See corresponding editorial on page 844.

Associations of dietary diversity with the gut microbiome, fecal metabolites, and host metabolism: results from 2 prospective Chinese cohorts

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ABSTRACT

Background: Dietary diversity is essential for human health. The gut ecosystem provides a potential link between dietary diversity, host metabolism, and health, yet this mechanism is poorly understood.

Objectives: Here, we aimed to investigate the relation between dietary diversity and the gut environment as well as host metabolism from a multiomics perspective.

Methods: Two independent longitudinal Chinese cohorts (a discovery and a validation cohort) were included in the present study. Dietary diversity was evaluated with FFQs. In the discovery cohort ($n = 1916$), we performed shotgun metagenomic and 16S ribosomal ribonucleic acid (rRNA) sequencing to profile the gut microbiome. We used targeted metabolomics to quantify fecal and serum metabolites. The associations between dietary diversity and the microbial composition were replicated in the validation cohort ($n = 1320$).

Results: Dietary diversity was positively associated with α diversity of the gut microbiota. We identified dietary diversity–related gut environment features, including the microbial structure (β diversity), 68 microbial genera, 18 microbial species, 8 functional pathways, and 13 fecal metabolites. We further found 332 associations of dietary diversity and related gut environment features with circulating metabolites. Both the dietary diversity and diversity-related features were inversely correlated with 4 circulating secondary bile acids. Moreover, 16 mediation associations were observed among dietary diversity, diversity-related features, and the 4 secondary bile acids.

Conclusions: These results suggest that high dietary diversity is associated with the gut microbial environment. The identified key microbes and metabolites may serve as hypotheses to test for preventing metabolic diseases. *Am J Clin Nutr* 2022;116:1049–1058.

Keywords: dietary diversity, gut microbiota, metabolomics, gut environment, bile acids, host metabolism

Introduction

A healthy diet is critical for preventing metabolic diseases (1). A most important aspect of a healthy diet is consuming diverse foods, which is universally recommended by various dietary guidelines (2, 3). Dietary diversity is associated with a lower risk of metabolic diseases such as obesity and type 2 diabetes (T2D) (4, 5). A randomized controlled trial suggested that increasing healthy dietary diversity facilitates long-term weight loss among adults with overweight or obesity (6). Although previous studies have indicated that dietary diversity is beneficial for human health, its underlying mechanism is poorly understood.

The intestinal tract is a complex ecological system that links dietary exposures and host metabolic health. The intestinal tract contains a mix of food substrates, digestive juices, digested

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products, and microbes (7, 8). Diet plays a key role in shaping the gut microbiome; the gut microbiome in turn affects food digestion and absorption, producing functional metabolites for the host (7, 9). However, little is known about how dietary diversity may affect the gut environment (including microbes, functional pathways, and metabolites) and its subsequent impact on host metabolic health. The system involving the interconnections among dietary diversity, the gut ecosystem, and host metabolism remains a black box.

Therefore, we systematically examined the longitudinal associations between dietary diversity and gut environment profiles in a deeply phenotyped prospective cohort: the Guangzhou Nutrition and Health Study (GNHS) (10). First, we identified dietary diversity–related gut environment features, including microbial diversity, composition, functions, taxa, and fecal metabolites. Second, we evaluated the associations of dietary diversity and gut environmental features with host circulating metabolites. Last, we validated the associations between dietary diversity and the α diversity, structure, and genera of the gut microbial community with data from another population-based longitudinal cohort study: the China Health and Nutrition Survey (CHNS) (11).

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Supplemental Figures 1–5, Supplemental Methods, and Supplemental Tables 1–9 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn/.

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Abbreviations used: ARGININE-SYN4-PWY, L-ornithine de novo biosynthesis; CHEI, Chinese Healthy Eating Index; CHNS, China Health and Nutrition Survey; DDS, dietary diversity score; DFS, dietary diversity score fecal metabolite feature score; DGLA, dihomo-γ-linolenic acid; DMS, dietary diversity score microbiota feature score; DPS, dietary diversity score pathway feature score; FBG, fasting blood glucose; FDR, false discovery rate; GDCA, glycodeoxycholic acid; GLCA-3S, glycolithocholic acid 3-sulfate; GNHS, Guangzhou Nutrition and Health Study; HbA1c, glycated hemoglobin; ICC, intraclass correlation coefficient; LEfSe, linear discriminant analysis effect size; NAD-BIOSYNTHESIS-II, NAD salvage pathway II; NorDCA, nordeoxycholic acid; OTU, operational taxonomic unit; PDI, plant-based diet index; PERMANOVA, permutational multivariate analysis of variance; rRNA, ribosomal ribonucleic acid; TDCA, taurodeoxycholic acid; TRPSYN-PWY, L-tryptophan biosynthesis; T2D, type 2 diabetes; WGCNA, weighted correlation network analysis.

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Methods

Study participants

We included data from 2 ongoing population-based cohorts in the present study: the GNHS and the CHNS (10, 11). In the GNHS, 4048 participants were recruited from Guangzhou, China during 2008–2013 and then followed up every 3 y. Fecal and blood samples for multiomics assessments were collected during a follow-up visit between 2014 and 2018. We excluded participants who reported antibiotic use within 1 wk preceding the sample collection day, those with cancer, and those with T2D at baseline, because these diseases' status or medications may have a substantial influence on the gut microbiome (12, 13). Thus, a total of 1916 GNHS participants were included in our analyses (the discovery cohort) (**Supplemental Figure 1**). The CHNS included participants recruited from 8 provinces across China and all participants have been followed up every 4 y since 1989; thereafter, 8 more provinces/megacities were included up to 2018 (11). A total of 1320 CHNS participants were included in our analyses (the validation cohort), with FFQs administered in 2011 and 2015 and the gut microbiome profiled in 2015 (Supplemental Figure 1, **Supplemental Methods**).

The GNHS was approved by the Ethics Committee of the School of Public Health at Sun Yat-sen University (2018048) and the Ethics Committee of Westlake University (20190114ZJS0003). The CHNS was approved by the Ethics Committee of the National Institute of Nutrition and Food Safety of the Chinese Center for Disease Control and Prevention (no. 201524) and the Institutional Review Board of the University of North Carolina at Chapel Hill (no. 07-1963). All participants provided written informed consent.

