Supplementary Information (SI) for Food & Function.
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1 **Supplementary Methods**

2 **Method S1 Fecal sample collection**

3 Participants collected their own fecal samples following instructions provided by staff at the 4 recruitment site. Stool samples were obtained either the night before or the morning of the physical 5 examination. These samples were divided into two 30-mL cryogenic tubes. The samples were 6 transported to our laboratory on dry ice within four hours and stored in freezers at -80°C until analysis.

7 **Method S2 Fecal microbial DNA extraction and 16S rRNA gene sequencing**

8 Fecal microbial DNA was extracted from each sample using the QIAamp® DNA Stool Mini Kit 9 (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The amplification procedure 10 of 16S ribosomal RNA (rRNA) gene was divided into two PCR steps. In the first PCR reaction, 16S 11 rRNA gene from extracted genomic DNA was amplified using the primes 341F(5'- 12 CCTACGGGNGGCWGCAG-3') and 805R (5'-GACTACHVGGGTATCTAATCC-3') directionally 13 targeting the V3 and V4 hypervariable region. Amplification was performed in triplicate 20 μL mixture 14 containing 4 μL FastPfu Buffer, 2 μL dNTPs, 0.1 μM primer 341 F, 0.1 μM primer 805 R and 10 ng of 15 template DNA. Reactions were run in a ABI GeneAmp® 9700 PCR instrument according to the 16 following cycling program: denaturation at 95 °C for 2 min, followed by 25 cycles of 95 °C for 30s 17 (denaturing), 55 °C for 30s (annealing), and 72 °C for 30s (elongation), and a final extension at 72 °C 18 for 5 min. Subsequently, the amplified products were checked by 2% agarose gel electrophoresis and 19 ethidium bromide staining. Amplicons were quantified using a QuantiFluor™-ST quantification system 20 (Promega, USA) according to the manufacturer's instructions. In the second PCR step, sequencing 21 primers and adaptors were added to the ends of the amplicon products to generate indexed libraries 22 ready for downstream sequencing on Illumina platfrom. The concentration of the pooled libraries was 23 determined by the QuantiFluor™-ST quantification system. Finally, DNA libraries were multiplexed 24 and loaded onto the Illumina MiSeq platform (Illumina Inc., CA, USA) according to manufacturer's 25 instructions. Sequencing was performed using paired-end; image analysis and base calling were 26 conducted by the Control Software embedded in the instrument. Raw paired-end fastq files were 27 processed by using QIIME2 2022.2

28 **Method S3 16S rRNA gene sequencing bioinformatics**

29 We utilized the Quantitative Insights into Microbial Ecology 2 (QIIME 2, 2022.2) platform for
30 bioinformatics analysis of gut microbiota[1]. Initially, raw data were demultiplexed and imported into
31 QIIME2 using the q2-demux plugin, followed by denoising with DADA2[2]. The DADA2 pipeline (q2-
32 dada2) was employed to generate amplicon sequence variant (ASV) feature tables and representative
33 sequences by filtering out low-quality and duplicate sequences, learning error rates, merging paired
34 reads, and removing primers and chimeras. Taxonomic classification of each ASV was performed
35 using the classify-sklearn algorithm from the q2-feature-classifier plugin, referencing the Silva-138-99
36 database[3]. All ASVs were aligned and used to construct a phylogenetic tree with mafft (q2-alignment)
37 and fasttree2 (q2-phylogeny). Gut microbial α-diversity (observed species, Chao 1 index, Shannon
38 index, and Simpson index) and β-diversity (Bray-Curtis distance) were calculated by q2-diversity
39 based on rarefied ASV counts (minimum 20,000 sequences). Finally, genus-level absolute abundance
40 tables were extracted from the pipeline and converted to relative abundance tables by normalizing to
41 the total counts of each genus for comparative analysis of gut microbiota discrepancies.

