (4) was a linear mixed-effect model with adipose tissue metabolites as independent variable and plasma metabolites as dependent variable. Notably (Table 3), eight out of the 19 adipose tissue metabolites were associated with longitudinal alterations in plasma metabolites. They were 5-hydroxytryptophan (5-HTP), acetoacetic acid, benzyl alcohol, galactosamine, glycochenodeoxycholic acid, ketoglutaric acid, lysine, and methylamylacetic acid. The 36 associated plasma metabolites comprised eight amino acids and derivatives, 13 fatty acids, one

nucleotide derivative, one acyl-carnitine, one hormone, and one bile acid. Among these, 5-HTP was linked to the highest number of plasma metabolites. Given its negative association with both DDE exposure levels and the majority of fatty acids, 5-HTP may offer a protective effect against DDE-induced increases in plasma fatty acid levels, potentially mitigating adverse metabolic outcomes in post-bariatric surgery patients.

Targeted gene expression analysis

Our plasma and adipose tissue metabolomics analyses highlighted tryptophan metabolism as a key pathway in DDE's effects on weight loss. We examined 42 genes involved in this pathway, quantifying 26 after quality control (Table S10). Notably, ACAT2 expression decreased by 20% per unit increase in adipose tissue *p,p'*-DDE, while ALDH3A2 expression increased by 10%, both with raw *p*-values < 0.05 (Table S11).

Discussion

This study investigated the impact of adipose tissue DDE accumulation on metabolic profiles in adolescents and its effect on weight loss post-bariatric surgery. In our previous publication, we demonstrated that higher DDE exposure in visceral adipose tissue was associated with more weight regain after bariatric surgery in the long term.⁹ In the present study, we found that this obesogenic effect could be mediated by plasma methylbutyryl-carnitine. Additionally, DDE exposure was associated with reduced adipose 5-HTP and negatively affected plasma tryptophan levels, influencing the kynurenine pathway, which is linked to obesity-related inflammation. These findings suggest that DDE exposure may impair weight loss post-surgery by disrupting local adipose tissue metabolism and systemic inflammatory pathways.

Methylbutyryl-carnitine is a type of branched-chain acylcarnitine which arise from the conjugation of fatty acids with L-carnitine and function in fatty acid oxidation.⁵³ Weight loss intervention, such as dietary change and exercise,^{54, 55} was observed to be associated with a decrease in blood acylcarnitines. A systematic review on the metabolomic signatures after bariatric surgery also demonstrated that acylcarnitines derived from the branched-chain amino acids catabolism decreased after bariatric surgery.⁵⁶ Moreover, among a healthy pediatric population, Hirschel et al. reported a positive association between acylcarnitines in dried blood spots and BMI. Our observation of positive association of four acylcarnitines, including methylbutyryl-carnitine, with BMI among adolescents after bariatric surgery were consistent with these prior reports. The body of evidence suggested that acylcarnitines may be involved in the mechanisms of weight regulation or serve as biomarkers of BMI changes; however, the exploration of the impact of DDE, a model lipophilic obesogen, on acylcarnitine profile is relatively limited in human studies. Hu et al. reported the associations of eight acylcarnitines with perinatal exposure to *p,p'*-DDE in children living in California, USA, but the directions of effect were inconsistent.⁵⁷ Yan et al. reported negative associations of several acylcarnitines with organochlorines among an adult population in California.⁵⁸ Our results suggested that DDE

exposure may influence weight regulation through a mediating role of methylbutyryl-carnitine (Figure 5). Further research is warranted, for example to explore possible proinflammatory activation by acetylcarnitines.⁵³

The associations of DDE exposure with plasma metabolites could be initiated by the local impact of DDE on adipose tissue (Figure 5). We identified a pathway linking the accumulated *p,p'*-DDE concentration in adipose tissue to BMI changes after bariatric surgery with tryptophan metabolism as the critical mediator. Tryptophan is an essential amino acid that participates in various metabolic functions.⁵⁹ In addition to its role in protein synthesis, tryptophan can be catabolized into kynurenines, serotonin, and indoles, and the tryptophan-kynurenine pathway holds special significance in obesity.⁶⁰ In tryptophan-kynurenine pathway, indoleamine 2,3-dioxygenase (IDO) catalyzes the conversion of tryptophan to kynurenine in extrahepatic tissues, and the expression of IDO is modulated by pro-inflammatory cytokines.⁶¹ DDE was found in vivo to contribute to inflammation in adipose tissue via induction of pro-inflammatory cytokine.⁶² Lischka et al. found among pediatric patients with severe obesity that BMI and body fat mass were positively associated with kynurenine and the activity of indoleamine 2,3-dioxygenase that converts tryptophan to kynurenine.⁶³ Similarly, increased levels of kynurenine and tryptophan in fasting blood were also observed in an adult population to be associated with higher BMI and metabolic syndrome.⁶⁴ In the current study, we established the possible relationship between DDE exposure and tryptophan-kynurenine pathway, partially via adipose tissue 5-HTP. The body of evidence implied a critical role for tryptophan metabolism in the obesogenic mechanisms of DDE. However, relevant human studies remained limited, warranting further research.

