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Circulating linoleic acid and its interplay with gut microbiota during pregnancy for gestational diabetes mellitus

Jiahui Qiu[1†], Ping Hu[2†], Fan Li[3], Yichao Huang[4], Yunhaonan Yang[3], Fengjiang Sun[5], Ping Wu[6], Yuwei Lai[6], Yi Wang[7], Xiangwang He[3], Yidan Dong[3], Peiqi Zhang[8], Shanshan Zhang[3], Nianwei Wu[3], Tianlei Wang[3], Shizhuo Yang[3], Shuo Li[3], Jiaying Yuan[9], Xiaojuan Liu[10], Gang Liu[11], Yayi Hu[12], Jason H. Y. Wu[13], Da Chen[5*], An Pan[6*] and Xiong-Fei Pan[3,14,15*]

Abstract

Background Circulating linoleic acid (LA) levels have been reported to be associated with various metabolic outcomes. However, the role of LA and its interplay with gut microbiota in gestational diabetes mellitus (GDM) remains unclear. This study aimed to investigate the longitudinal association between circulating LA levels during pregnancy and the risk of GDM, and the potential role of gut microbiota.

Methods A nested case–control study was conducted within the ongoing Tongji-Huaxi-Shuangliu Birth Cohort in Chengdu, China. Blood and fecal samples were collected during early and middle pregnancy from 807 participants. GDM was diagnosed in middle pregnancy using the International Association of Diabetes and Pregnancy Study Groups criteria. Plasma LA levels were measured using gas chromatography-mass spectrometry, and gut microbiota was analyzed through 16S rRNA gene sequencing and shotgun metagenomic sequencing. A two-sample Mendelian randomization study was conducted using data from the IEU OpenGWAS database and the FinnGen consortium.

Results Elevated plasma LA levels were associated with a lower risk of GDM in both early (P for trend = 0.002) and middle pregnancy (P for trend = 0.02). Consistently, Mendelian randomization analysis revealed that each unit increase in LA was associated with a 16% reduction in GDM risk (odds ratio: 0.84, 95% confidence interval: 0.72, 0.95). In early pregnancy, higher plasma LA levels were correlated with higher adiponectin levels ($P < 0.001$) and lower levels of triglycerides ($P < 0.001$), HbA1c ($P = 0.04$), and C-peptide ($P = 0.04$). The LA-accociated microbiota mediated the relationship between LA and C-peptide ($P = 0.01$). Additionally, the inverse association between LA and GDM was modified by *Bilophila* (P for interaction = 0.03), with a stronger association observed in participants with lower *Bilophila* levels in early pregnancy. Metagenomic analyses further showed that the LA-associated pathway (D-galacturonate degradation I) and its key enzyme (EC 4.2.1.7) were associated with metabolic traits.

†Jiahui Qiu and Ping Hu contributed equally to this work.

*Correspondence:
Da Chen
dachen@jnu.edu.cn
An Pan
panan@hust.edu.cn
Xiong-Fei Pan
pxiongfei@scu.edu.cn
Full list of author information is available at the end of the article

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Conclusions Our study provides evidence for an inverse causal association between plasma LA levels during pregnancy and GDM risk, which is both mediated and modified by gut microbiota.

Keywords Gestational diabetes mellitus, Linoleic acid, Gut microbiota, Cohort, Pregnancy

Background

Gestational diabetes mellitus (GDM) is characterized by the onset of hyperglycemia during pregnancy and poses a significant health challenge worldwide [1]. GDM is associated with both short-term and long-term adverse health effects for mothers and their offspring, including obesity, impaired glucose metabolism, and cardiovascular disease [1]. With the prevalence of GDM increasing dramatically over the past two decades by more than 30% in numerous countries, there is a pressing need to understand the underlying mechanisms of GDM and identify modifiable risk factors [2].

Linoleic acid (LA; 18:2n6), distinguished as a primary omega- 6 fatty acid, was extensively investigated for its role in metabolic health. Since LA cannot be synthesized in human body and is primarily obtained through diet, its circulating levels are a reliable biomarker for dietary intake of LA, thus providing a more accurate measure than traditional questionnaires [3, 4]. Recent studies suggested a relationship between fatty acids, particularly LA, and glucose metabolism. Specifically, studies have reported an inverse association between elevated concentrations of plasma LA and risk of type 2 diabetes [5–7]. However, the exact role of LA in GDM remains unclear, with conflicting results from various studies [8–14]. Our previous nested case–control study among 1618 pregnant women, conducted within two prospective Chinese cohorts, showed an inverse association between circulating LA levels in early pregnancy and the risk of GDM [9]. Consistently, lower levels of LA were observed in GDM women at 18–22 weeks of pregnancy, as compared to non-GDM controls in a study from India [10]. In contrast, another study found that women with GDM showed a high level of LA compared to those without GDM even in early pregnancy [13]. Moreover, within the NICHD Fetal Growth cohort, analyses showed no significant associations between LA levels at gestational weeks 10–14 and 15–26, and the risk of GDM [14]. Several factors contribute to these discrepancies, including differences in study design, sample size, and population characteristics. Most studies were cross-sectional or based on small sample sizes, which limits their ability to reach a reliable conclusion. Furthermore, most prior studies focus on single time measurements during pregnancy, failing to capture the longitudinal changes in LA levels and their potential cumulative effect on GDM risk.

Gut microbiota could interact with the host's diet for maternal health. Women diagnosed with GDM experience significant shifts in their gut microbiota during pregnancy, typically characterized by a reduction in beneficial bacteria and an increase in potentially harmful bacteria [15, 16]. These imbalances tend to evolve throughout the entire pregnancy, affecting insulin sensitivity and glucose metabolism [16]. The composition and function of the gut microbiota are profoundly shaped by the diversity and quality of the host's diet. The intake of dietary fatty acids significantly influences the composition of gut microbiota in both animal models and humans [17]. A previous study showed that a LA-rich diet worsens metabolic responses and exacerbates gut microbiota dysbiosis in obese rats with diabetes [18]. However, the intricate interplay between LA and the gut microbiota in GDM remains an underexplored area.

To bridge this knowledge gap, we utilized data from the prospective Tongji-Huaxi-Shuangliu Birth Cohort (THSBC) in China to investigate the longitudinal association of plasma LA levels with GDM risk during both early and middle pregnancy. Additionally, a two-sample Mendelian randomization (MR) approach was applied to assess causality between plasma LA levels and GDM risk. We also explored the potential role of gut microbiota in the relationship between LA and GDM.

Methods

Study design

The THSBC is an ongoing birth cohort to assess risk factors and consequences of major maternal and neonatal outcomes in Chengdu, China. Pregnant women aged 18–40 years were invited to participate when they received the first antenatal care (6–15 weeks of pregnancy) at Shuangliu Maternal and Child Health Hospital. The following women were excluded: (1) women who had received infertility treatments such as in vitro fertilization and intrauterine insemination; (2) women who reported severe chronic or infectious diseases; and (3) women who were unable to or refused to complete the baseline questionnaire. A total of 6143 pregnant women were recruited in 2017–2019, and followed up throughout the pregnancy. GDM cases were diagnosed using a 75-g oral glucose tolerance test conducted between 24 and 28 weeks of pregnancy, based on the criteria set by the International

Association of Diabetes in Pregnancy Study Groups: fasting glucose ≥5.1 mmol/L, 1-h glucose ≥10.0 mmol/L, or 2-h glucose ≥8.5 mmol/L [19]. Our study was based on available linked data from 3 nested case–control studies within the THSBC [9, 20, 21]. A total of 269 GDM cases were included and matched with 538 non-GDM controls in a 1:2 ratio, based on maternal age (± 3 years) and gestational age (± 4 weeks). All GDM cases or controls did not have pre-existing type 1 or type 2 diabetes. Blood and fecal samples were collected during both early (10.93 ± 2.18 weeks of gestation) and middle pregnancy (24.71 ± 1.55 weeks of gestation), aliquoted, and stored at −80 °C for future analysis. Among the 807 individuals, 16S rRNA gene sequencing was conducted on fecal samples collected during early and mid-pregnancy from 96 matched pairs (1:1 ratio), matched by maternal age (± 3 years), gestational age (± 3 weeks), and sample collection date (± 4 weeks). Additionally, shotgun metagenomic sequencing was conducted on 56 matched pairs (1:1 ratio) with fecal samples from early pregnancy, using the same matching criteria. The study design and analytical framework are illustrated in Fig. 1.