42 **Method S4 Blood sample collection**

43 Venous blood samples were collected by licensed nurses after an overnight fast of at least 12
44 hours. Within 1 hours of collection, the blood samples were 154 transported to the laboratory,
45 fractionated, and stored at -80℃ until further use.

46 **Method S5 Metabolomics testing**

47 Chromatographic conditions: Chromatographic separation was performed using an ACQUITY
48 UPLC HSS T3 column (100 mm × 2.1 mm, 1.8 μm, Waters); Mobile phase A is 5 mmol/L ammonium
49 acetate + 5 mmol/L acetic acid aqueous solution, and mobile phase B is acetonitrile; The gradient
50 elution procedure is: 0-0.5 min, 1% B; 0.5~6.5 min, 1% ~ 99% B; 6.5~8.0 min , 99% B; 8.0~8.5 min ,
51 99% B; 8.5~10.0 min, 1% B； Flow rate of 0.35 mL/min; Injection volume 4μL; The chromatographic
52 column temperature is 40°C.

53 Mass spectrum conditions: use the electric spray ion source, collect once respectively in the
54 positive and negative electric spray mode. The temperature of the ion source is 350°C; The capillary

55 voltage is+3.8 kV in positive mode and -3.4 kV in negative mode; the purge gas flow rate is 1 Arb;
56 The auxiliary airflow speed is 12 Arb; The sheath gas flow rate is 50 Arb. The data acquisition mode
57 is full scan and data dependent acquisition (DDA). In one acquisition cycle, the full scan range is 70-
58 1050 Da, the resolution is 60000, automatic gain control (AGC) is the standard, and the maximum ion
59 implantation time (Maximum IT) is automatic; Select the top 4 ions with a response intensity higher
60 than 5000 in the full scan for secondary scanning. The resolution of the secondary scanning is 15000,
61 the automatic gain control (AGC) is customized, and the maximum ion implantation time (Maximum
62 IT) is customized; The dynamic exclusion time is set to 6 seconds.
63 Non targeted metabolomics analysis was conducted using participant plasma samples and
64 Metabolon's HD4 Discovery untargeted metabolomics platform. All samples are processed using the
65 automatic MicroLab STAR system ® Prepared by Hamilton Company, Reno, NV, USA, and detected
66 using ultra-high performance liquid chromatography and high-resolution mass spectrometry (UPLC-
67 HRMS). Add quality control standards to each sample extract to monitor instrument performance and
68 assist in chromatographic comparison. Metabolites are identified by comparing them with purified
69 standards or Metabolon libraries of repeated unknown entities based on retention time and index, mass
70 to charge ratio, and chromatographic data.
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Supplementary Table S1 Baseline characteristics of the participants from WELL-China cohort

Characteristics	Low DDS *N* = 3181	Medium DDS *N* = 2962	High DDS *N* = 1960	*p* value[a]
Age, years	49.8 (13.9)	54.8 (12.7)	57.1 (12.4)	*<0.001*
Female (%)	1859 (58.4)	1893 (63.9)	1364 (69.6)	*<0.001*
Education level (%)				*<0.001*
Elementary or less	644 (20.3)	713 (24.1)	300 (15.3)	
Secondary school	1560 (49.1)	1609 (54.4)	1200 (61.3)	
Higher	974 (30.6)	638 (21.6)	459 (23.4)	
Missing	3	2	1	
Annual income, yuan (%)				*<0.001*
＜5000 ¥	1886 (59.4)	1976 (66.8)	1242 (63.4)	
5000 – 10,000 ¥	1000 (31.5)	785 (26.5)	597 (30.5)	
＞10,000 ¥	291 (9.16)	199 (6.72)	119 (6.08)	
Missing	4	2	2	
Physical activity (%)				*<0.001*
Low	538 (18.5)	355 (12.6)	193 (10.4)	
Moderate	1377 (47.3)	1373 (48.9)	951 (51.2)	
High	997 (34.2)	1079 (38.4)	712 (38.4)	
Missing	269	155	104	
Smoking status (%)				*<0.001*
Nonsmokers	2269 (71.5)	2260 (76.4)	1614 (82.4)	
Current smokers	906 (28.5)	699 (23.6)	345 (17.6)	
Missing	6	3	1	
Alcohol consumption status (%)				
Nondrinkers	1643(51.7)	1678(56.7)	1217(62.1)	*<0.001*
Current drinkers	1538(48.3)	1284(43.3)	743(37.9)	
Total energy intake, kcal/d	1235 (490)	1379 (512)	1594 (539)	*<0.001*
Antibiotic use (%)	524 (16.5)	535 (18.1)	347 (17.7)	*0.225*
BMI, kg/m^2	23.5 (3.34)	23.6 (3.14)	23.6 (3.15)	*0.686*