In plasma, six other metabolites were associated with adipose tissue DDE concentrations including indole-3-methyl acetate, lactate, mevalolactone, orotate, thyroxine, and undecylenic acid. Lactate is a hydroxycarboxylic acid mainly metabolized from glucose (65%) and alanine (16-20%) intracellularly, playing an important role in energy metabolism.⁶⁵ In vivo evidence

demonstrated that a release of DDT from adipose tissue could inhibit gluconeogenesis from lactate between pyruvate and phosphoenolpyruvate.⁶⁶ This indicated that the suppression effect of DDT and its metabolites on gluconeogenesis could partly explain the variations in weight loss among adolescents receiving bariatric surgery.⁶⁶ Orotate is well known as a precursor in de novo pyrimidine biosynthesis which is particularly active in proliferating cells to fulfill the increased demand of nucleic acid precursors and other cellular components.⁶⁷ There is limited evidence that DDE exposure increases orotate or de novo pyrimidine biosynthesis in human and animals. Although Chapados et al. reported increased proliferative capacity of human preadipocytes exposed to DDE for 48 hours in vitro,⁶⁸ more research is warranted to replicate the association and explore the impact of DDE exposure on DNA metabolism and cell proliferation.

Tyrosine metabolism is the only pathway we found to be associated with both DDE exposure and BMI or waist circumference. Tyrosine is a non-essential amino acid and can be catabolized or transformed to generate a variety of metabolites, such as neurotransmitters and hormones, that participate in obesity-related pathways.⁶⁹ In a study involving 80 children with obesity undergoing a year-long lifestyle intervention, plasma tyrosine was associated with insulin resistance, as observed in their longitudinal metabolomic profiles.⁷⁰ Among an asymptomatic adult population at higher risk for metabolic syndrome, serum tyrosine was found to be associated with waist circumference, body shape index, and insulin resistance, underscoring its vital role in metabolic diseases.⁷¹ Few evidence has been reported for the association between DDE and tyrosine so far, and our findings highlighted the potential role of tyrosine as a mediator in the association of DDE exposure with changes in anthropometric measures.

In addition to DDE, other lipophilic persistent organic pollutants (POPs) may also be released from adipose tissue during rapid weight loss following bariatric surgery. These compounds include polychlorinated biphenyls, polybrominated diphenyl ethers, and other

organochlorine pesticides, which can similarly accumulate in adipose tissue due to their lipophilic properties and long half-lives.⁷² It is plausible that the simultaneous release of these compounds could influence metabolic outcomes, potentially interacting with DDE or acting through independent mechanisms.^{73, 74} The present study builds upon a previous publication from our group, where we specifically identified the obesogenic effect of DDE and demonstrated that higher DDE exposure in visceral adipose tissue was associated with less improvement in BMI and waist circumference.⁹ Importantly, in that study, we evaluated the effect of DDE after adjusting for a few other lipophilic POPs, and the association between DDE and weight change remained significant, suggesting that the effect of DDE is independent of those pollutants.⁹ However, mixture analyses coupled with omics analysis are necessary to fully understand the effects of lipophilic pollutant mixture on obesity. This remains an important direction for future research.

The present study exhibits several strengths. The prospective Teen-LABS cohort provided a unique opportunity to examine the health impact of DDE exposure on changes in metabolic profiles. The rapid release of DDT/DDE from adipose tissue into circulation after bariatric surgery initiated a “natural experiment” that magnifies the potential effects of DDT/DDE among participants in the cohort. To our knowledge, this is the first study to examine the impact of *p,p'*-DDE concentrations measured in the visceral adipose tissue on the longitudinal metabolic profiles among adolescents. The study provided insights into the risks posed by DDE exposure and its chronic adverse effects on obesity. The repeated measures of plasma metabolomics over a 3-year period after bariatric surgery enabled us to evaluate longitudinal effects on metabolic changes among adolescents. Moreover, we used a discovery-oriented hypothesis-free approach, untargeted metabolomics, to identify as many metabolites as possible, which allowed us to discover novel biomarkers and pathways that have not been studied using targeted approaches. Moreover, the Teen-LABS study specifically targeted adolescents with severe obesity, with most selected medical centers being free-standing tertiary care pediatric

hospitals located in major metropolitan areas and dedicated to adolescent-only weight loss surgery (WLS) programs.²⁸ Given the standardized recruitment process and the nature of these specialized facilities, we believe that the recruited participants represented a representative sample of the target population, minimizing the potential for selection bias. Finally, all plasma samples were collected in a fasting state, which minimized the impact of dietary intake on metabolic profiles.

The study also has some limitations. First, the sample size is relatively small given the high dimensionality of metabolomics data, which may limit statistical power to detect significant associations after multiple comparison correction. This could partially explain why we did not observe any adipose tissue metabolites significantly associated with DDE exposure after multiple comparison correction. While we conducted a series of sensitivity analyses to assess robustness, the results should be interpreted with caution, and further studies with larger sample sizes are warranted to validate these findings. Second, previous literature has suggested that sex-specific differences in tryptophan metabolism may exist.⁷⁵ Given that our study cohort was predominantly composed of females, it is possible that sex-specific effects could influence the observed associations. However, due to the small sample size and the limited representation of males, we were not able to conduct stratified analyses to assess potential sex differences. Future studies with larger and more balanced cohorts are needed to further investigate the potential for sex-specific effects of DDE exposure. Third, all participants had severe obesity at baseline (i.e., before bariatric surgery), so the present findings should be generalized to the broader population with caution. Fourth, as the adipose tissue *p,p'*-DDE concentration and adipose tissue metabolomics were measured concurrently, establishing a temporal relationship is challenging. However, given the long half-life of DDE in fat mass, it is plausible that the observed changes in adipose tissue metabolites were induced by DDE exposure, rather than the reverse. Fifth, while the hypothesis of DDE re-distribution is supported by previous literature, we were unable to directly assess this assumption due to the lack of post-surgical DDE