Assessment of plasma LA

Fifty microliters of plasma from fasting blood samples was spotted onto Whatman SG81 ion exchange paper, air-dried, and washed with acetone to remove neutral lipids. Fatty acid methyl esters (FAMEs) were then extracted using anhydrous methanol with 1% H_2SO_4 at 70 °C for 3 h. The FAMEs were analyzed by gas chromatography–mass spectrometry using an Agilent 7890B GC with a J&W DB- 23 column and an Agilent 5977B mass spectrometer under electron impact ionization. LA levels were expressed as a relative weight percentage of total FAMEs in each sample, which has been widely used in population studies examining the effects of blood fatty acids [7, 22, 23, 24]. All analyses were conducted blindly, with matched case–control samples processed in the same batch in a randomized order to minimize systematic errors. Quality control measures, including procedural blanks and quality control samples, were used to monitor assay performance across batches. Details on assessment of plasma LA can be found in a previous study [9].

16S rRNA gene sequencing for fecal samples

Details on fecal sample collection and 16S rRNA gene sequencing have been provided previously [20]. Briefly, DNA was extracted using TIANamp Stool DNA kit. Bacterial 16S rRNA gene sequences (V3–V4 region) were PCR amplified, followed by a 2-step nested PCR to generate community amplicons. These were pooled equimolarly to form a barcode-PCR library, quantified using a Qubit 2.0 fluorometer, and sequenced with paired-end reads (300 bp ×2) on the Illumina MiSeq platform. Quality control included a standard mixture of genomic DNA from various bacterial species and a negative control. Paired-end reads were denoised, merged via the DADA2 pipeline, and classified using a naïve Bayes classifier trained on the V3–V4 region of the 99% identity Greengenes reference database (version 13_8). To examine associations between individual microbial genus and LA levels, 391 amplicon sequence variants (ASVs) with mean relative abundance >0.01% in at least 10% of samples were retained, which were then collapsed into 67 genera. Relative abundances were arcsine square root transformed, followed by a *z*-score normalization.

Shotgun metagenomic sequencing for fecal samples

Shotgun metagenomic sequencing was performed using the methods described previously [21]. Fecal sample collection and DNA extraction followed the same protocol as 16S rRNA gene sequencing. Sequencing libraries (paired-end, insert size: 350 bp) were prepared using the Tn5 DNA Library Prep Kit for Illumina and sequenced on the Illumina NovaSeq 6000 platform (read length: 150 bp). Quality control of the whole-genome shotgun sequencing data was performed using KneadData (v0.7.2), Trimmomatic (v0.33), and Bowtie2 (v2.3.4.3). Reads aligning to the human genome and ribosomal DNA (rDNA) were filtered out by mapping them to the human reference genome (GRCh37) and the SILVA 128 database. Additionally, non-human reads shorter than 75 bp were discarded. Taxonomic profiling was carried out using MetaPhlAn (v3.0.3). Microbial functional profiling, including MetaCyc pathways and Enzyme Commission

(See figure on next page.)

Fig. 1 Study design and analytical framework. The study was based on the Tongji-Huaxi-Shuangliu Birth Cohort, with data collected during early and middle pregnancy. Participants included GDM cases and controls matched in a 1:2 ratio for fatty acid assessments, and in a 1:1 ratio for 16S rRNA gene sequencing and shotgun metagenomic sequencing. Data acquisition encompassed demographic and clinical information through questionnaires, along with plasma and fecal samples for biochemical and microbiota analyses. Statistical analyses included conditional logistic regression, linear mixed-effects models, and Mendelian randomization, with mediation and interaction analyses to assess the role of microbiota. GDM, gestational diabetes mellitus; OGTT, 75-g oral glucose tolerance test; SNPs, single-nucleotide polymorphisms

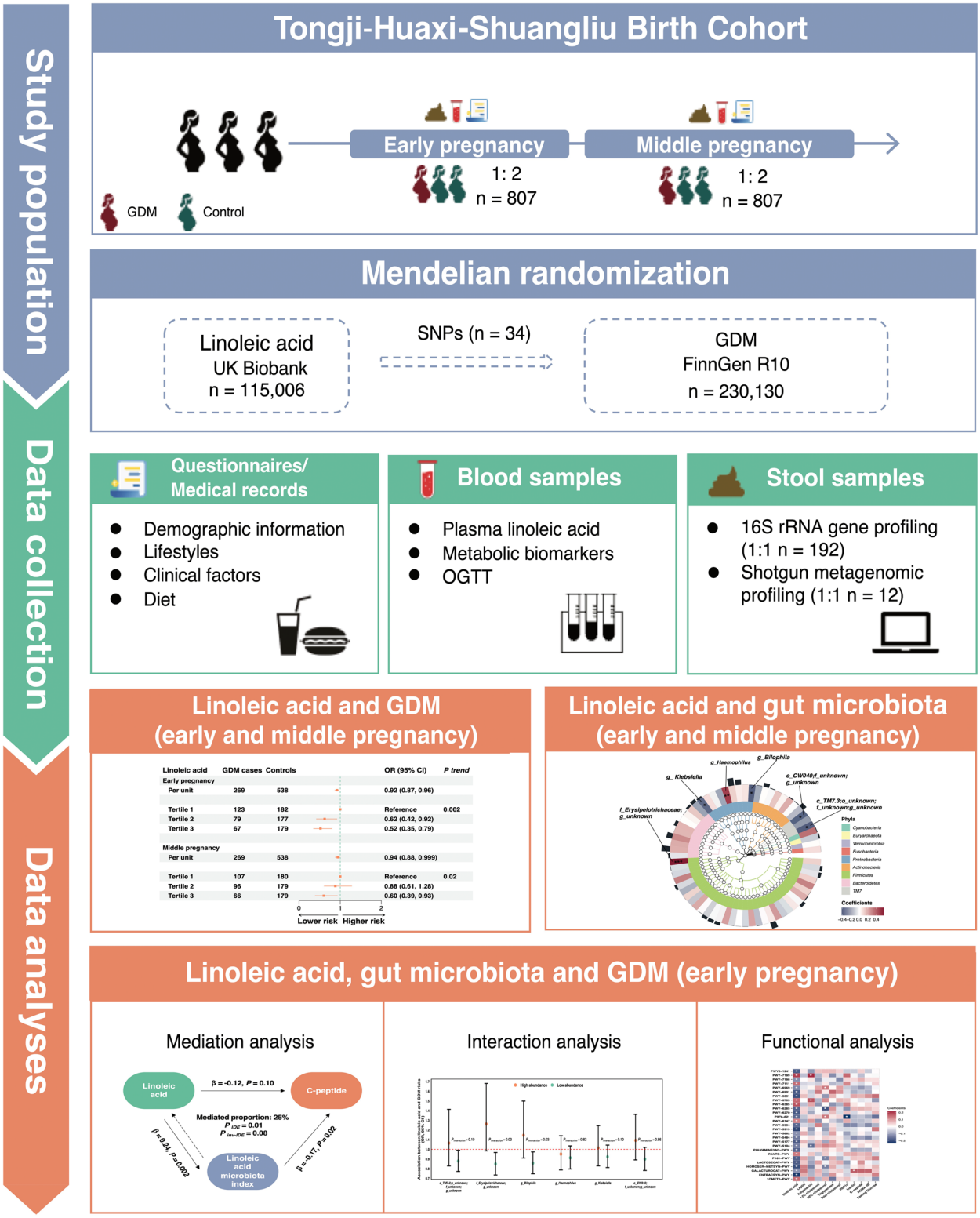

Fig. 1 (See legend on previous page.)

gene families, was conducted with HUMAnN (v3.0.0-alpha.3). For downstream analyses, species were filtered to exclude markers with low overall abundance (< 0.01%) and those present in fewer than 10% of samples. Microbial pathway filtration was performed through a multi-step selection process as previously described [25]. In brief, pathways with the top 50% of mean abundance and the top 50% of variance were retained. The remaining pathways were clustered using the R function cutree at a height of 0.6, and a representative pathway was selected for each cluster. Ultimately, 66 pathways were included in the final analyses. All the microbial features were arcsine square root transformed and were subsequently converted into *Z*-score before the analyses.

Assessment of covariates

Maternal demographics (age and education), lifestyles (dietary intake, cigarette smoking, alcohol drinking, and physical activity), and clinical factors (pre-pregnancy BMI, parity, GDM history, and family history of diabetes) were collected via structured questionnaires and medical records. Physical activity was assessed using the Chinese Pregnancy Physical Activity Questionnaire, with energy expenditure quantified in terms of metabolic equivalent of task (MET) hours per week, based on the types of activities and their respective durations [26]. Self-reported dietary intake categories were as follows: meat (0, ≤50, ≤100, >100 g/day), vegetables and fruits (≤50, ≤100, ≤150, >150 g/day), eggs (0, ≤1, >1/day), and dairy product (0, <250, ≥250 ml/day). Metabolic biomarkers were evaluated using fasting blood samples from early pregnancy among 269 GDM cases and 538 controls as previously described [9], including fasting plasma glucose, serum insulin, C-peptide, hemoglobin A1 C (HbA1c), total cholesterol, triglycerides, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, adiponectin, and leptin. The homeostatic model assessment for insulin resistance (HOMA-IR) was calculated as fasting glucose (mmol/L) × fasting insulin (mIU/L)/22.5.