Dietary assessment and calculation of the dietary diversity score

Dietary information was collected via semiquantitative FFQ at baseline and at a follow-up visit. In the discovery cohort, we used a validated FFQ with 79 food items (14, 15). In the validation cohort, we used another validated FFQ with 74 food items (16, 17). We converted food items to standard servings according to the Chinese Dietary Guidelines in both cohorts (2). According to the literature and local dietary habits, we evaluated dietary diversity with the dietary diversity score (DDS) (4, 5, 18). Briefly, the food items were aggregated into 6 major food groups: grains, vegetables, fruits, dairy and dairy products, legumes and legume products, and meat and alternatives (**Figure 1**, **Supplemental Table 1**). If a participant consumed $\geq$2 servings of a particular food group per week, intake of this food group was considered habitual and assigned 1 point (5). Otherwise, the score for that food group was 0. The total DDS was calculated by summing the scores of all 6 major food groups, ranging from 0 (low dietary diversity) to 6 (high dietary diversity). We classified participants into 2 groups by their DDS: the high DDS (sufficient dietary diversity, DDS = 6) and low DDS groups (insufficient dietary diversity, DDS $\leq$ 5), because the number of participants with a total DDS $\leq$ 4 was small. Moreover, we further classified participants according to DDS stability, namely, into a stable high DDS group (high total DDS at both baseline and follow-up) and a stable low DDS group (low total DDS at both baseline

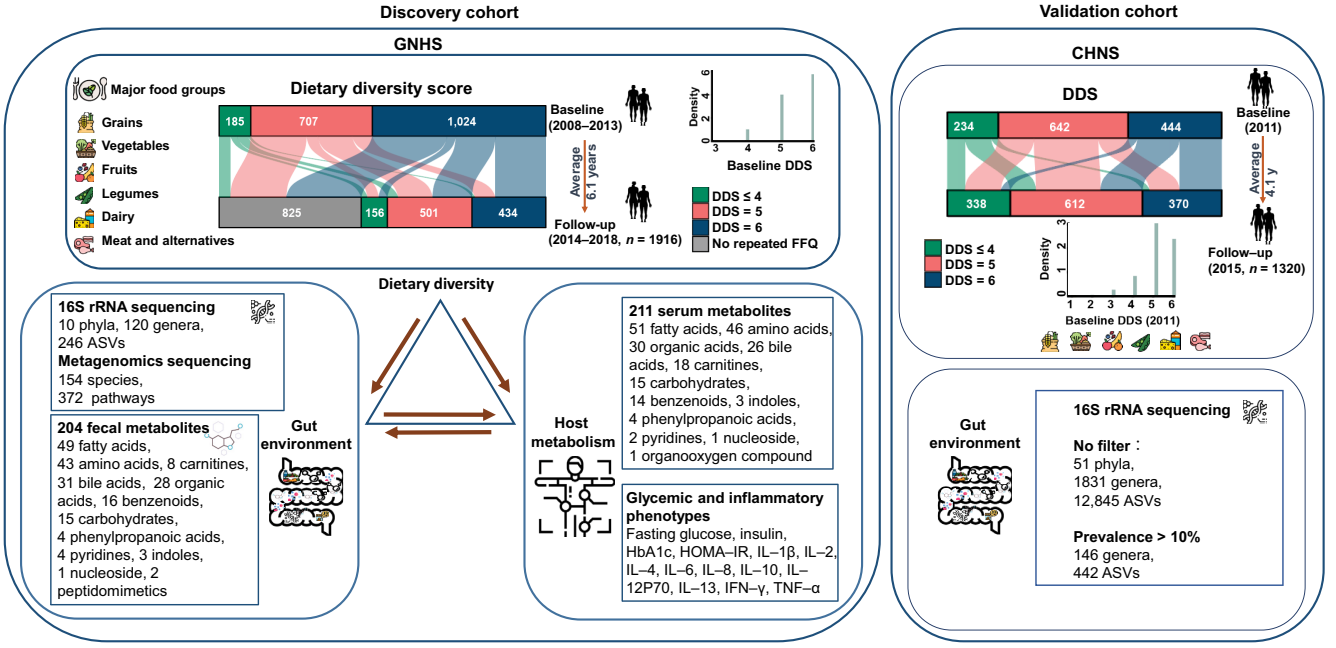

FIGURE 1 Study design: associations of dietary diversity with the gut microbiome, fecal metabolites, and host metabolism. We profiled the fecal metagenome, performed 16S rRNA sequencing of fecal samples, and conducted targeted metabolomics of fecal and serum samples from the discovery cohort (GNHS, $n = 1916$). We also performed fecal 16S rRNA sequencing in the validation cohort (CHNS, $n = 1320$). In both cohorts, dietary information was collected via FFQs administered at baseline and at a follow-up visit (2014–2018 for the GNHS and 2015 for the CHNS) when fecal samples were collected. We classified the FFQ items into 6 major food groups to calculate the DDS: grains, vegetables, fruits, legumes and legume products, dairy and dairy products, and meat and alternatives. ASV, amplicon sequence variant; CHNS, China Health and Nutrition Survey; DDS, dietary diversity score; GNHS, Guangzhou Nutrition and Health Study; HbA1c, glycated hemoglobin; rRNA, ribosomal RNA.

and follow-up). We also calculated DDSs within the 6 individual food groups using the same method (Supplemental Methods). According to the literature, we additionally calculated the plant-based diet index (PDI) and Chinese Healthy Eating Index (CHEI) (19, 20).

16S ribosomal ribonucleic acid gene sequencing and shotgun metagenomic sequencing

In the GNHS, the V3–V4 hypervariable region of the 16S ribosomal RNA (rRNA) gene was amplified and sequenced. Moreover, we performed shotgun metagenomic sequencing among 1148 participants in the GNHS. In the CHNS, the V4 region of the 16S rRNA gene was sequenced (Supplemental Methods).

Measurement of fecal and serum metabolites

We quantified the concentrations of 204 fecal metabolites and 211 serum metabolites among 957 GNHS participants. Targeted metabolomics measurements were performed by a triple quadrupole system (Supplemental Methods) (21).

Measurement of glycemic and inflammatory phenotypes

Glycemic phenotypes included fasting blood glucose (FBG), fasting insulin, glycated hemoglobin (HbA1c), HOMA-IR, and glucose metabolic status (22). For glucose metabolic status, participants were divided into 3 groups: normal glucose tolerance ($n = 825$), impaired glucose regulation (FBG $\geq$ 6.1 and $<$7.0 mmol/L; $n = 51$), and T2D (FBG $\geq$ 7.0 mmol/L, HbA1c concentration $\geq$ 6.5%, or self-reported use of medication for diabetes; $n = 81$) (22); and these T2D cases were incident cases of T2D between the baseline and follow-up visits. In addition, we randomly selected 300 GNHS participants to assess blood inflammatory biomarkers (using blood samples collected at the same time point as those for measuring blood metabolites), including IFN-γ, TNF-α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, and IL-13 (Supplemental Methods).