quantification in plasma. Future studies are warranted to directly measure circulating DDE levels to confirm the mobilization of DDE following bariatric surgery. Sixth, we observed unbalanced variability (i.e., CVs) of DDE concentrations in different exposure groups. While we used standardized protocols to minimize measurement error, the inherent variability in exposure levels among participants might have influenced the CV estimates. Future studies should consider employing additional analytical techniques to further validate these findings. Seventh, we did not have information on household size, which prevented adjustment of parental income level as a socioeconomic status (SES) indicator. As a result, the SES classification may not fully capture the financial burden on families, particularly in larger households. Eighth, medical center was included as a covariate in the models to mitigate potential confounding from environmental factors, such as neighborhood deprivation and food security, and surgical practices. However, this adjustment may not fully eliminate residual confounding, as unmeasured geographical variability could still influence the results. Ninth, we used a universal knot at one year to model phase-dependent changes in weight loss outcomes. While this approach effectively captures the overall trend in weight reduction and stabilization, it may not fully account for individual variability in weight change patterns, particularly among participants with higher initial BMI. Further investigation with more flexible modeling approaches is warranted. However, due to the limited sample size in this study, we did not have sufficient power to implement more complex models. Finally, while widely used in observational studies,⁴⁴ the meet-in-the-middle approach is considered inadequate for strong inference of a causal effect of the independent variable on the dependent variable through the intermediate variable.⁷⁶ More research is warranted to validate the mediating role of the metabolites reported in the present study.

In summary, *p,p'*-DDE levels in visceral adipose tissue were linked to changes in local and systemic metabolites in adolescents post-bariatric surgery, potentially compromising weight loss outcomes. Specifically, alterations in methylbutyryl-carnitine and tryptophan metabolism may mediate the indirect effects of DDE on BMI and waist circumference over three years. These

findings enhance our understanding of environmental obesogens and their impact on metabolic pathways. From a public health perspective, our results underscore the importance of integrating environmental health considerations into clinical practice, particularly when evaluating weight loss outcomes in adolescents undergoing bariatric surgery. Healthcare providers may consider assessing environmental exposures in vulnerable populations to optimize post-surgical care. Furthermore, public health policies aimed at reducing exposure to persistent organic pollutants (POPs) could mitigate adverse metabolic outcomes. Future research using advanced systems biology approaches, such as multi-omics, could further elucidate the mechanisms behind metabolic dysregulation caused by environmental pollutants.

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Tables and Figures

Table 1. Descriptive characteristics by exposure groups for covariates for the 60 participants included in the present analysis from the Teen-LABS cohort, 2007-2012.

	Low (≤ 13.6 ng/g) (N=20)	Moderate (13.6-18.4 ng/g) (N=20)	High (> 18.4 ng/g) (N=20)	Overall (N=60)
Age at baseline, years [mean (SD)]	16.5 (1.5)	16.5 (1.6)	17.3 (1.3)	16.8 (1.5)
Sex [N (%)]				
Male	4 (20.0)	6 (30.0)	6 (30.0)	16 (26.7)
Female	16 (80.0)	14 (70.0)	14 (70.0)	44 (73.3)
Race [N (%)]				
Others ^a	5 (25.0)	8 (40.0)	10 (50.0)	23 (38.3)
White	15 (75.0)	12 (60.0)	10 (50.0)	37 (61.7)
Parents income category [N (%)]				
Less than \$25,000	11 (57.9)	5 (29.4)	8 (44.4)	24 (44.4)
\$25,000 to \$74,000	5 (26.3)	10 (58.8)	6 (33.3)	21 (38.9)
\$75,000 or more	3 (15.8)	2 (11.8)	4 (22.2)	9 (16.7)
Missing	1	3	2	6
Site of medical center ^b [N (%)]				
A	19 (95.0)	10 (50.0)	5 (25.0)	34 (56.7)
B	1 (5.0)	10 (50.0)	15 (75.0)	26 (43.3)
<i>p,p'</i> -DDE, ng/g				
Mean (SD)	10.0 (3.1)	16.4 (1.4)	28.3 (8.0)	18.2 (9.1)
Median (min, max)	10.8 [1.0, 13.3]	16.7 [13.8, 18.4]	25.8 [18.5, 55.2]	16.7 [0.1, 55.2]
BMI, kg/m ² [mean (SD)]				
Baseline	53.1 (8.9)	54.9 (11.2)	54.3 (9.6)	54.1 (9.77)
0.5 years	39.9 (7.3)	43.0 (10.5)	42.1 (8.8)	41.7 (8.88)

	Low (≤13.6 ng/g) (N=20)	Moderate (13.6-18.4 ng/g) (N=20)	High (>18.4 ng/g) (N=20)	Overall (N=60)
1 year	36.6 (8.2)	39.1 (10.2)	39.8 (10.1)	38.4 (9.35)
3 years	37.7 (10.5)	49.5 (13.1)	41.0 (11.2)	43.1 (12.5)
Waist circumference, cm [mean (SD)]				
Baseline	144 (16.3)	145 (16.8)	141 (17.9)	144 (16.8)
0.5 years	119 (16.9)	119 (17.4)	117 (19.4)	118 (17.7)
1 year	110 (16.9)	114 (22.1)	113 (19.8)	112 (19.1)
3 years	106 (11.3)	133 (15.4)	113 (20.9)	119 (20.0)

Teen-LABS, teen longitudinal assessment of bariatric surgery; SD, standard deviation; min, minimum; max, maximum.