Statistical analysis

Associations between LA and GDM

Univariable conditional logistic regression models were employed to compare baseline characteristics between GDM cases and their matched controls. In the single time-point analysis, odds ratios (ORs) and corresponding 95% confidence intervals (CIs) were estimated using multivariable conditional logistic regression models to assess the association between LA levels during early and middle pregnancy and the risk of GDM. Adjustments were made for maternal age (years, continuous), education (primary or middle school, high school, and college or above), gestational age at baseline blood collection (weeks, continuous), parity (nulliparous and multiparous), cigarette smoking (ever and never), alcohol drinking (ever and never), physical activity (continuous, MET-hours/week), pre-pregnancy BMI (< 18.5, 18.5–24, 24–28, and ≥28 kg/m^2 based on the Chinese criteria for overweight and obesity) [27], family history of diabetes (yes and no), and history of GDM (yes and no). LA levels were analyzed both as a categorical variable, divided into tertiles based on the distribution among controls, and as a continuous variable, expressed as the percentage increase by weight of total fatty acids. To examine linear trends across tertiles of LA, pregnant women were assigned the median value in each tertile, which was then modeled as a continuous variable. To explore the longitudinal association between plasma LA levels and the risk of GDM, we used linear mixed models to examine the joint association, incorporating LA data from both early and middle pregnancy and accounting for participant-specific random intercepts and random effects for the matched case–control pairs, with adjustments for the above covariates and stage of pregnancy (i.e., early and middle pregnancy). Partial Spearman's correlation coefficients were calculated between LA measured in early pregnancy and multiple metabolic biomarkers including fasting plasma glucose, serum insulin, C-peptide, HbA1c, total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol, adiponectin, leptin, and HOMA-IR after adjusting for the aforementioned covariates. Sensitivity analyses were conducted by (1) including BMI as a continuous variable; (2) further adjusting for dietary intake of meat, vegetables, fruits, eggs, and dairy products; (3) excluding women with history of GDM or family history of diabetes during early and middle pregnancy, and (4) exluding women with a history of smoking and drinking. We also performed exploratory subgroup analyses by age (< 30 and ≥30 years), BMI (< 24 and ≥24 kg/m^2), and physical activity(< 116.68 and ≥116.68 MET-hours/week), with 116.68 representing the median physical activity level. *P* values for interaction were evaluated using a likelihood ratio test, comparing models with and without the interaction term between LA and stratifying variables.

Mendelian randomization analyses

Two-sample MR analyses were conducted to examine the causal relationship between plasma LA and GDM using genome-wide association study (GWAS) data. GWAS data for LA were obtained from the IEU OpenGWAS database (https://gwas.mrcieu.ac.uk/, GWAS IDs: ebi-a-GCST90092881), comprising 115,006 participants from European ancestry [28]. GWAS data for GDM outcomes were from the FinnGen consortium, specifically from

Release 10 (https://finngen.gitbook.io/documentation), with the dataset for 14,718 GDM cases and 215,592 controls. Forty-one single-nucleotide polymorphisms (SNPs) associated with LA were selected based on their genome-wide significance ($P < 5 \times 10^{-8}$) and the absence of linkage disequilibrium ($R^2 < 0.001$ within a window size of 10,000 kilobases). Linkage disequilibrium was assessed using the PLINK clumping method with data from the 1000 Genomes European panel. The SNP (rs1260326) showed a significant association with GDM ($P < 5 \times 10^{-5}$). To reduce bias and control for type 1 error rates, this SNP was removed from the analysis, ensuring that the SNPs used as instrumental variables do not have a direct association with the outcome [29]. Moreover, four SNPs that were not present in the outcome GWAS summary data were omitted. During the harmonization process of exposure and outcome data, two palindromic SNPs with intermediate allele frequencies were identified and subsequently removed. Ultimately, a total of 34 instrumental variables were carefully selected for our Mendelian randomization analyses. For each variant incorporated into the genetic instruments, F-statistics were subsequently computed according to literature and an F-statistic below 10 implies the presence of weak instrument bias [30, 31].

The random-effects multiplicative inverse-variance weighted (IVW) method, which allows for heterogeneity for the SNPs used in the instruments, was used as the primary MR method. MR-Egger, weighted median (WM), simple mode, and weighted mode approaches were utilized as complementary methods. To evaluate heterogeneity, Cochran's *Q* test was employed. If the Cochran's *Q* test yielded a $P < 0.05$, a random-effects model would be employed in the subsequent analyses. Conversely, if the *P* was ≥ 0.05, a fixed-effects model would be utilized [32]. Subsequently, the MR-Egger intercept test was conducted to assess horizontal pleiotropy. Mendelian randomization pleiotropy residual sum and outlier (MR-PRESSO) was employed to identify outliers (i.e., potentially pleiotropic SNPs) and estimate the causal effect after their exclusion, with 10,000 simulations performed [33]. Finally, a leave-one-out analysis was conducted to ensure the robustness of the combined effect estimate, thus confirming that the removal of any single SNP did not unduly influence the MR analyses [34].

Associations between LA and gut microbiota

We calculated α-diversity and β-diversity metrics for early and middle pregnancy based on the relative abundance of ASVs for each sample using the vegan package in R. α-diversity, quantified by the Shannon index, Simpson index, and Chao 1 index, was compared across LA tertiles based on the distribution among controls using a linear mixed-effects model to account for repeated measures within participants. β-diversity was evaluated via permutational multivariate analysis of variance (PERMANOVA) utilizing a Bray–Curtis dissimilarity matrix to assess overall community composition differences across LA tertiles. To examine associations between individual microbial genus and LA levels, linear mixed-effects models (for joint association) were constructed, incorporating participant-specific identifiers (IDs) and matched case–control IDs as random effects to account for intra-individual variability and the paired study design based on data collected from early and middle pregnancy. For the genera that showed significant associations in the joint association model, additional analyses were performed to assess single time-point associations using linear models. Dynamic associations (i.e., delta associations) were estimated based on temporal changes in LA from early to middle pregnancy and microbiota abundance in middle pregnancy. The associations between LA and gut microbiota in early and middle pregnancy were also assessed separately. All models were adjusted for maternal age, education level, gestational age at baseline blood collection, pre-pregnancy BMI, sampling time, dietary intake (meat, vegetables, fruits, eggs, and dairy products), and GDM status. *P* values were adjusted for multiple comparisons using the Benjamini–Hochberg procedure to control the false discovery rate (FDR), with a corrected *P* value threshold of < 0.25 considered statistically significant [35]. This threshold was used to balance sensitivity and specificity in hypothesis generation, as exploratory analyses prioritize minimizing missed biological relationships (Type II errors) over strict control of false positives (Type I errors). By using this lenient FDR-adjusted threshold, we aimed to systematically identify plausible biological pathways for future mechanistic studies while maintaining statistical transparency in high-dimensional data exploration.

Associations between LA-associated microbial genera and metabolic biomarkers

LA microbiota index (LAMI) was calculated based on microbiota data. First, genera showing significant positive correlations (abundance increasing with LA levels) and negative correlations (abundance decreasing with LA levels) with plasma LA were identified through linear mixed-effects models. Subsequently, for each individual, the mean abundance of negatively correlated genera was subtracted from that of positively correlated genera. Finally, this score was standardized across the cohort using *z*-score normalization [36]. To verify the reliability of the established LAMI, we employed a linear mixed-effects models to analyze the relationship between LA levels and LAMI based on data from early and middle pregnancy, adjusting for the same covariates as those

used in the initial LA-microbiota association study. Additionally, we used multivariable linear regression models to assess the associations of LAMI with various metabolic biomarkers (fasting plasma glucose, serum insulin, C-peptide, HbA1c, total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol, adiponectin, leptin, and HOMA-IR), adjusting for the same covariates as in the previous conditional logistic models. *Z*-score normalization was performed for metabolic biomarkers.

Mediation analyses for gut microbiota in the association between LA and metabolic biomarkers in early pregnancy

For biomarkers significantly associated with both LAMI and LA, we conducted a bidirectional mediation analysis using the mediate function from the R package mediation to explore the mediating role of gut microbiota in the relationship between plasma LA and metabolic biomarkers. Here, the levels of plasma LA, gut microbiota, and metabolic biomarkers were all from the early pregnancy. Prior to the mediation analysis, we performed *z*-score normalization for plasma LA levels. The mediation models were adjusted for maternal age, education, gestational age at baseline blood collection, parity, cigarette smoking, alcohol drinking, physical activity, pre-pregnancy BMI, family history of diabetes, and history of GDM.