Statistical analysis

All statistical analyses were performed using Stata 15.0 (Stata Corp) or R version 4.0.3 (R Foundation for Statistical Computing). The intraclass correlation coefficient (ICC) (R package "irr") was calculated to estimate the associations of total DDS between baseline and follow-up in both cohorts. We used Spearman correlation analysis to investigate the cross-sectional correlations of the dietary diversity scores with the PDI and CHEI. In the GNHS, linear regression models were used to investigate the associations between each DDS (total DDS or the DDS of each food subgroup) and the α-diversity of the gut microbiota, adjusted for age, sex, BMI, total energy intake, physical activity, education level, household income, smoking status, alcohol drinking status, use of antihypertensive drugs, and lipid-lowering drugs. A permutational multivariate analysis of

variance (PERMANOVA) (R package "vegan") was performed to estimate the β-diversity dissimilarity based on the Bray–Curtis distance.

In the GNHS, we identified DDS-related microbial genera (based on 16S rRNA sequencing) and species (based on metagenomics data) by 2 steps, because the relative abundances of bacteria were not normally distributed (23, 24). We included taxa present in >10% of the samples and with a relative abundance > 0.01% in the analysis. First, we used linear discriminant analysis effect size (LEfSe) to identify taxa across the high and low DDS groups (23). Then we used linear and logistic regression to assess the associations of the DDS with the ln-transformed relative abundances (linear regression) and prevalence (logistic regression) of the identified microbial taxa (24). $P < 0.05$ was considered statistically significant for either linear or logistic regression. For the diversity of individual food groups, we used LEfSe to identify discrepant genera between the highest and lowest scores at baseline. Moreover, we identified DDS-related microbial functional pathways by linear regression. P values were corrected using the Benjamini–Hochberg false discovery rate (FDR). FDR < 0.25 was considered statistically significant for the pathway analysis. All linear/logistic regression analyses in the GNHS were adjusted for the same covariates as the α-diversity analysis.

To better understand the impact of dietary diversity on the functional readout of the gut microbiota, we then characterized the associations between dietary diversity and the fecal metabolome. We used weighted correlation network analysis (WGCNA) to obtain the coexpressed metabolite modules and reduce the number of tests conducted with the metabolomics data (25). In addition, we used linear regression to identify DDS-related metabolite features, including WGCNA modules and specific metabolites. Statistically significant associations were reported at $P < 0.05$ and FDR < 0.25. To gain deeper insights into the contribution of dietary diversity to host–gut interactions, we explored the associations between the DDS, DDS-related features, and circulating metabolites using similar strategies as the aforementioned fecal metabolome analysis.

We calculated the DDS microbiota feature score (DMS), DDS fecal metabolite feature score (DFS), and DDS pathway feature score (DPS), which represented the corresponding DDS-related gut environment features (Supplemental Methods). Then we used bidirectional mediation analyses to investigate the associations among the DDS, DDS-related features, and circulating metabolites (26). The analysis was performed with the "RMediation" package (27). We used Spearman correlation analysis to investigate the associations of identified circulating metabolites with glycemic and inflammatory phenotypes, adjusted for age and sex.

In the CHNS, multivariate mixed-effects linear regression was used to investigate the associations between each DDS variable and α-diversity metrics. The random effects in these mixed models were the sample collection provinces or megacities, and the fixed effects were age, sex, BMI, total energy intake, physical activity, education level, household income, smoking status, alcohol drinking status, use of antihypertensive drugs, and the urbanization index. We adjusted for the urbanization index and sample collection regions in our model because both factors showed a strong association with variation in the gut microbiome (28). We examined the associations between the baseline or stable total DDS and the significant microbial genera identified in the GNHS by using mixed-effects linear or logistic regression models, adjusted for the same list of potential confounders as the aforementioned α-diversity analyses in the CHNS (details in the Supplemental Methods).

Results

Characteristics of the study populations

Table 1 and Figure 1 show the characteristics of the included participants. The GNHS participants were middle-aged and elderly adults, with a mean ± SD age of 59.2 ± 6.7 y at baseline, and 6.1 y of follow-up (Table 1, Figure 1). The participants habitually consumed a mean ± SD 5.4 ± 0.67 groups of foods at baseline; no participant scored < 3 (Figure 1). Between the 2 time points (baseline and follow-up), the ICC (presented with the 95% CI) of the total DDS was 0.43 (0.36, 0.50); 297 individuals maintained a stable (i.e., DDS did not change) high DDS (DDS = 6) and 367 individuals maintained a stable low DDS (DDS < 6) between the 2 time points.

In the CHNS, the mean ± SD age of the participants in 2011 was 48.2 ± 12.3 y (Table 1). Most participants habitually consumed 6 (34%) or 5 (49%) groups of foods, and some habitually consumed <5 groups of foods (17%) (Figure 1). The ICC (95% CI) of the total DDS between the 2 visits (2011 and 2015) was 0.42 (0.36, 0.48). A total of 194 individuals maintained a stable high DDS and 700 individuals maintained a stable low DDS between the 2 time points.

Correlation of dietary diversity with other dietary patterns

In the discovery cohort (GNHS), we found that the diversities of fruits, vegetables and legumes were positively correlated with the PDI, whereas the diversities of dairy and meat and alternatives were inversely correlated with the PDI (**Supplemental Table 2**) (all $P < 0.01$). Moreover, the total DDS and the diversity scores of all 6 individual food groups were positively correlated with the CHEI (Supplemental Table 2) (all $P < 0.01$).

Dietary diversity was associated with gut microbial diversity and overall composition

In the discovery cohort (GNHS), we found that both baseline and stable total DDS were associated with α-diversity. A higher baseline total DDS was associated with higher microbial α-diversity metrics [**Figure 2**A, P-trend = 0.02 for observed operational taxonomic units (OTUs) and Shannon index]. The α-diversity between the baseline high DDS and low DDS groups remained significantly different (observed OTUs, $P = 0.02$; Shannon index, $P = 0.03$). We also found significant associations between the stable DDS groups and α-diversity metrics (**Supplemental Figure 2**A, P-trend = 0.04 for observed OTUs; P-trend = 0.01 for Shannon index and Pielou's evenness). In the validation cohort, we observed positive associations between the baseline total DDS and 2 microbial α-diversity parameters (Figure 2A, DDS = 5 compared with DDS = 4, Shannon index, $P = 0.01$; Pielou's evenness, $P = 0.002$) but not the other 2 parameters (Figure 2A, observed OTUs or Faith's phylogenetic diversity).