^a Others including Black or African American, Asian, American Indian or Alaska Native, Native Hawaiian or Other Pacific Islander, Unknown, and multi-race.

^b Location is denoted as A and B to maintain confidentiality in accordance with Institutional Review Board requirements.

Table 2. Significance of selected ^a metabolic signature in plasma associated with *p,p'*-DDE exposure by chromatography columns and ionization modes.

Significance pattern ^b			Number of metabolite features				Total
Sig. of coefficients ^c		Sig. of interaction ^d	C18 column		HILIC column		
1 st year	1 st -3 rd year		ESI-	ESI+	ESI-	ESI+	
Negative	-	No	-	1	2	6	9
Positive	-	No	-	1	-	13	14
-	Negative	No	-	1	-	1	2
-	Positive	No	4	-	-	1	5
-	-	Yes	-	2	7	20	29
Negative	-	Yes	-	-	6	25	31
Positive	-	Yes	-	5	27	62	94
-	Negative	Yes	-	4	-	5	9
Negative	Negative	Yes	-	9	1	22	32
-	Positive	Yes	3	4	4	10	21
Positive	Positive	Yes	-	-	-	22	22
Total			7	27	47	187	268

DDE, dichloro-diphenyldichloro-ethylene; Sig., significance; C18, reverse-phase C18 liquid chromatography; HILIC, hydrophilic interaction liquid chromatography; ESI, electrospray ionization.

^a The significance pattern with only one metabolite feature was not included.

^b The significance pattern takes into account both the individual significance of each coefficient of the interaction term (β_3 and β_5) by t-test and the joint significance test by ANOVA in Eq. (1).

^c The individual significance is summarized by time since surgery (q -value < 0.05). A positive significant coefficient (either β_3 for 1st year or β_5 for 1st-3rd year) in Eq. (1) is denoted as “Positive”, and vice versa.

^d The joint significance testing on the overall interaction is tested at a q -value threshold of 0.05.

Table 3. The systemic impact of *p,p'*-DDE in adipose tissue on plasma metabolomics mediated via adipose tissue metabolomics.