Interaction analyses between gut microbiota and LA for GDM in early pregnancy

To explore the statistical interaction between plasma LA and gut microbiota for GDM, we utilized a standard statistical approach to examine the interaction between LA and individual genera based on data from early pregnancy [37, 38]. We categorized low and high abundance groups using the second tertile of the relative abundance of LA-related microbial genera. Multivariable conditional logistic models were constructed, incorporating LA levels, the abundances of LA-related microbial genera, and the interaction term between these variables, with adjustments for maternal age, education, gestational age at baseline, parity, cigarette smoking, alcohol drinking, physical activity, pre-pregnancy BMI, family history of diabetes, and history of GDM. *P* values for interaction were determined using the likelihood ratio test by comparing models with and without the interaction term. Furthermore, we analyzed the relationships between LA levels and GDM within subgroups defined by different abundance levels of the specified genera using logistic regression models, adjusting for the aforementioned covariates and matched case–control IDs.

Species-level and functional analyses

Based on the genus-level associations identified from 16S rRNA data, we conducted species-level analysis using metagenomic data. Multivariable linear regression models were applied to early pregnancy data to examine the relationships between metabolic biomarkers and LA-associated microbiota at the species level. In subsequent functional analyses, LA-related pathways were identified and further evaluated for their associations with metabolic biomarkers, with statistical significance defined as $P < 0.25$ corrected using the Benjamini–Hochberg procedure. Additionally, key enzymes within significant pathways were analyzed for their associations with metabolic markers using multivariable linear regression models. All models were adjusted for the same covariates as those used in the genus-level analyses.

All statistical analyses were conducted using RStudio software (version 2021.09.1) or STATA 18.0 (Stata Corporation). A two-tailed $P < 0.05$ was considered statistically significant unless otherwise specified.

Results

Characteristics of study participants

Of the 807 participants, the mean maternal age (standard deviation) was 27.78 years (3.88) and the mean gestational age was 10.38 weeks (1.95) at baseline (Table 1). Pregnant women with GDM exhibited a significantly higher pre-pregnancy BMI compared to controls (22.25 $kg/m^2 \pm 3.27$ vs. 21.14 $kg/m^2 \pm 2.98$, $P < 0.001$). While most baseline characteristics showed no significant differences between the two groups, pregnant women with GDM were more likely to have a family history of diabetes (10.78% vs. 5.76%, $P = 0.001$) and a history of GDM (5.58% vs. 1.30%, $P = 0.01$), compared to controls. To understand potential confounding, we quantitatively assessed the relationships of circulating LA and microbiota diversity with pre-pregnancy BMI, family history of diabetes, and a history of GDM using linear mixed-effects modeling (Additional file 1: Fig. S1). Baseline characteristics were similar between 192 pregnant women with 16S profiling and 615 without (Additional file 1: Table S1).

Association between LA and GDM in observational analyses

Elevated LA levels were associated with a lower risk of GDM in multivariable conditional logistic models (Fig. 2A). During early pregnancy, adjusted ORs for GDM significantly decreased across LA tertiles: for Tertile 1 (≤ 23.10), Tertile 2 (23.10–26.02), and Tertile 3 (> 26.02), ORs were 1.00, 0.62 (95% CI, 0.42–0.92), and 0.52 (95% CI, 0.35–0.79), respectively (P trend = 0.002). Each unit increase in the LA percentage was associated with an OR of 0.92 (95% CI, 0.87–0.96). During middle pregnancy, similar but slightly weaker associations were observed: for Tertile 1 (≤ 19.96), Tertile 2 (19.96–22.38), and Tertile 3 (> 22.38), ORs were 1.00, 0.88 (95% CI, 0.61–1.28),

Table 1 Baseline characteristics of study participants

	Total ($N=807$)[a]	GDM cases ($n=269$)[a]	Controls ($n=538$)[a]	*P*values
Maternal age (years)	27.78 ± 3.88	27.89 ± 4.02	27.73 ± 3.82	NA
Gestational age (weeks)	10.38 ± 1.95	10.30 ± 2.08	10.42 ± 1.89	NA
Pre-pregnancy BMI (kg/m^2)	21.51 ± 3.12	22.25 ± 3.27	21.14 ± 2.98	< 0.001
College education or above	332 (41.14)	106 (39.41)	226 (42.01)	0.65
Ever smoking	42 (5.20)	17 (6.32)	25 (4.65)	0.32
Ever drinking	147 (18.22)	53 (19.70)	94 (17.47)	0.45
Physical activity, MET-hours/week	129.55 ± 79.30	124.00 ± 77.23	132.33 ± 80.24	0.17
Nulliparous	403 (49.94)	140 (52.04)	263 (48.88)	0.34
Family history of diabetes	60 (7.43)	29 (10.78)	31 (5.76)	0.001
History of GDM	22 (2.73)	15 (5.58)	7 (1.30)	0.01

[a] Data are reported as mean ± standard deviation, or number (percentage)

BMI body mass index, *GDM* gestational diabetes mellitus, *MET* metabolic equivalent task

and 0.60 (95% CI, 0.39–0.93), respectively (P trend = 0.02). Each unit increase in LA percentage was associated with an OR of 0.94 (95% CI, 0.88–0.999). The linear mixed-effects model showed lower LA levels in GDM patients compared to controls ($\beta = -0.66$; 95% CI, −1.03 to −0.29). Higher LA levels were correlated with a more favorable metabolic profile, characterized by higher adiponectin ($P < 0.001$) and lower triglycerides ($P < 0.001$), HbA1c ($P = 0.04$), and C-peptide ($P = 0.04$) (Fig. 2B). Sensitivity analyses confirmed these associations (Additional file 1: Table S2). Subgroup analyses showed no statistical heterogeneity by age, pre-pregnancy BMI, and physical activity (all P for interaction ≥ 0.19, Additional file 1: Table S3-S5).

Association between LA and GDM in MR analyses

The selected 34 SNPs for MR analyses explained approximately 3.6% of the variance in plasma LA levels, with a mean F-statistic of 127.08. In primary MR analyses using the MRE-IVW method, each unit increase of LA was associated with a 16% reduction in GDM risk (OR 0.84, 95% CI 0.72, 0.95). Consistent results were obtained from the weighted median, MR Egger, weighted mode, and simple mode methods (Fig. 2C, D). Both the IVW method ($Q = 54.10$, $P = 0.01$) and MR-Egger method ($Q = 54.10$, $P = 0.01$) indicated potential heterogeneity among the instrumental variables, leading to the use of a random effects model for reliable estimates. MR-Egger regression (intercept = − 0.0002, $P = 0.97$) showed no potential pleiotropy, and MR-PRESSO did not identify any outliers (all $P > 0.1$). Stability of the findings was confirmed through the leave-one-out analysis, which showed no significant change when any single SNP was excluded (Additional file 1: Fig. S2).

Association between LA and gut microbiota

The α-diversity and microbial community composition showed no significant differences across different LA levels (Fig. 3A, B, Additional file 1: Fig. S3). At the genus level, *Haemophilus* and an unidentified genus within the family *Erysipelotrichaceae* were positively associated with increased LA levels. Conversely, *Klebsiella*, *Bilophila*, an unidentified genus within the class *TM7 -3*, and an unidentified genus within the order *CW040* were negatively associated with LA levels (Fig. 3C, E). Four LA-associated genera were further associated with changes in LA levels from early to middle pregnancy (delta association). Additionally, three genera were associated with LA levels in early pregnancy, and five were associated with LA levels in middle pregnancy (Fig. 3D). The LAMI based on these 6 genera, was positively associated with plasma LA levels across all 192 pregnant women ($P < 0.001$), within GDM cases ($n = 96$) ($P = 0.04$), and non-GDM controls ($n = 96$) ($P = 0.004$) using data from both early and middle pregnancy (Additional file 1: Fig. S4).

Association between LA and metabolic health and the mediating role of gut microbiota

LAMI was found to be inversely correlated with several metabolic biomarkers, including HOMA-IR ($P = 0.02$), insulin ($P = 0.03$), and C-peptide ($P = 0.01$) in early pregnancy (Fig. 4A). Given the significant correlations between LAMI and C-peptide, and the association between LA and C-peptide, we hypothesized that the gut microbiome might mediate the effects of LA on host metabolic health. In the bidirectional mediation analysis using data collected from early pregnancy, LAMI was found to mediate the association of the plasma LA levels with C-peptide (mediation proportion = 25%; $P = 0.01$) (Fig. 4B).