TABLE 1 Characteristics of the participants according to the baseline DDS[1]

	GNHS		CHNS	
	High DDS (score = 6)	Low DDS (score < 6)	High DDS (score = 6)	Low DDS (score < 6)
Participants, *n*	1024	892	444	876
Age, y	59.1 ± 6.6	59.3 ± 6.8	45.9 ± 13.5	49.4 ± 11.5
Men, n (%)	277 (27.1)	332 (37.2)	199 (44.8)	423 (48.3)
BMI, kg/m^2	23.0 ± 2.8	23.4 ± 3.0	24.3 ± 4.5	24.3 ± 4.9
Total energy intake, kcal/d	1788 ± 514	1716 ± 513	2223 ± 1066	1964 ± 1390
Physical activity, MET-h/d	45.8 ± 25.2	44.9 ± 25.7	27.5 ± 23.3	34.5 ± 27.4
Current alcohol drinking, n (%)	66 (6.4)	73 (8.2)	175 (39.4)	324 (37.0)
Current smoking status, n (%)	108 (10.5)	182 (20.4)	106 (23.9)	243 (27.7)
Antihypertensive drug users, n (%)	40 (3.9)	51 (5.7)	44 (9.9)	81 (9.2)
Lipid-lowering drug users, n (%)	39 (3.8)	57 (6.4)		

[1] Values are mean ± SD for continuous variables and *n* (%) for dichotomous variables. CHNS, China Health and Nutrition Survey; DDS, dietary diversity score; GNHS, Guangzhou Nutrition and Health Study; MET, metablic equivalent.

The PERMANOVA indicated that β-diversity between the high and low DDS groups also significantly differed (Figure 2B, $P = 0.005$ for baseline DDS; Supplemental Figure 2B, $P = 0.007$ for stable DDS). In the validation cohort, the gut microbial community significantly differed between the high and low DDS groups (Figure 2B, $P = 0.001$ for baseline DDS; Supplemental Figure 2B, $P = 0.001$ for stable DDS).

Regarding the diversity of individual food groups, we found that the baseline fruits diversity score was positively associated with microbial α-diversity in the discovery cohort (Figure 2C,

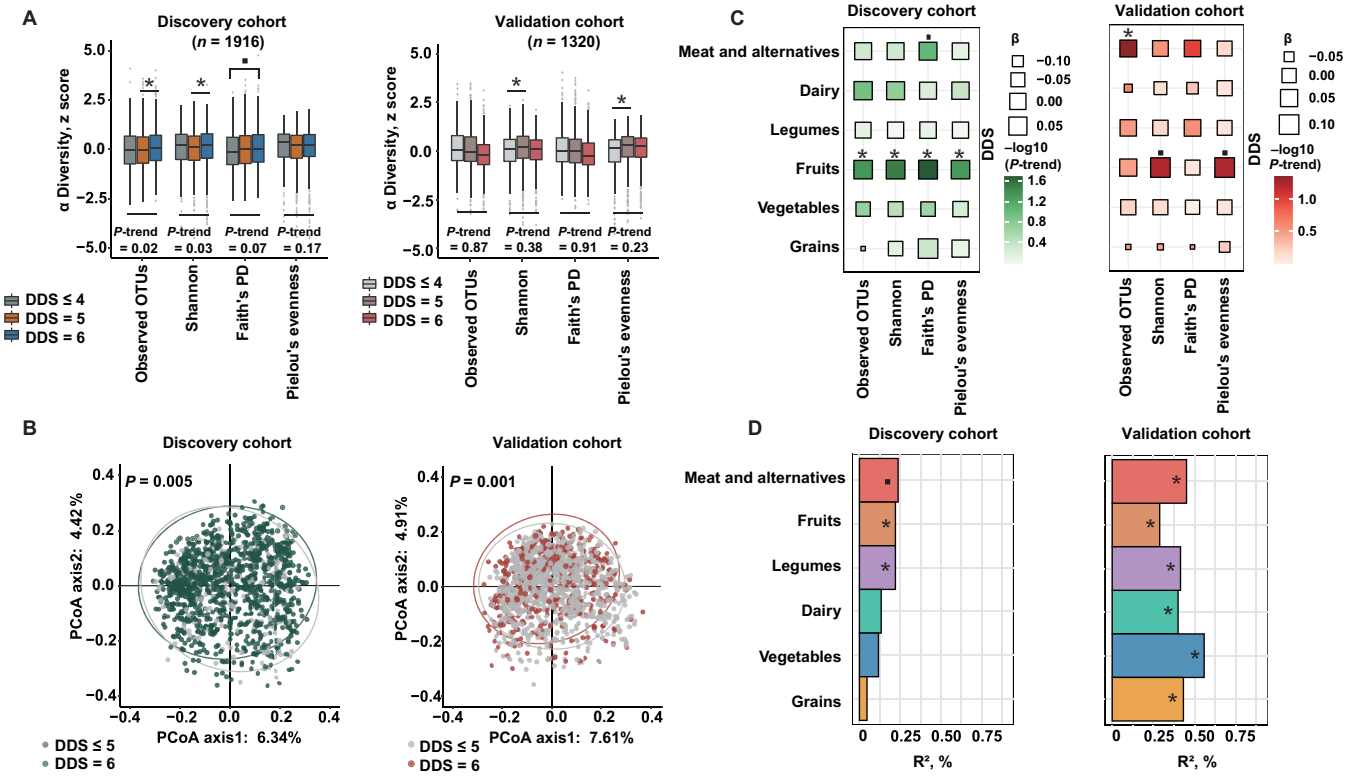

FIGURE 2 Dietary diversity was associated with the diversity of the gut microbiota in the discovery and validation cohorts. (A) Prospective associations of the total DDS with α diversity. (B) ASV-level PCoA between the baseline high DDS and low DDS groups, based on Bray–Curtis distance. (C) Prospective associations between the diversities of the 6 major food groups and α diversity. (D) Gut microbial variations according to the diversity of 6 major food groups, based on the ASV-level Bray–Curtis distance. (A) For box plots, the center line is the median, and the limits represent the upper and lower quartiles. (A, C) *P* values were calculated by linear regression models (discovery cohort) and multilevel mixed-effects linear regression models (validation cohort) adjusted for sociodemographic, lifestyle, and medication factors. (B, D) Permutational multivariate ANOVA was used to calculate the β-diversity dissimilarities. In the Guangzhou Nutrition and Health Study, $n = 1024$ for DDS = 6, $n = 707$ for DDS = 5, and $n = 185$ for DDS ≤ 4. In the China Health and Nutrition Survey, $n = 444$ for DDS = 6, $n = 642$ for DDS = 5, and $n = 234$ for DDS ≤ 4. $^*P < 0.05$; ■ $P = 0.06$. ASV, amplicon sequence variant; DDS, dietary diversity score; OTU, operational taxonomic unit; PCoA, principal coordinate analysis; PD, phylogenetic diversity.