Metabolites in AT associated with DDE ^a	Effect estimates with DDE ^b	Metabolites in plasma associated with those in AT ^c	Metabolite class	Effect estimates with AT metabolites ^d	
				1 st year	1 st -3 rd year
5-HTP	-0.51 (-0.90, -0.12)	Tryptophan	Amino acid and derivatives	-0.55 (-0.90, -0.22)	-0.44 (-0.78, -0.12)
		N-Acetylphenylalanine	Amino acid and derivatives	-0.70 (-1.18, -0.26)	-0.93 (-1.39, -0.49)
		Myristic acid	Fatty acids	0.05 (-0.23, 0.33)	0.44 (0.17, 0.72)
		10-Hydroxydecanoate	Fatty acids	-0.36 (-0.67, -0.05)	-0.51 (-0.82, -0.21)
		Margaric acid	Fatty acids	0.05 (-0.23, 0.36)	-0.64 (-0.92, -0.34)
		Palmitoleic acid	Fatty acids	0.03 (-0.29, 0.37)	-0.53 (-0.84, -0.19)
		Decenoic acid	Fatty acids	-0.11 (-0.42, 0.20)	-0.55 (-0.86, -0.25)
		Arachidonic acid	Fatty acids	0.08 (-0.22, 0.40)	-0.54 (-0.83, -0.23)
		Docosatrienoic acid	Fatty acids	0.12 (-0.18, 0.46)	-0.45 (-0.75, -0.12)
		17-Octadecynoic acid	Fatty acids	0.11 (-0.23, 0.47)	-0.53 (-0.86, -0.18)
		Palmitic acid	Fatty acids	0.08 (-0.24, 0.43)	-0.48 (-0.79, -0.14)
		Nonadecenoic acid	Fatty acids	0.12 (-0.22, 0.49)	-0.49 (-0.82, -0.13)
		Eicosatrienoic acid	Fatty acids	0.20 (-0.11, 0.55)	-0.46 (-0.76, -0.12)
		11-Octadecenoic acid	Fatty acids	0.12 (-0.20, 0.48)	-0.52 (-0.84, -0.17)
		Orotate	Nucleotide derivatives	-0.65 (-1.04, -0.26)	-0.47 (-0.85, -0.09)
		Indole-3-propionic acid	Others	-0.54 (-0.85, -0.23)	-0.42 (-0.73, -0.12)
		3-Methylcrotonylglycine	Others	-0.79 (-1.24, -0.33)	-0.64 (-1.09, -0.20)
		Nutriacholate	Others	-0.57 (-0.96, -0.16)	-0.74 (-1.12, -0.34)
		Glyceraldehyde	Others	-0.47 (-0.74, -0.21)	-0.42 (-0.68, -0.15)
		Glycerol-Myristate	Others	0.14 (-0.17, 0.48)	-0.44 (-0.75, -0.12)
Acetoacetic acid	-0.41 (-0.79, -0.02)	ACC	Amino acid and derivatives	-0.01 (-0.32, 0.29)	0.54 (0.25, 0.83)
		4-Acetamidobutanoate	Amino acid and derivatives	-0.03 (-0.45, 0.39)	0.73 (0.33, 1.12)
		Allothreonine	Amino acid and derivatives	0.00 (-0.29, 0.30)	0.49 (0.21, 0.77)
		Nonadecenoic acid	Fatty acids	-0.11 (-0.48, 0.27)	-0.67 (-1.02, -0.31)
		Docosatrienoic acid	Fatty acids	-0.12 (-0.46, 0.22)	-0.60 (-0.92, -0.27)
		Hydroxybutyrylcarnitine	Acylcarnitines	-0.27 (-0.60, 0.08)	-0.68 (-1.01, -0.36)
		Glycoursodeoxycholic acid	Bile acids	-0.20 (-0.59, 0.19)	0.57 (0.19, 0.94)
		Allose	Others	-0.93 (-1.28, -0.57)	-0.54 (-0.88, -0.20)
		2-Hydroxybutyrate	Others	-0.09 (-0.43, 0.26)	-0.59 (-0.92, -0.25)
Benzyl Alcohol	-0.54 (-0.90, -0.18)	Hydroxyoctanoic acid	Others	-0.16 (-0.53, 0.23)	-0.78 (-1.14, -0.42)
		Undecylenic acid	Fatty acids	-0.47 (-0.70, -0.23)	-0.36 (-0.59, -0.13)
		Glyceraldehyde	Others	-0.54 (-0.79, -0.27)	-0.23 (-0.48, 0.03)
		Sedoheptulose	Others	-0.37 (-0.63, -0.11)	-0.49 (-0.75, -0.23)
		Oxalic acid	Others	-0.44 (-0.64, -0.22)	-0.24 (-0.44, -0.03)
Galactosamine	-0.50 (-0.93, -0.08)	Serine	Amino acid and derivatives	-0.51 (-0.76, -0.26)	0.03 (-0.25, 0.30)
		Palmitoleic acid	Fatty acids	-0.03 (-0.24, 0.18)	0.42 (0.19, 0.64)

		Undecylenic acid	Fatty acids	-0.01 (-0.29, 0.27)	0.59 (0.28, 0.88)
Glycochenodeoxycholic acid	-0.42 (-0.82, -0.02)	Thyroxine	Hormones	0.24 (-0.02, 0.51)	0.68 (0.41, 0.94)
		4-Acetamidobutanoate	Amino acid and derivatives	-0.15 (-0.50, 0.19)	0.66 (0.32, 1.00)
Ketoglutaric acid	-0.42 (-0.83, 0.00)	Myristic acid	Fatty acids	0.05 (-0.20, 0.29)	0.49 (0.25, 0.72)
		Allose	Others	-0.82 (-1.12, -0.52)	-0.43 (-0.73, -0.13)
		Indole-3-Acetamide	Amino acid and derivatives	0.78 (0.36, 1.19)	0.70 (0.32, 1.09)
Lysine	-0.46 (-0.87, -0.05)	Tyrosine	Amino acid and derivatives	0.47 (0.21, 0.73)	0.48 (0.24, 0.73)
Methylamylacetic acid	-0.42 (-0.83, 0.00)	2-Methylbutanal	Others	0.15 (-0.33, 0.62)	1.05 (0.55, 1.54)

^a The metabolites, extracted from adipose tissue metabolomics, that were both associated with *p,p'*-DDE concentration in adipose tissue at *p*-value < 0.05 and plasma metabolites at *q*-value < 0.05.

^b The estimated effect of DDE on adipose tissue metabolites.

^c The metabolites, extracted from plasma metabolomics, that were associated with adipose tissue metabolites at *q*-value < 0.05. The metabolites were colored by their chemical structures and primary biological functions.

^d The estimated effect of adipose tissue metabolites on plasma metabolites, with adjustment of DDE exposure, for the 1st year and the 1st-3rd year after bariatric surgery, respectively. The significant estimates after multiple comparison correction are in bold.

Figure Legends

Figure 1:

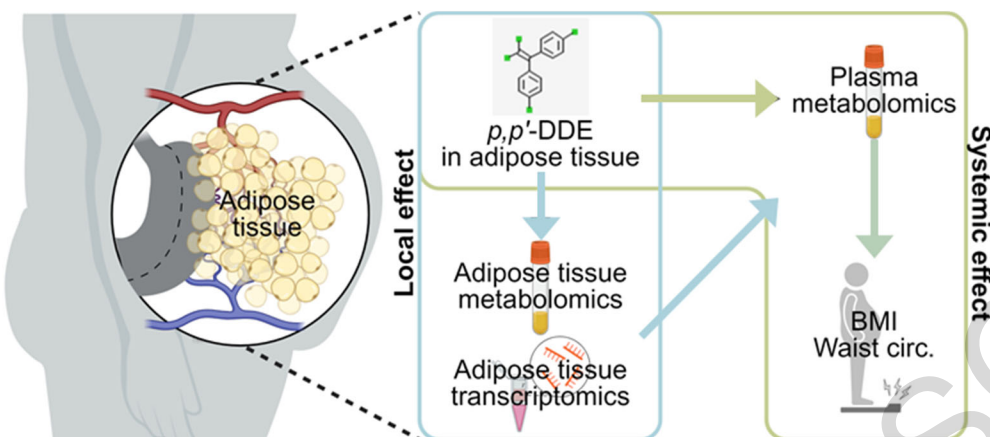


Figure 1. Overview of analysis design for an integrative approach via metabolomics that evaluated both the local and systemic effects of DDE exposure among an adolescent population with obesity. The *p,p'*-dichloro-diphenyldichloro-ethylene (*p,p'*-DDE) concentration was measured in the adipose tissue collected at bariatric surgery as a proxy for long-term cumulative exposure to DDE. We hypothesized a local effect estimated by adipose tissue metabolomics and a systemic effect revealed by plasma metabolomics of DDE exposure on participants metabolism which could lead to detrimental effect on weight loss. The adipose tissue transcriptomics served to strengthen the understanding of local alterations. The weight loss was quantified by body mass index (BMI) and waist circumference (circ.). Two main hypothesized mechanisms were denoted by colored arrows. The green arrows denote a systemic pathway linking DDE exposure and weight loss, and the blue arrows denote a similar pathway partially mediated by the local effect of DDE on adipose tissue. The figure created with BioRender.com.

Figure 2:

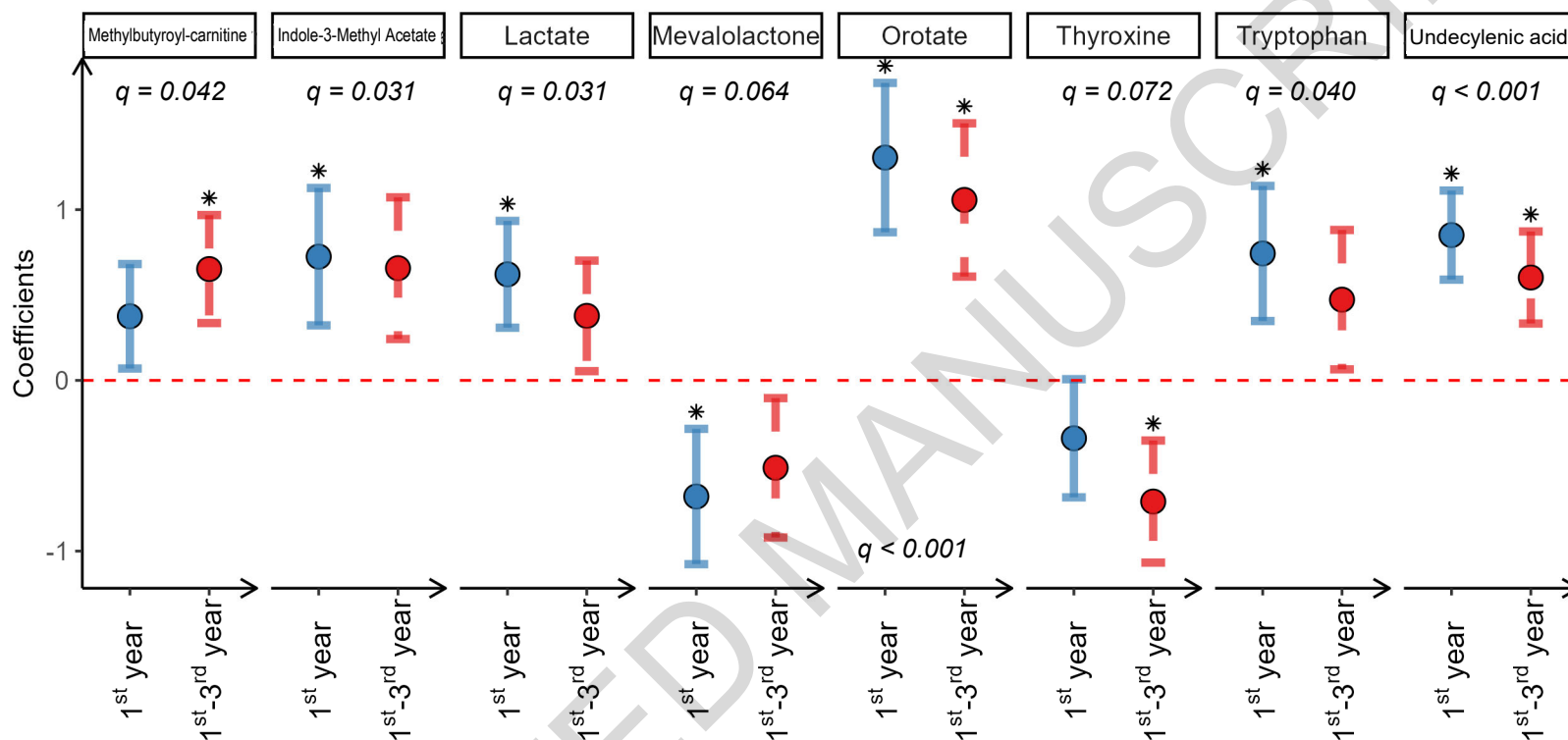


Figure 2. Significance pattern of confirmed metabolites whose temporal trend during the three years after bariatric surgery was significantly (q -values < 0.05) modified by p,p' -DDE concentration in visceral adipose tissues collected at the surgery. The coefficients of the interaction term between DDE exposure and time since surgery are colored in blue for the 1st year after surgery, and the 1st-3rd year was colored in red. The point estimates and 95% confidence interval are plotted on y-axis. The q -values of the joint significance test are showed for each metabolite, and the significant interaction terms (q -values < 0.05) are labelled with asterisks. Numeric data can be found in Excel Table S5.