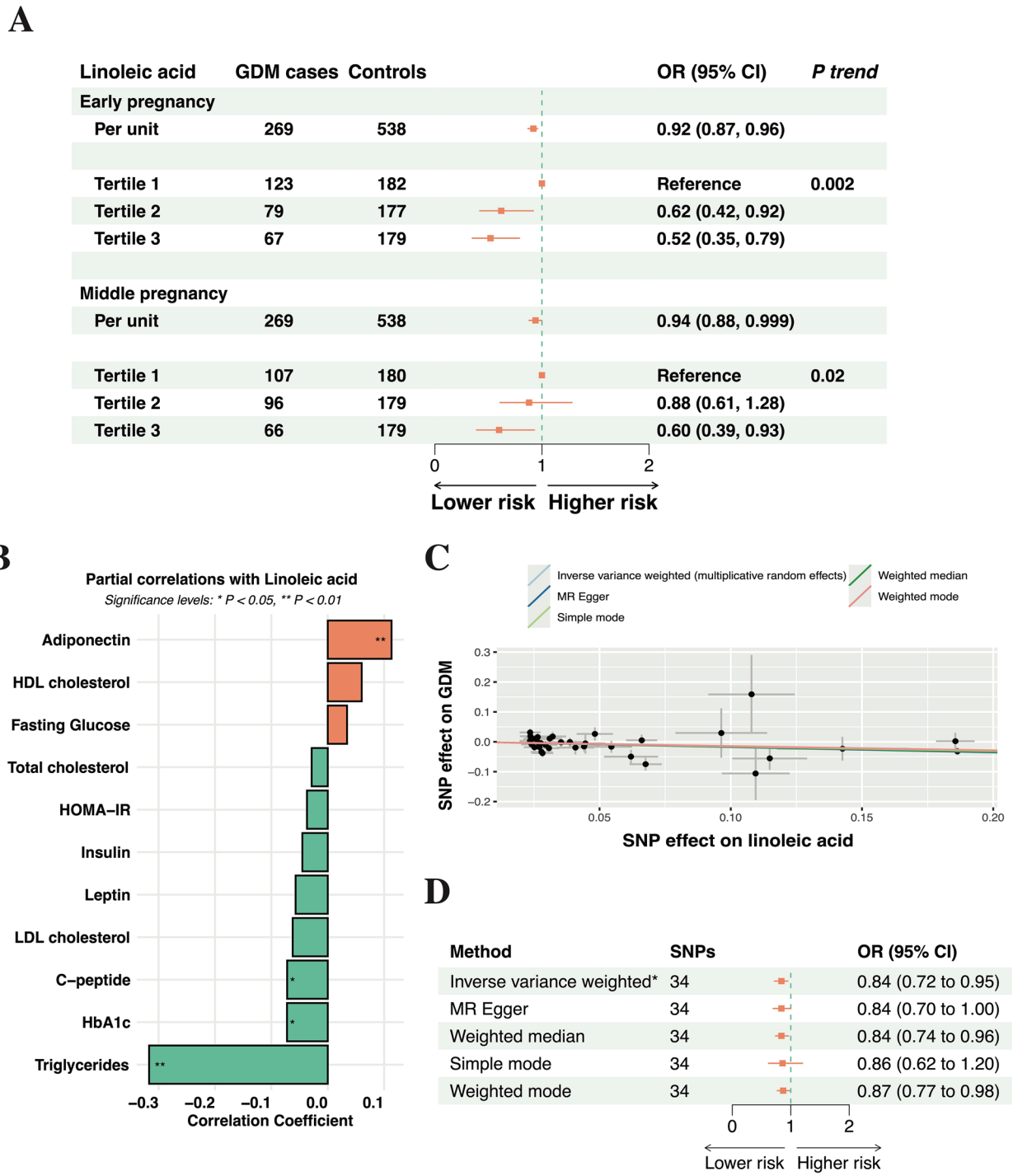

Fig. 2 Association and causal analyses for linoleic acid with GDM. **A** Association of linoleic acid levels with GDM risk across pregnancy. Adjusted for maternal age (years, continuous), education (primary or middle school, high school, and college or above), gestational age at baseline blood collection (weeks, continuous), parity (nulliparous and multiparous), cigarette smoking (ever and never), alcohol drinking (ever and never), physical activity (continuous, MET-hours/week), pre-pregnancy BMI (< 18.5, 18.5–24, 24–28, and ≥ 28 kg/m^2), family history of diabetes (yes and no), and history of GDM (yes and no). **B** Correlations of linoleic acid levels with metabolic biomarkers in early pregnancy. Partial Spearman's correlation coefficients were calculated, adjusting for the same covariates mentioned above. **C** Scatter plot of causal association between plasma linoleic acid and GDM. The slope of each line represents the MR effect estimate from different methods. **D** Forest plot for MR results. The "*" refers to random-effects multiplicative inverse-variance weighted method. BMI, body mass index; GDM, gestational diabetes mellitus; HbA1c, hemoglobin A1 C; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment for insulin resistance; LDL, low-density lipoprotein; MET, metabolic equivalent task; MR, mendelian randomization; SNPs, single-nucleotide polymorphisms

Association of LA with GDM modified by gut microbiota

A significant interaction was detected between LA levels and the abundance of *Bilophila* in early pregnancy in relation to GDM (*P* interaction = 0.03, Fig. 4C). Among pregnant women with low relative *Bilophila* abundance (≤ 0.067%), an inverse relationship was observed between LA levels and GDM (OR 0.86, 95% CI 0.75, 0.97). In contrast, no significant correlation was found in women with high *Bilophila* abundance (> 0.067%) (OR 1.15, 95% CI 0.91, 1.50) (Fig. 4C). Similarly, a significant interaction was observed between LA levels and the abundance of *f_Erysipelotrichaceae; g_unknown* in relation to GDM (*P* interaction = 0.03, Fig. 4C). Among women with low *f_Erysipelotrichaceae; g_unknown* abundance (≤ 0.613%), an inverse relationship was found between LA levels and GDM (OR 0.85, 95% CI 0.74, 0.97). No significant correlation was noted in women with high *f_Erysipelotrichaceae; g_unknown* abundance (> 0.613%) (OR 1.26, 95% CI 0.98, 1.68).

Species-level and functional analyses

Species-level analyses showed significant positive associations between *Bilophila wadsworthia* (average relative abundance = 0.10%) and several metabolic biomarkers, including positive associations with C-peptide, insulin, and HOMA-IR (Fig. 5A). With multivariable adjustments, 27 microbial functional pathways were associated with LA (FDR-adjusted *P* < 0.25, Fig. 5B). Nine of these pathways related to carbohydrate degradation, nucleotide synthesis, amino acid and secondary metabolite production, and energy generation were linked to changes in metabolic markers such as HbA1c, adiponectin, HDL cholesterol, and insulin (FDR-adjusted *P* < 0.25, Fig. 5B). Among the pathways identified, GALACTUROCAT-PWY (D-galacturonate Degradation I), which belongs to the carbohydrate degradation category, was positively associated with insulin levels. This key pathway is involved in breaking down D-galacturonate, an important component of pectin found in plant cell walls. It plays a crucial role in carbohydrate utilization by gut microbiota, converting D-galacturonate into intermediates that can be further metabolized for energy production and other biosynthetic processes. Moreover, the key enzyme EC 4.2.1.7 (D-altronate dehydratase) within this pathway was positively associated with leptin and fasting glucose, but was negatively associated with HDL cholesterol (Fig. 5C). Among the microbial contributors to this enzyme, *Klebsiella* ranked third in overall contribution, which was also found to be negatively associated with LA levels (Fig. 5D).

Discussion

In our prospective study, higher plasma LA levels in both early and middle pregnancy were inversely associated with the risk of GDM. The LA associated microbiota mediated the relationship between LA and metabolic biomarkers. Notably, *Bilophila* modified the inverse association between LA and GDM risk, which was stronger in pregnant women with lower *Bilophila* abundance. The GALACTUROCAT-PWY pathway may play a significant role in the metabolic effect of LA. These findings highlight the intricate interplay among LA, gut microbiota, and GDM risk.

Previous studies on the association between LA and GDM reported neutral or inconsistent effects [8–10, 14]. Compared with previous studies, our study uniquely measured LA levels at two distinct stages of pregnancy and applied MR analysis to validate the causal relationship. This methodological approach provides stronger evidence for the protective effect of LA against GDM. Interestingly, the only US-based study that explored longitudinal shifts in LA levels during pregnancy failed to establish a negative association between LA and GDM risk [14]. This discrepancy may be due to overlooked factors like the regulatory role of the gut microbiota. Our study offers novel insights into this aspect, demonstrating

(See figure on next page.)