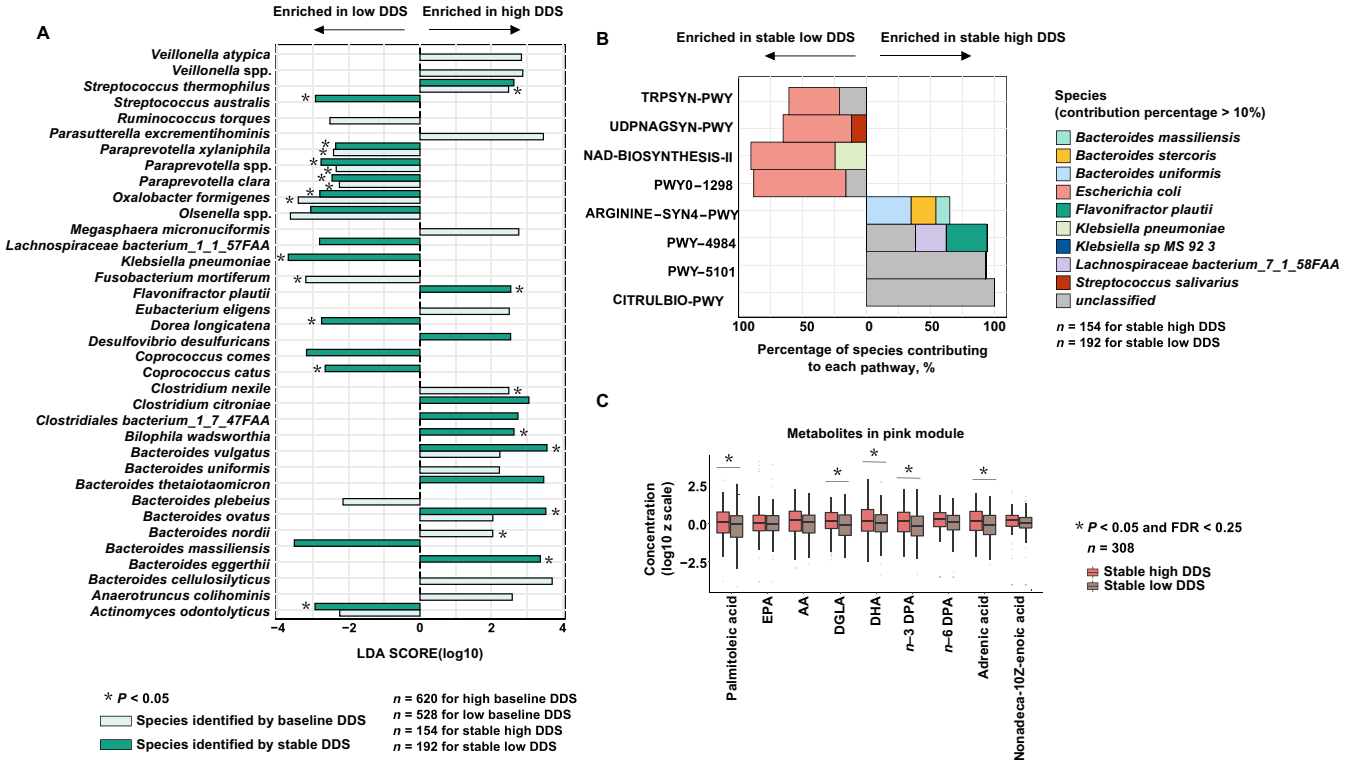

FIGURE 3 Dietary diversity–related microbial species, functional pathways, and fecal metabolites in Guangzhou Nutrition and Health Study participants. (A) Distribution of identified microbial species between the baseline or stable high DDS and low DDS groups. (B) Enriched metagenomic functional pathways in the stable DDS groups and the bacterial species (contribution percentage > 10%) contributing to each pathway. (C) Concentrations (z score) of fecal metabolites in the stable DDS groups ($n = 308$). (A) The species were identified by 2 steps. First, we used linear discriminant analysis effect size to screen for discrepant species. Second, we used linear and logistic regression to further assess the association of each total DDS with the relative abundance and prevalence of the microbial taxa identified in the first step, respectively. (B) We performed a linear regression analysis to estimate the associations between the total DDS and the microbial functional pathways. (C) We used linear regression models to identify the associations between the DDS and specific fecal metabolites. $n = 620$ for the high baseline DDS group, $n = 528$ for the low baseline DDS group, $n = 154$ for the stable high DDS group, and $n = 192$ for the stable low DDS group. (A) $^*P < 0.05$ for either linear or logistic regression. (C) $^*P < 0.05$ and FDR < 0.25. AA, arachidonic acid; ARGININE-SYN4-PWY, L-ornithine de novo biosynthesis; CITRULBIO-PWY, L-citrulline biosynthesis; DDS, dietary diversity score; DGLA, dihomo-γ-linolenic acid; DPA, docosapentaenoic acid; FDR, false discovery rate; LDA, linear discriminant analysis; NAD-BIOSYNTHESIS-II, NAD salvage pathway II; PWY0-1298, superpathway of pyrimidine deoxyribonucleosides degradation; PWY-4984, urea cycle; PWY-5101, L-isoleucine biosynthesis II; TRPSYN-PWY, L-tryptophan biosynthesis; UDPNAGSYN-PWY, UDP-N-acetyl-D-glucosamine biosynthesis I.

P-trend < 0.05 for all metrics). In the validation cohort, we found a similar trend (Figure 2C, Shannon index and Pielou's evenness). Moreover, we found significant associations of the baseline legumes diversity ($r^2 = 0.13\%$, $P = 0.01$) and fruits diversity ($r^2 = 0.22\%$, $P = 0.001$) with overall microbial composition in the GNHS (Figure 2D). In the validation cohort, diversity in all 6 food groups was significantly associated with microbial β-diversity (Figure 2D, all $P = 0.001$). The diversity of baseline grains, vegetables, dairy, legumes, fruits, and meat and alternatives explained 0.43%, 0.56%, 0.41%, 0.40%, 0.29%, and 0.45% of the variation in the structure of the gut microbiota, respectively (Figure 2D).

Dietary diversity, microbial taxa, and functional pathways

We identified 68 microbial genera that were correlated with the total DDS or specific food subgroup DDSs in the GNHS (**Supplemental Figure 3**A, **Supplemental Table 3**). Baseline total DDS was positively associated with the genera *Anaerotruncus* and *Veillonella* and an unclassified genus within the Pasteurellaceae family but inversely associated with the genus *Paraprevotella* (Supplemental Figure 3A). In the subsequent analysis (stable high compared with low DDS), the genus *Anaerotruncus* was enriched in the stable high DDS group. An unclassified genus within the Burkholderiaceae family was enriched in the stable low DDS group. These associations between baseline DDS and the genera *Anaerotruncus* and *Veillonella* were replicated in the CHNS data (Supplemental Figure 3B, C).