Figure 3:

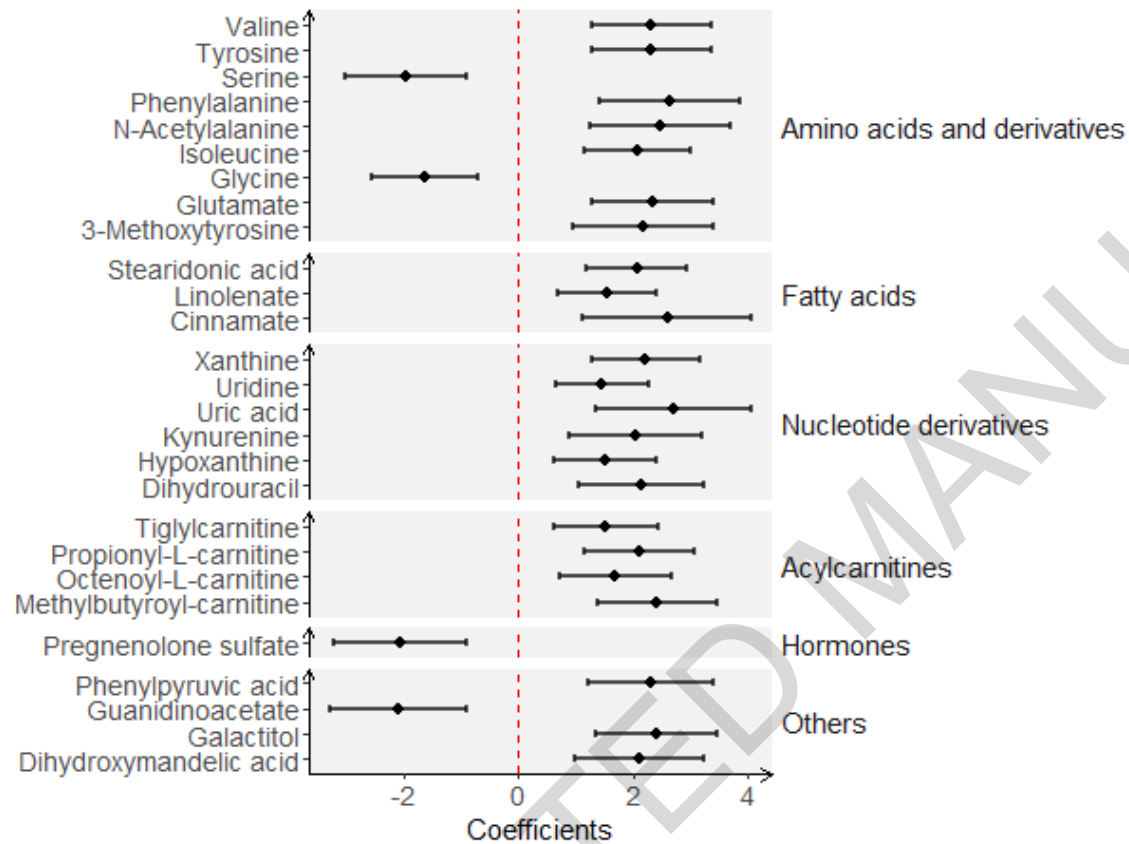


Figure 3. Significant confirmed metabolites in plasma associated with longitudinal changes in body mass index after multiple comparison correction. The point estimate and 95% confidence intervals are plotted on x-axis. Numeric data can be found in Excel Table S6.

Figure 4:

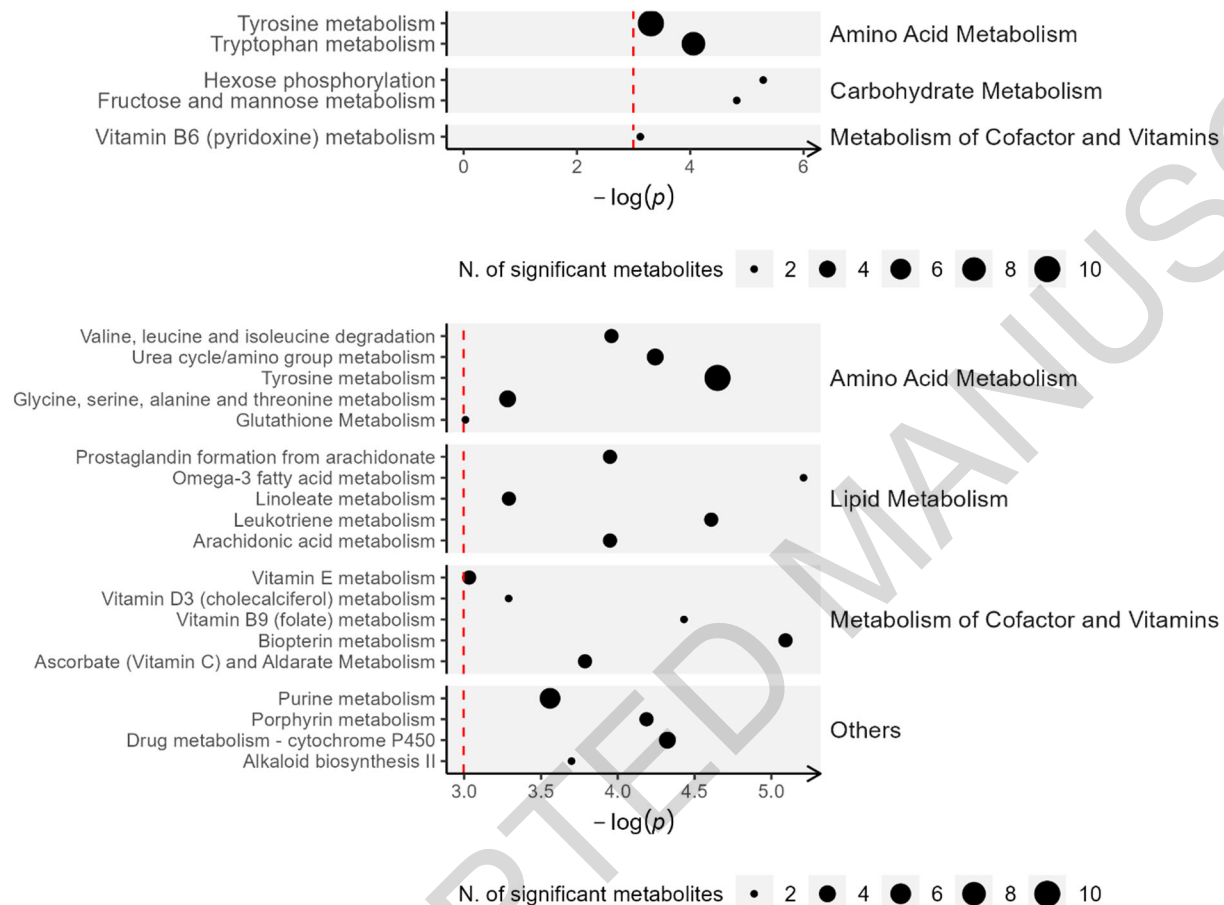


Figure 4. Biological pathways enriched by the metabolite features (A) whose temporal trend during the three years after bariatric surgery was significantly (q -values < 0.05) modified by p,p' -DDE concentration in visceral adipose tissues collected at the surgery or (B) longitudinally associated (q -values < 0.05) with body mass index (BMI) during the three years after bariatric surgery. The -

$\log_{10}(p\text{-values})$ computed by mummichog are plotted on the x-axis. The points are sized according to the number of significant metabolites that the pathway contained. The red dashed line denoted a p -value threshold of 0.05.

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Figure 5:

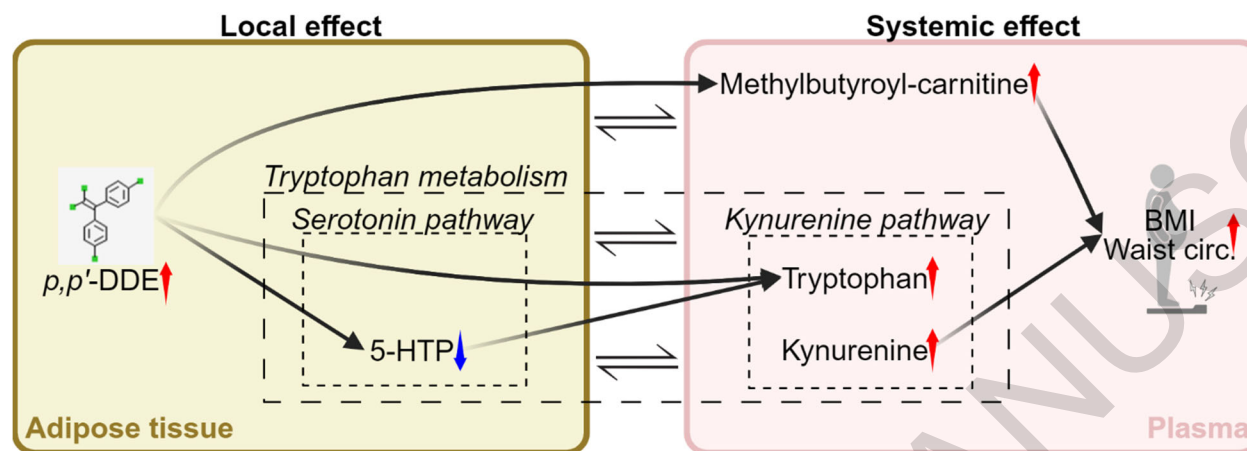


Figure 5. Mechanistic interpretation of results showing. DDE exposure effecting changes in BMI among adolescents both directly via systemic effect and indirectly via a local effect on adipose tissue. Abbreviations: 5-HTP, 5-hydroxytryptophan.