Fig. 3 Effect of linoleic acid on gut microbiota diversity and specific bacterial genera. **A** Shannon index across linoleic acid tertiles. Median lines, boxes (25 th–75 th percentiles), and whiskers (1.5 times the box length) show data distribution, with outliers marked. *P* values were from the linear mixed-effects model. Sample sizes (*n*) are provided for each group. **B** Principal coordinates analysis of beta diversity index changes during pregnancy across linoleic acid tertiles. *P* values were from the PERMANOVA. Sample sizes (*n*) are provided for each group. **C** Associations of linoleic acid levels with individual gut microbial genera. Significant associations of linoleic acid levels with microbial genera (FDR-adjusted *P* < 0.25) are presented, which are overlaid onto their taxonomic information. The innermost ring and phylogenetic trees use colors to distinguish major phyla. The height of the outer bars corresponds to the mean relative abundance of each microbial genus. **D** Associations of genera identified in the joint association analysis with linoleic acid in early and middle pregnancy. **E** Significant associations between linoleic acid levels and microbial genera are highlighted. All models were adjusted for GDM status (yes and no), maternal age (years, continuous), education (primary or middle school, high school, and college or above), gestational age at baseline blood collection (weeks, continuous), pre-pregnancy BMI (< 18.5, 18.5–24, 24–28, and ≥ 28 kg/m^2), and intake of meat (0, ≤ 50, ≤ 100, and > 100 g/day), vegetables and fruits (≤ 50, ≤ 100, ≤ 150, and > 150 g/day), eggs (0, ≤ 1, and > 1/day), and dairy product (0, < 250, and ≥ 250 ml/day). FDR, false discovery rate; GDM, gestational diabetes mellitus; PERMANOVA, permutational multivariate analysis of variance

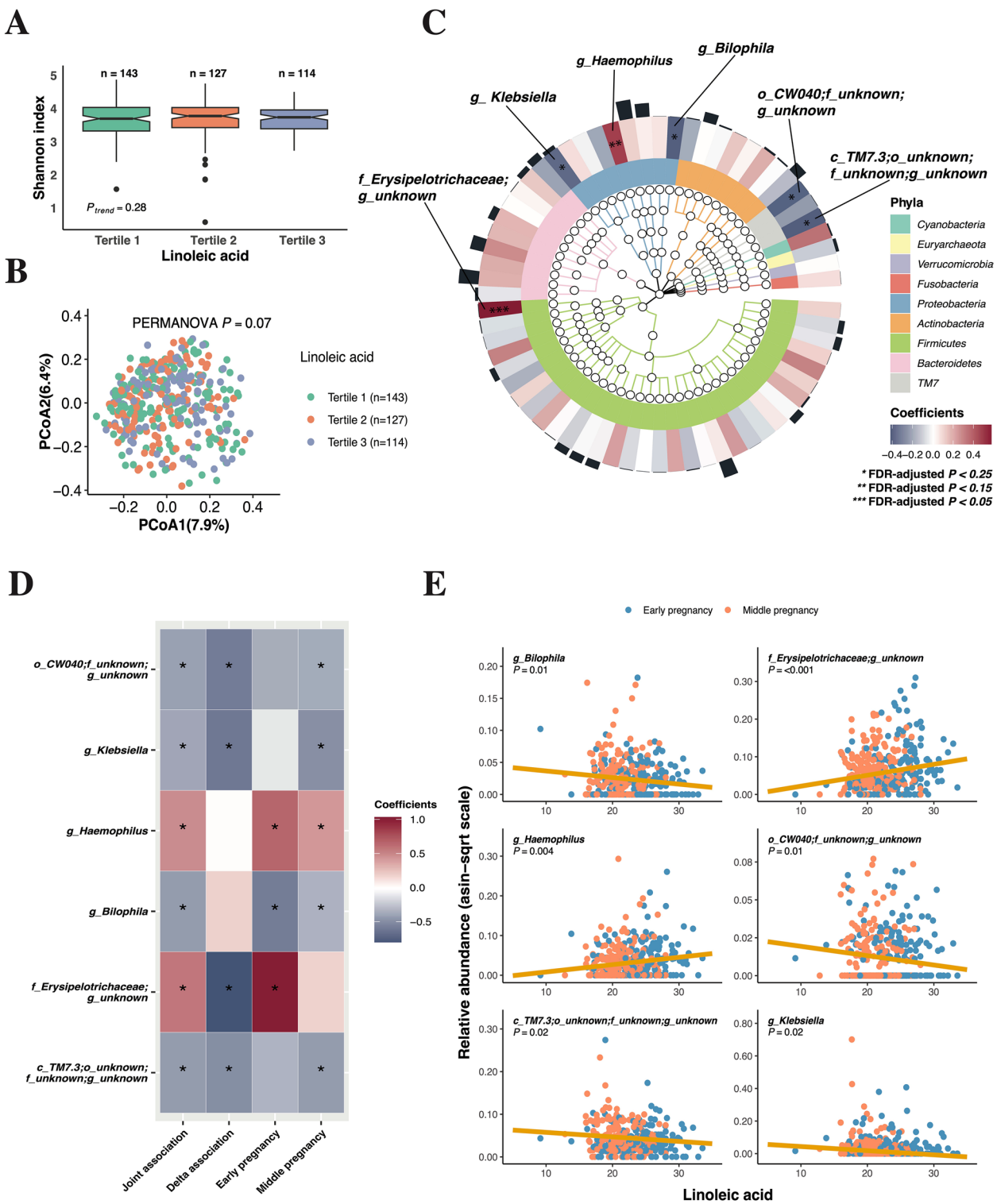

Fig. 3 (See legend on previous page.)

that the LA-associated microbiota mediated the relationship between plasma LA levels and the metabolic biomarker, C-peptide, suggesting an alternative mechanism for LA's protective effect on GDM. Furthermore, we found that *Bilophila* modified the association between LA and GDM, with a stronger inverse correlation in women with lower *Bilophila* abundance. This indicates that LA's protective effect on GDM risk is modified by