Among the diversities of the 6 individual food groups, fruits diversity was associated with the largest number of microbial genera (a total of 33 genera) in the GNHS, followed by the diversities of meat and alternatives, dairy, and legumes, at 16, 14, and 14 genera, respectively (Supplemental Table 3). Among all the identified genera (68 total), 17 shared an association with the diversities of ≥2 different food groups (Supplemental Table 3). The genera *Anaerostipes*, *Lachnospiraceae UCG-010*, and *Ruminococcaceae UCG-013* were enriched in participants who consumed a high diversity of fruits and dairy. The genus *Fusobacterium* was enriched in participants who consumed a low diversity of dairy, fruits, and vegetables (Supplemental Table 3).

Next, we used shotgun metagenomic sequencing data to identify DDS-related species and functional pathways in the GNHS participants. We found that 18 species ($P < 0.05$) and 8 pathways ($P < 0.05$ and FDR < 0.25) were significantly associated with the baseline or stable DDS (**Figure 3**A, B, **Supplemental Tables 4** and **5**); of these, 4 species were significantly associated with both baseline and stable DDSs (Figure 3A, $P < 0.05$). Three Bacteroides species (*Paraprevotella* spp., *Paraprevotella clara*, *Paraprevotella xylaniphila*) and 1 Proteobacteria species (*Oxalobacter formigenes*) were enriched in both the baseline low DDS and stable low DDS groups (Figure 3A, $P < 0.05$). Two Bacteroides species (*Bacteroides vulgatus* and *Bacteroides ovatus*) were enriched in the stable high DDS groups (Figure 3A). Within the 2 genera (i.e., *Anaerotruncus* and *Veillonella*) validated based on the 16S data, we also identified 2 species (*Veillonella atypica* and *Veillonella* spp.) within the *Veillonella* genus and 1 species (*Anaerotruncus colihominis*) within the *Anaerotruncus* genus enriched in the high DDS group by LEfSe (Figure 3A, Supplemental Table 4).

Three pathways, the L-ornithine de novo biosynthesis (ARGININE-SYN4-PWY), urea cycle (PWY-4984), and L-citrulline biosynthesis (CITRULBIO-PWY) pathways, involved in the function of the bacterial urea cycle were enriched in the stable high DDS group (Figure 3B, Supplemental Table 5). The L-isoleucine biosynthesis II (PWY-5101) pathway was enriched in the stable high DDS group. In addition, 4 pathways, including L-tryptophan biosynthesis (TRPSYN-PWY), UDP-N-acetyl-D-glucosamine biosynthesis I (UDPNAGSYN-PWY), the superpathway of pyrimidine deoxyribonucleosides degradation (PWY0-1298), and the NAD salvage pathway II (NAD-BIOSYNTHESIS-II), were enriched in the stable low DDS group (all $P < 0.05$ and FDR < 0.25, Figure 3B, Supplemental Table 5). Notably, we did not find any pathway with FDR < 0.05. Seven microbial species related to DDS contributed to these identified pathways (Figure 3B), such as *Bacteroides uniformis* for pathway ARGININE-SYN4-PWY and *Klebsiella pneumoniae* for pathway NAD-BIOSYNTHESIS-II.

Dietary diversity was associated with fecal fatty acid, organic acid, and pyridine metabolism

We clustered the 204 fecal metabolites into 12 WGCNA modules (**Supplemental Figure 4**A, **Supplemental Table 6**). Supplemental Figure 4A and B show the associations of each DDS variable with the WGCNA modules and metabolites not clustered by WGCNA (gray module). We found that the stable DDS was positively associated with the pink module (Supplemental Figure 4A, $P = 0.02$). This module contained 9 long-chain unsaturated fatty acids (Figure 3C, Supplemental Table 6), of which the concentrations of palmitoleic acid (16:1n–7), dihomo-γ-linolenic acid (DGLA, 20:3n–6), Docosahexaenoic acid (DHA, 22:6n–3), n–3 docosapentaenoic acid (n–3 DPA, 22:5n–3), and adrenic acid (ADA, 22:4n–6) were significantly higher in the stable high DDS group than in the stable low DDS group (Figure 3C, $P < 0.05$). For metabolites within the nonclustered module, we observed that 1 pyridine (picolinic acid, $P = 0.003$), 1 organic acid (α-hydroxyisobutyric acid, $P = 0.04$), and 2 saturated fatty acids [myristic acid (14:0), $P = 0.01$; capric acid (10:0), $P = 0.02$] were enriched in the high baseline total DDS group (Supplemental Figure 4B).

Dietary diversity and gut environment features were linked to host secondary bile acid metabolism

In total, 211 serum metabolites were clustered in 11 WGCNA modules (**Supplemental Figure 5**, **Supplemental Table 7**). We observed 91 associations between the DDS-related features (including 18 identified microbial species, 8 functional pathways, and 13 fecal metabolites) and the 11 WGCNA modules ($P < 0.05$, Supplemental Figure 5A, B). For the DDS, we found an inverse correlation between the stable DDS and the red WGCNA module (Supplemental Figure 5A, $P = 0.04$). For the DDS-related features of the gut environment, 19 DDS-related features were linked with the red WGCNA module (Supplemental Figure 5B, all $P < 0.05$).

The red WGCNA module mainly contained conjugated secondary bile acids and aromatic amino acid derivatives (**Figure 4**A, Supplemental Table 7). Furthermore, we found 332 associations between the stable DDS or DDS-related features of the gut environment and specific circulating metabolites enriched in the red WGCNA module. The stable DDS was inversely associated with 4 circulating secondary bile acids: glycodeoxycholic acid (GDCA; $P = 0.01$), taurodeoxycholic acid (TDCA; $P = 0.04$), glycolithocholic acid 3-sulfate (GLCA-3S; $P = 0.02$), and nordeoxycholic acid (NorDCA; $P = 0.01$) (Figure 4A). Most DDS-associated features, such as fecal DGLA concentration, DHA concentration, *B. ovatus* abundance, and *B. vulgatus* abundance, were inversely associated with the 4 secondary bile acids (Figure 4A, $P < 0.01$ and FDR < 0.01). Moreover, many features linked to low DDS, such as the abundances of *P. clara* and *Coprococcus catus*, were positively associated with the concentrations of GDCA, TDCA, NorDCA, and GLCA-3S (Figure 4A, $P < 0.01$ and FDR < 0.01 for all).