Note: Continuous variables are presented as mean (SD), and categorical variables are presented as n (percentage).

Abbreviations: DDS, dietary diversity score; BMI, body mass index.

[a]*p* values were calculated by using one-way ANOVA for continuous variables and the $\chi 2$ test for categorical variables.

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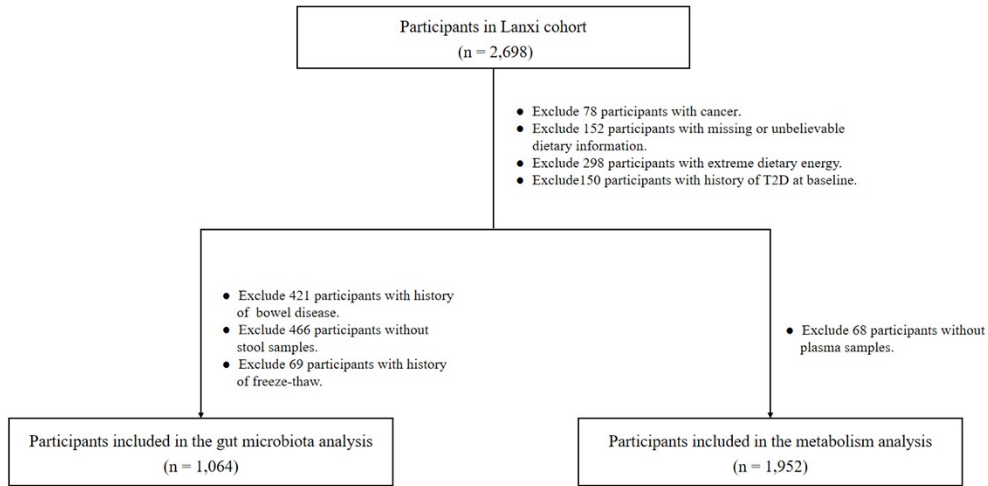

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81 **Supplementary Figure S1 Selection process of the study population in Lanxi cohort**

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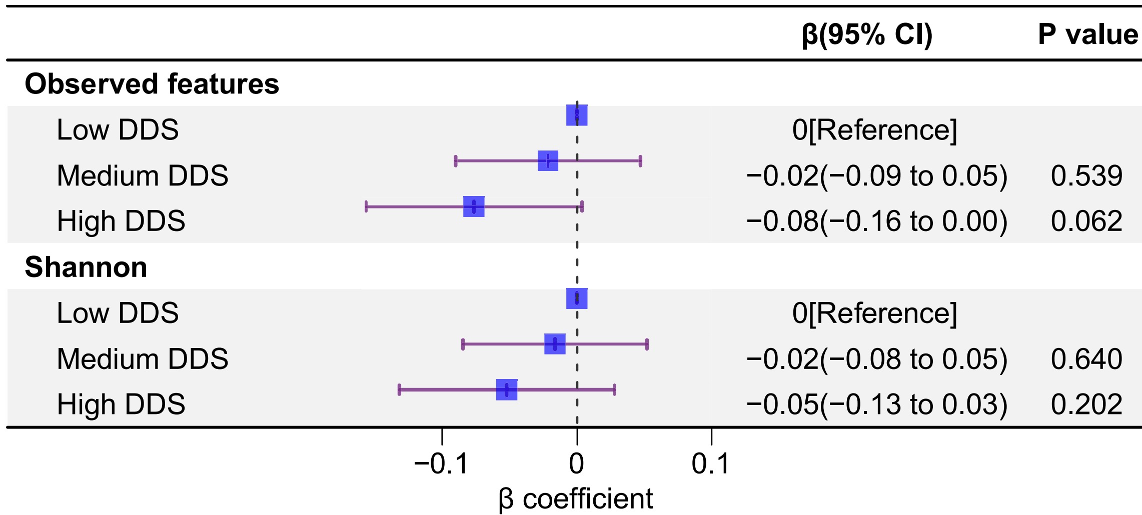