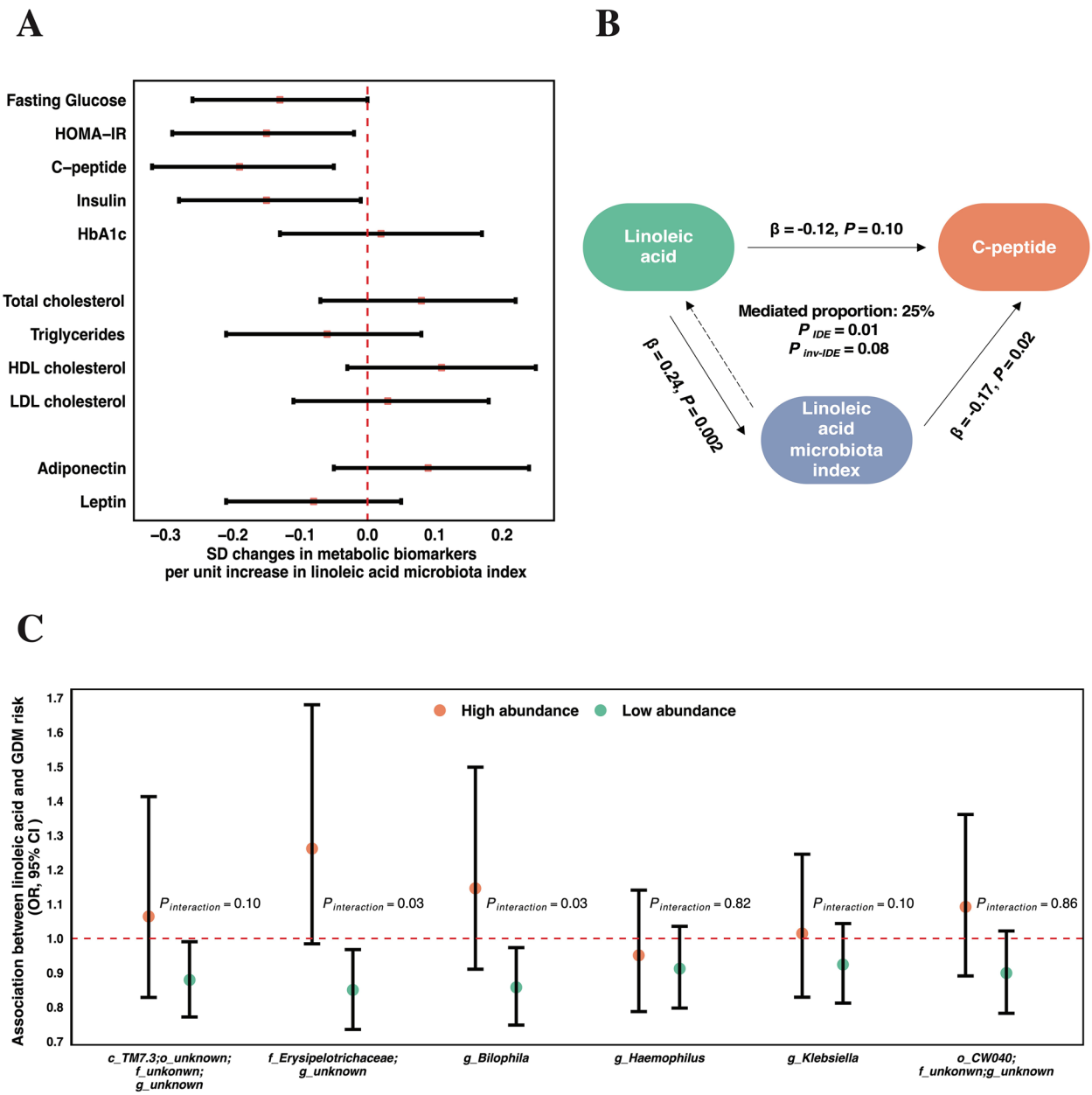

Fig. 4 Mediation and interaction analyses for linoleic acid, gut microbiota, and metabolic health in early pregnancy. **A** Effect of linoleic acid microbiota index on metabolic biomarkers. **B** Mediation analysis of linoleic acid microbiota index for the association of linoleic acid with C-peptide. P_{IDE} and $P_{inv\text{-}IDE}$ were estimated by the bidirectional mediation analysis. P_{IDE} indicates the *P* value for the indirect effect and $P_{inv\text{-}IDE}$ indicates *P* value for the inverse indirect effect. The full arrowed lines show the effect of linoleic acid on C-peptide mediated by linoleic acid microbiota index. Inverse mediation was performed to check whether linoleic acid microbiota index can influence C-peptide through linoleic acid. **C** Interaction between linoleic acid levels and specific gut microbiota for GDM risk. Low and high abundance groups were defined based on the second tertile of relative abundance for LA-related microbial genera. The *P* value for interaction was derived from the interaction term between LA-related microbial genera and LA levels in the multivariable conditional logistic regression models. All models were adjusted for maternal age (years, continuous), education (primary or middle school, high school, and college or above), gestational age at baseline blood collection (weeks, continuous), parity (nulliparous and multiparous), cigarette smoking (ever and never), alcohol drinking (ever and never), physical activity (continuous, MET-hours/week), pre-pregnancy BMI (< 18.5, 18.5–24, 24–28, and ≥ 28 kg/m^2), family history of diabetes (yes and no), and history of GDM (yes and no). GDM, gestational diabetes mellitus; HbA1c, hemoglobin A1 C; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment for insulin resistance; IDE, indirect effect; inv-IDE, inverse indirect effect; LDL, low-density lipoprotein

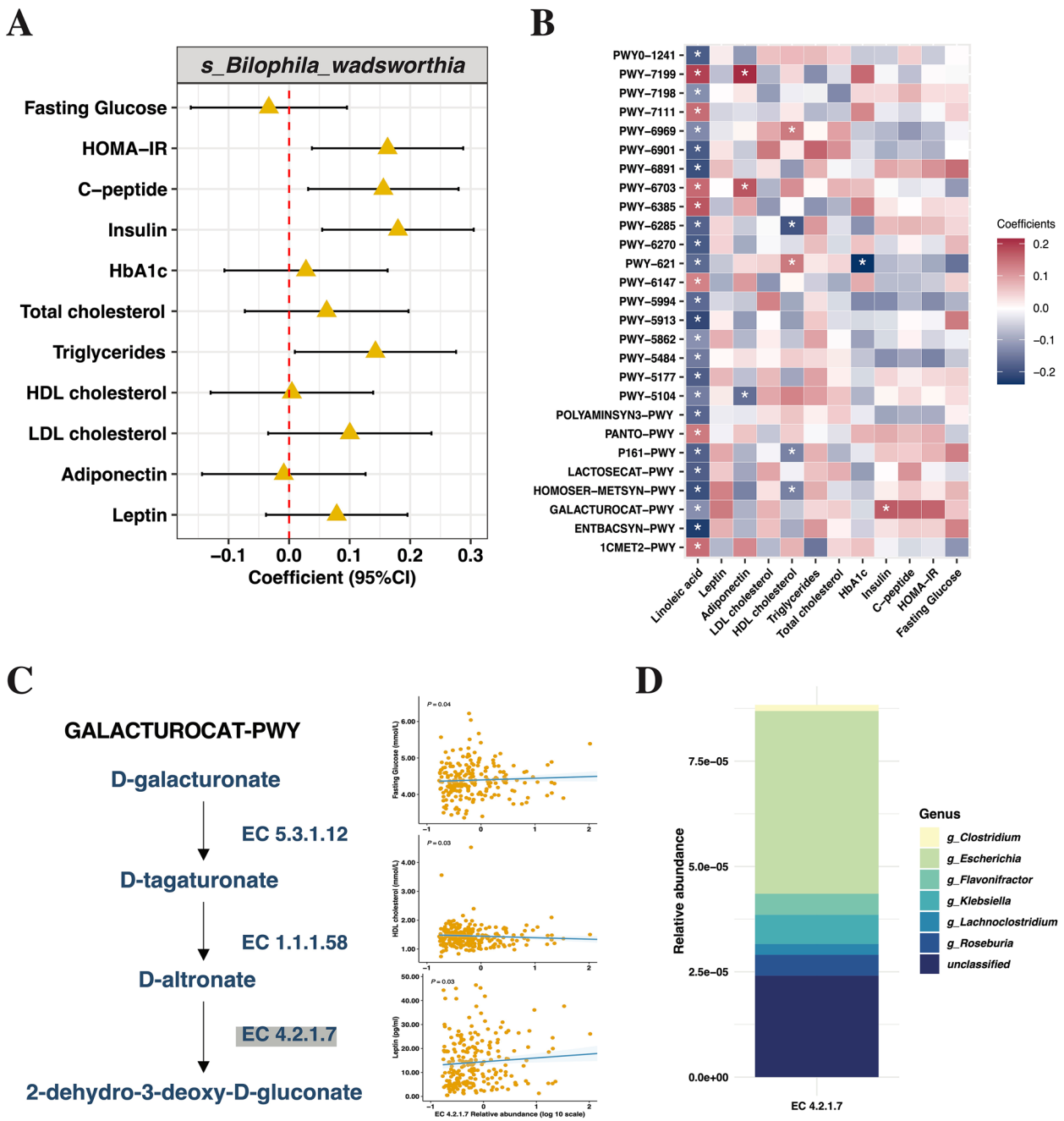

Fig. 5 Association of linoleic acid-related microbiota and pathways with metabolic markers in early pregnancy. **A** Associations between *Bilophila wadsworthia* (average relative abundance = 0.10%) and metabolic markers. All models were adjusted for maternal age (years, continuous), education (primary or middle school, high school, and college or above), gestational age at baseline blood collection (weeks, continuous), parity (nulliparous and multiparous), cigarette smoking (ever and never), alcohol drinking (ever and never), physical activity (continuous, MET-hours/week), pre-pregnancy BMI (< 18.5, 18.5–24, 24–28, and ≥ 28 kg/m^2), family history of diabetes (yes and no), and history of GDM (yes and no). **B** Associations of linoleic acid and metabolic markers with metagenomic pathways. All models were adjusted for GDM status, maternal age, education, gestational age at baseline blood collection pre-pregnancy BMI, and intake of meat, vegetables and fruits, eggs, and dairy product. **C** Key steps in the D-GALACTUROCAT-PWY are shown with corresponding enzymes. Scatter plots show the correlations between the relative abundance of key enzyme (EC 4.2.1.7) and metabolic markers. **D** Microbiota contributing to EC 4.2.1.7 enzyme. HbA1c, hemoglobin A1 C; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment for insulin resistance; LDL, low-density lipoprotein

the gut microbiota, particularly the abundance of *Bilophila*. Prior studies have shown that diets high in animal products increase *Bilophila* abundance [39, 40]. *Bilophila wadsworthia*, a species within this genus, has been linked to higher inflammation, intestinal barrier dysfunction, and bile acid dysmetabolism, all of which contribute to glucose dysregulation [41]. Consistent with these findings, our study also observed that higher *Bilophila wadsworthia* abundance was associated with elevated levels of C-peptide and insulin. Our findings may collectively explain why significant associations between LA and GDM were not observed in populations that generally adhered to a meat-rich diet, such as those in the USA [14].

While the evidence regarding LA and its association with GDM is still limited, findings from type 2 diabetes research provide a compelling basis for understanding LA's potential role in metabolic health. Extensive studies, including comprehensive meta-analyses and observational studies, consistently showed that high dietary intake and elevated circulating LA were significantly associated with lower risks of type 2 diabetes [6, 42, 43]. Moreover, a recent study using MR also found a causal relationship between high levels of LA and a lower risk of type 2 diabetes, as well as lower fasting blood glucose and glycated HbA1c levels [44]. Consistently, high plasma phospholipid LA levels were favorably linked with lower serum LDL cholesterol, triglycerides, and fasting plasma glucose in a Norwegian general population [45]. These findings align with our study, where we also observed that higher LA levels were associated with decreased triglycerides, HbA1c, and C-peptide levels. Such consistent associations across different populations highlight the robustness of LA's beneficial effects on metabolic health.