We calculated the DMS, DFS, and DPS, which represented the global features of DDS-related gut microbiota, fecal metabolites, and functional pathways, respectively. We found a complex, bidirectional relation among the DDS, DFS/DMS, and the 4 circulating secondary bile acids (Figure 4B, C, **Supplemental Tables 8** and **9**). On the one hand, the DFS and DMS mediated the effects of the DDS on the 4 circulating bile acids (all P-mediation < 0.05). On the other hand, GDCA, TDCA, NorDCA, and GLCA-3S mediated the effect of the DDS on the DFS or DMS (P-mediation < 0.05 for all) (Figure 4B, C, Supplemental Tables 8, 9).

Circulating secondary bile acids were linked to host glycemic and inflammatory phenotypes

In the cross-sectional analysis, we found that GDCA was positively associated with HOMA-IR ($P = 0.02$), fasting insulin ($P = 0.01$), and TNF-α ($P = 0.048$, Figure 4D). TDCA was positively associated with fasting insulin ($P = 0.04$), IL-8 ($P = 0.048$), and TNF-α ($P = 0.02$). NorDCA was positively associated with glucose metabolic status (normal status to disease status, $P = 0.046$, Figure 4D).

Discussion

In this large-scale multiomics study, we found that dietary diversity was positively associated with α-diversity of the human

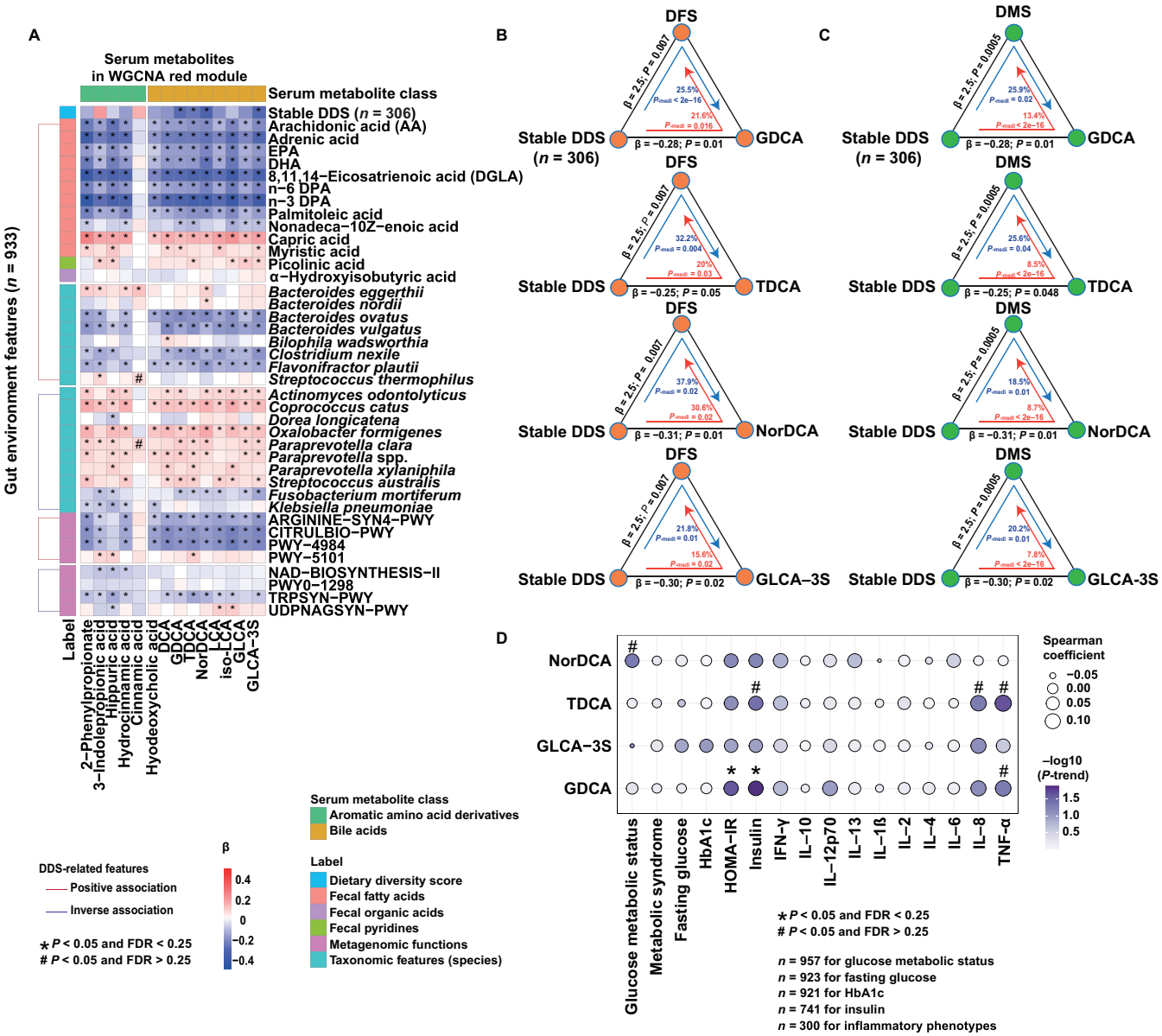

FIGURE 4 Associations among dietary diversity, features of the gut environment, and host circulating metabolites in Guangzhou Nutrition and Health Study participants. (A) Associations of the stable DDS and DDS-related gut environment features with host circulating metabolites within the WGCNA red module. (B, C) Relations between the stable DDS, scores on gut environment features, and secondary bile acids according to bidirectional mediation analysis ($n = 306$). (D) Cross-sectional associations between secondary bile acids and glycemic and inflammatory phenotypes. (A) We used linear regression models to investigate the associations of the stable DDS ($n = 306$) and DDS-related gut environment features ($n = 933$) with host circulating metabolites. (B, C) We separately calculated the DFS and DMS. Bidirectional mediation analysis was conducted with the "RMediation" package in R. At each edge, the β coefficient and P value labels between the 2 points were determined via linear regression. The proportions and P values of indirect effects are labeled at the center of the triangle charts. (D) We adjusted for age and sex in the Spearman correlation analysis of bile acids and phenotypes. (D) $n = 957$ participants; among them, 81 had type 2 diabetes, 51 were prediabetic. $^{*}P < 0.05$ and FDR < 0.25; $^{\#}P < 0.05$ and FDR > 0.25. ARGININE-SYN4-PWY, L-ornithine de novo biosynthesis; CITRULBIO-PWY, L-citrulline biosynthesis; DCA, deoxycholic acid; DDS, dietary diversity score; DFS, dietary diversity score fecal metabolite feature score; DMS, dietary diversity score microbiota feature score; DPA, docosapentaenoic acid; FDR, false discovery rate; GLCA, glycolithocholic acid; GLCA-3S, glycolithocholic acid 3-sulfate; G/TDCA, glyco-/tauro-conjugated deoxycholic acid; iso-LCA, isolithocholic acid; LCA, lithocholic acid; NAD-BIOSYNTHESIS-II, NAD salvage pathway II; NorDCA, nordeoxycholic acid; PWY0-1298, superpathway of pyrimidine deoxyribonucleosides degradation; PWY-4984, urea cycle; PWY-5101, L-isoleucine biosynthesis II; TRPSYN-PWY, L-tryptophan biosynthesis; UDPNAGSYN-PWY, UDP-N-acetyl-D-glucosamine biosynthesis I; WGCNA, weighted gene coexpression network analysis.