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86 **Supplementary Figure S2 Gut microbiota α- diversity alterations in the WELL-China cohort**

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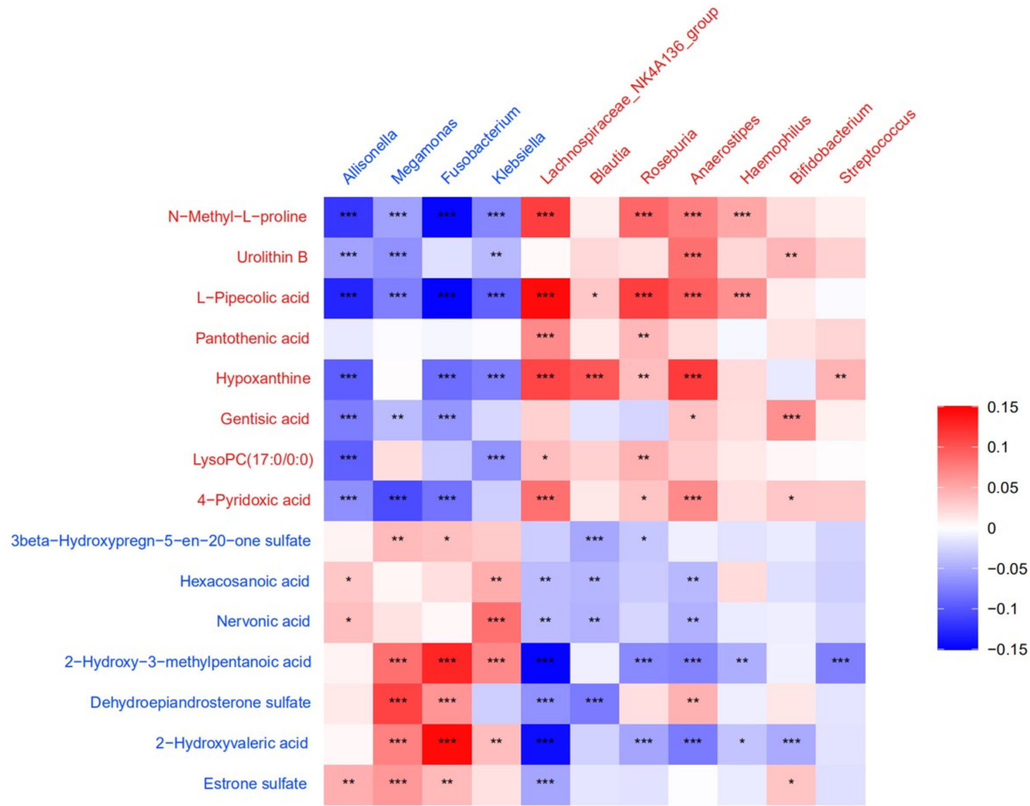

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90 **Supplementary Figure S3 Associations between dietary diversity score -related gut**

91 **microbial genera and plasma metabolites in Lanxi cohort**

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