Our data suggest that the GALACTUROCAT-PWY may play a role in the protective effect of LA on metabolic health. We observed that the relative abundance of the GALACTUROCAT-PWY was inversely associated with LA levels and positively associated with insulin levels. This aligns with recent evidence that D-galacturonate degradation I was elevated in insulin-resistant children, highlighting its potential involvement in metabolic dysregulation [46]. Notably, the key enzyme EC 4.2.1.7 in this pathway was also linked to unfavorable metabolic biomarkers, and the relative abundance of the bacteria (such as *Klebsiella*) associated with this enzyme decreased as LA levels increased in our work. Consistently, high LA levels were shown to associate with higher concentrations of adiponectin, a factor associated with insulin sensitivity, which was also demonstrated in other studies [47, 48]. These findings suggest that LA may enhance insulin sensitivity through its effects on the microbiota, thereby contributing to an improved metabolic profile.

Our findings have strong clinical implications for personalized nutrition strategies that incorporate gut microbiota to more effectively prevent or manage GDM. The bidirectional relationship between dietary components and the gut microbiota is widely recognized [40, 49]. Previous studies showed that LA supplementation increased the abundance of bacterial species capable of metabolizing LA, and thus led to an increase of several metabolites improving metabolic conditions [50]. During pregnancy, the gut microbiota dynamically correlates with host glucose metabolism and plays a crucial role in preserving the host's metabolic function [21]. Similar analyses in the context of erythrocyte n- 6 polyunsaturated fatty acids and type 2 diabetes also reported an important role of gut microbiota [51]. Our findings highlight the importance of considering individual differences in gut microbiota when assessing the impact of dietary interventions on metabolic health.

Our study has several strengths that ensure the validity and reliability of its findings. The longitudinal design, tracking participants from early to middle pregnancy, allows us to investigate longitudinal association between LA levels and GDM risk over time. The integration of multi-omics data and the use of MR analyses enrich the breadth and depth of our research, enabling us to reach valid conclusions about the relationship between LA and GDM. However, we acknowledge inherent limitations. First, as our study used existing linked data from three nested case–control studies in the THSBC, certain analyses only had moderate sample sizes that may lack statistical power to detect subtle associations. Future large-scale studies from diverse populations are needed to corroborate our findings. Second, while the MR analyses confirmed a potential causal association between LA and GDM, the used GWAS data were mostly for Caucasians, and the MR finding may not be generalizable to Chinese. Third, the food questionnaire was limited to broad food categories, which restricted our ability to perform more detailed analyses based on specific nutrient intakes. Instead of directly assessing dietary LA intake through food questionnaires, we used plasma LA levels as a surrogate marker, which was supported by prior evidence [3, 4]. Notably, in a subset analysis of 146 participants with paired fecal and plasma LA measurements in the THSBC, we observed a significant positive correlation between these two biological compartments ($\beta = 0.18$; $P = 0.032$). While this association suggests possible communication between systemic circulation and gut microbial metabolism, the underlying biological mechanisms—whether mediated through intestinal absorption efficiency, microbial modification of LA, or alternative metabolic pathways—demand future mechanistic investigations. Fourth, the mediation analyses only used cross-sectional data for LA, gut

microbiota, and metabolic biomarkers due to data unavailability. Future studies with temporal data, assessing these variables at multiple time points throughout pregnancy, are necessary to better understand the temporal relationships and causal pathways between LA, gut microbiota, and maternal metabolic health.

Conclusions

Our study documented robust evidence for the inverse association between plasma LA levels during pregnancy and the risk of GDM. In addition, it offered novel insights into the potential role of microbiota in modulating LA to improve insulin sensitivity and reduce GDM risk. By uncovering the intricate interplay between LA and gut microbiota, our findings may offer new directions for future research on personalized nutritional strategies for GDM. However, future large-scale studies, along with animal and in vitro experiments, are needed to validate these findings and explore the underlying mechanisms in greater depth.

Abbreviations

ASVs	Amplicon sequence variants
BMI	Body mass index
FDR	False discovery rate
GDM	Gestational diabetes mellitus
HbA1c	Hemoglobin A1C
HDL	High-density lipoprotein
HOMA-IR	Homeostatic model assessment for insulin resistance
IDs	Identifiers
IDE	Indirect effect
inv-IDE	Inverse indirect effect
IVW	Inverse-variance weighted
LA	Linoleic acid
LAMI	LA microbiota index
LDL	Low-density lipoprotein
MET	Metabolic equivalent task
MR	Mendelian randomization
MR-PRESSO	Mendelian randomization pleiotropy residual sum and outlier
PERMANOVA	Permutational multivariate analysis of variance
SNPs	Single-nucleotide polymorphisms
WM	Weighted median

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12916-025-04061-7.

Additional file 1: Table S1 Baseline characteristics of study participants with and without 16S profiling. Table S2 ORs (95% CIs) for GDM comparing tertiles of linoleic acid in early and middle pregnancy in sensitive analysis. Table S3 ORs (95% CIs) for GDM comparing tertiles of linoleic acid in early and middle pregnancy by age. Table S4 ORs (95% CIs) for GDM comparing tertiles of linoleic acid in early and middle pregnancy by BMI. Table S5 ORs (95% CIs) for GDM comparing tertiles of linoleic acid in early and middle pregnancy by physical activity. Fig. S1 Boxplots for linoleic acid and Shannon index across different groups by pre-pregnancy BMI, family history of diabetes and history of GDM. Fig. S2 The plot of the "leave-one-out" analysis to show the influence of individual SNP on the causal effect. Fig. S3 Simpson index and Chao1 index across linoleic acid tertiles. Fig. S4. Relationship between linoleic acid and the linoleic acid microbiota index in different participants.

Additional file 2.

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Authors' contributions

X-FP, AP, and DC conceived the study concept and design. YD, PZ, SZ, NW, TW, SY, JY, and XL contributed to the biospecimen collection. PH, FS, PW, YL, and GL conducted major experiments. JQ, FL, YY, XH, and SL analyzed the data. YH, YW, and JYHW interpreted the data. JQ wrote the first draft of the manuscript. JQ and PH contributed equally to the work. All authors contributed to the critical revision of the manuscript and approved the final version for publication. X-FP, AP, and DC are the guarantors of this work and, as such, had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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Data availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

This study involves human participants and was approved by the Ethics Committee of the Tongji Medical College, Huazhong University of Science and Technology (2017-S225). Participants gave informed consent to participate in the study before taking part.

Consent for publication

All authors have approved the publication of this manuscript.

Competing interests

The authors declare no competing interests.

Author details

[1]Center for Reproductive Medicine, Department of Obstetrics and Gynecology & Section of Epidemiology and Population Health, Ministry of Education Key Laboratory of Birth Defects and Related Diseases of Women and Children, West China Second University Hospital, Sichuan University, Chengdu, Sichuan, China. [2]Ministry of Education Key Laboratory of Environment and Health, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, China. [3]Section of Epidemiology and Population Health & Department of Gynecology and Obstetrics, Ministry of Education Key Laboratory of Birth Defects and Related Diseases of Women and Children & Children's Medicine Key Laboratory of Sichuan Province, West China Second University Hospital, Sichuan University, Chengdu, Sichuan, China. [4]Department of Toxicology, School of Public Health, Anhui Medical University, Hefei, Anhui, China. [5]School of Environment and Guangdong Key Laboratory of Environmental Pollution and Health, Jinan University, Guangzhou, Guangdong, China. [6]Department of Epidemiology and Biostatistics, Ministry of Education Key Laboratory of Environment and Health, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, China. [7]Department of Epidemiology and Population Health, Albert Einstein College of Medicine, Bronx, NY, USA. [8]Department of Epidemiology and Biostatistics, School of Public Health, Chengdu Medical College, Chengdu, Sichuan, China. [9]Department of Science and Education & Shuangliu Institute of Women's and Children's Health, Shuangliu Maternal and Child Health Hospital, Chengdu, Sichuan, China. [10]Department

of Laboratory Medicine, Ministry of Education Key Laboratory of Birth Defects and Related Diseases of Women and Children, West China Second University Hospital, Sichuan University, Chengdu, Sichuan, China. [11]Department of Nutrition and Food Hygiene, Hubei Key Laboratory of Food Nutrition and Safety, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, China. [12]Department of Obstetrics and Gynecology, Ministry of Education Key Laboratory of Birth Defects and Related Diseases of Women and Children, West China Second University Hospital, Sichuan University, Chengdu, Sichuan, China. [13]School of Population Health, The George Institute for Global Health, University of New South Wales, Sydney, NSW, Australia. [14]Shuangliu Institute of Women's and Children's Health, Shuangliu Maternal and Child Health Hospital, Chengdu, Sichuan, China. [15]Biomedical Big Data Center, West China Hospital, Sichuan University, Chengdu, Sichuan, China.

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