gut microbiome and associated with microbial community structure. We further revealed associations between dietary diversity and 68 microbial genera, 18 microbial species, 8 functional pathways, and 13 fecal metabolites in the gut. The findings related to α- and β- diversity of the gut microbiota and 2 microbial genera were validated in another independent prospective cohort. Furthermore, a total of 332 direct associations of dietary diversity and related features with circulating metabolites indicated a

complex interplay among diet, the gut microbiome, and host metabolism. To the best of our knowledge, this study is the largest population-based study to provide comprehensive insights into the associations of dietary diversity with gut environment features and host metabolism.

In the present study, we found that high dietary diversity–related features of the gut environment might play key roles in maintaining host health. In general, a higher α-diversity of the gut microbiota is associated with better health (29). Consistent with previous studies (30), we observed a positive association of total dietary diversity and fruits diversity with α-diversity of the gut microbiota (30). Notably, we did not find any significant association between vegetable diversity and α/β- diversity of the gut microbiota; the associated bacterial genera were also limited in the discovery cohort. These results differ from those in the European population (31), possibly because of differences in cooking habits between these populations, because vegetables are usually deeply cooked in the Chinese culture.

The benefits of high dietary diversity for cardiometabolic health may be partly attributed to specific gut microbial taxa and metabolites. For example, the 2 validated genera, *Anaerotruncus* and *Veillonella*, may lower inflammation and benefit cardiometabolic health (32, 33). *B. ovatus* is inversely associated with BMI (34), and *B. vulgatus* has been found to protect against coronary artery disease (35). In addition, dietary diversity was positively associated with fecal n–3 PUFAs, which are known anti-inflammatory substrates and help prevent chronic diseases, including cardiovascular disease and T2D (36).

Notably, previous studies have reported some harmful effects of these low DDS–related microbial features. For instance, *Dorea longicatena* is enriched in individuals with obesity and is positively associated with insulin resistance (34). *Fusobacterium mortiferum* belongs to the *Fusobacterium* genus, which is enriched in individuals with colon cancer and T2D (14, 37). Moreover, we found that dietary diversity was inversely associated with the microbial TRPSYN-PWY pathway. In previous studies, tryptophan was positively associated with T2D incidence (38).

Dietary diversity was closely correlated with 4 circulating secondary bile acids (GDCA, TDCA, NorDCA, and GLCA-3S). Secondary bile acids can regulate host glucose and lipid metabolism by activating farnesoid X receptor and G-protein-coupled bile acid receptor 1 (39). A recent study found that GDCA and TDCA were positively associated with T2D (40). GDCA, GLCA-3S, and TDCA are conjugated secondary bile acids (8), and previous studies have reported that conjugated deoxycholic acid and lithocholic acid are positively associated with insulin resistance (41). Consistent with these findings, we found a positive association between GDCA and insulin resistance in the present study. In addition, we found that high concentrations of NorDCA were associated with worse glucose metabolic status. TDCA was positively associated with IL-8 and TNF-α, which were positively associated with T2D in previous studies (42, 43). Taken together, these results highlight the potential of high dietary diversity in shaping the gut environment and circulating bile acid profiles for better health status.

Our study has several strengths. First, to the best of our knowledge, this study is among the first to provide a multiomics analysis of the associations between dietary diversity, gut environment features, and circulating metabolites. Second, the use of repeated measures of diet using FFQs minimized diet alterations during the long-term follow-up. Third, the results were replicated in an independent cohort with a wider age range (19–80 y old) and in wider regions of China, which suggests that the findings are robust and generalizable. However, our study also has several limitations. First, the observational study design prevented us from establishing definite causal relations among dietary diversity, features of the gut environment, and circulating metabolites and could not eliminate the influence of residual confounding factors. Second, dietary intake was assessed by FFQs, which are subject to the influence of recall bias and measurement error. The associations may be confounded by other dietary characteristics, such as the plant-based dietary pattern or the Healthy Eating Index. In addition, the ICCs of dietary diversity between the 2 time points were low, suggesting that diet may change over time; thus, a more frequent longitudinal profile of dietary diversity is needed. Given the potential for measurement errors on the FFQ, a more reliable instrument (such as a 7-d food diary) would help to accurately categorize routine dietary diversity over time. Third, our shotgun metagenomics and metabolomics analyses were exploratory, with less stringent thresholds (such as FDR < 0.25), and these results have not been replicated in another independent cohort.

In conclusion, our study highlighted the potential role of high dietary diversity in shaping and maintaining a healthy gut environment and regulating circulating metabolites. We determined key gut microbes, metabolites, and functional pathways that linked dietary diversity, the gut environment, and host metabolic health in 2 deeply phenotyped Chinese populations. The identified features of the gut microbiome may be used as biomarkers of a healthy gut ecosystem or as potential intervention targets for preventing metabolic diseases in the future.

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The authors' responsibilities were as follows—HW, Y-mC, and J-SZ: designed the research; CX, J-tW, CS, and ZM: conducted the research and performed the statistical analysis; CX, ZM, JT, and J-SZ: wrote the paper; YO, YY, ZJ, YF, MS, WG, FX, EY-WY, YL, XL, YT, JW, FH, BZ, HW, Y-mC, and J-SZ: provided essential materials; J-SZ: was primarily responsible for the final content; and all authors: read and approved the final manuscript. The authors report no conflicts of interest.

Data Availability

For the GNHS cohort, the raw data on the 16S rRNA gene sequences were deposited in the China National GeneBank Sequence Archive (CNSA, https://db.cngb.org/cnsa/) of the China National GeneBank DataBase (CNGBdb) under accession number CNP0000829. The metabolomics data are available at the Metabolomics Workbench (ST001669). Data on the sociodemographic and lifestyle factors as well as FFQ information from the CHNS cohort were deposited at the following website: https://www.cpc.unc.edu/projects/china. Other data sets are available upon reasonable request to the corresponding authors.

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