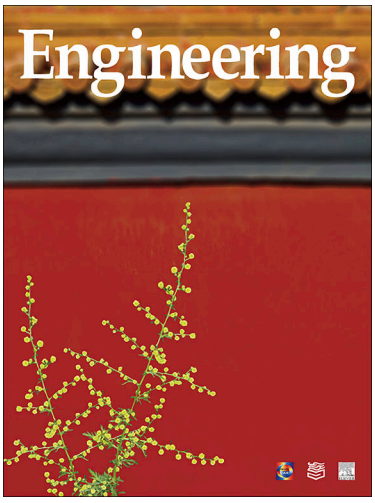
Journal Pre-proofs



Article

Food pairing pattern independently associated with cardiometabolic traits beyond dietary indices across eastern and western populations

Qiufeng Deng, Yuhao Sun, Mingxia Gu, Jingxiang Fu, Fengzhe Xu, Yuwen Jiao, Yan Zhou, Beining Ma, Lu Liu, Xiuchao Wang, Quanbin Dong, Tingting Wu, Huayiyang Zou, Jing Shi, Yifeng Wang, Yanhui Sheng, Liming Tang, Wei Sun, An Li, Shixian Hu, Jusheng Zheng, Yan He, Hongwei Zhou, Wei Wu, Xiangqing Kong, Lianmin Chen

PII: S2095-8099(25)00123-7
DOI: https://doi.org/10.1016/j.eng.2025.02.015
Reference: ENG 1835

To appear in: *Engineering*

Received Date: 17 October 2024
Revised Date: 10 December 2024
Accepted Date: 13 February 2025

Please cite this article as: Q. Deng, Y. Sun, M. Gu, J. Fu, F. Xu, Y. Jiao, Y. Zhou, B. Ma, L. Liu, X. Wang, Q. Dong, T. Wu, H. Zou, J. Shi, Y. Wang, Y. Sheng, L. Tang, W. Sun, A. Li, S. Hu, J. Zheng, Y. He, H. Zhou, W. Wu, X. Kong, L. Chen, Food pairing pattern independently associated with cardiometabolic traits beyond dietary indices across eastern and western populations, *Engineering* (2025), doi: https://doi.org/10.1016/j.eng.2025.02.015

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2025 THE AUTHORS. Published by Elsevier LTD on behalf of Chinese Academy of Engineering and Higher Education Press Limited Company

Food pairing pattern independently associated with cardiometabolic traits beyond dietary indices across eastern and western populations

Qiufeng Deng[a,b,#], Yuhao Sun[a,#], Mingxia Gu[c,#], Jingxiang Fu[d], Fengzhe Xu[e], Yuwen Jiao[b], Yan Zhou[b], Beining Ma[a], Lu Liu[a], Xiuchao Wang[a], Quanbin Dong[a], Tingting Wu[a], Huayiyang Zou[a], Jing Shi[a], Yifeng Wang[a], Yanhui Sheng[f], Liming Tang[b], Wei Sun[a], An Li[g], Shixian Hu[h], Jusheng Zheng[e], Yan He[d], Hongwei Zhou[d], Wei Wu[i,*], Xiangqing Kong[a,c,*], Lianmin Chen[a,b,*]

[a] *Department of Cardiology, The First Affiliated Hospital of Nanjing Medical University, Nanjing Medical University, Nanjing, China*

[b] *Changzhou Medical Center, The Third Affiliated Hospital of Nanjing Medical University, Nanjing Medical University, Changzhou, China*

[c] *Department of Cardiology, Nanjing Central Hospital, Nanjing, China*

[d] *Microbiome Medicine Center, Department of Laboratory Medicine, Zhujiang Hospital, Southern Medical University, Guangzhou, China*

[e] *School of Life Sciences, Westlake University, Hangzhou, China*

[f] *Cardiovascular Research Center, The Affiliated Suzhou Hospital of Nanjing Medical University, Nanjing Medical University, Suzhou, China*

[g] *Department of Periodontology, Stomatological Hospital, School of Stomatology, Southern Medical University, Guangzhou, China*

[h] *Institute of Precision Medicine, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, China*

[i] *Guangdong Provincial Institute of Public Health, Guangdong Provincial Center for Disease Control and Prevention, Guangzhou, Guangdong, China*

[#] These authors contributed equally to this work.
*Correspondence authors
E-mail addresses: cnnjwuwei@163.com (W. Wu), kongxq@njmu.edu.cn (X. Kong), lianminchen@njmu.edu.cn (L. Chen)

Abstract

Cardiometabolic health is closely linked to diet. Both specific food choices and dietary indices are commonly used to guide cardiometabolic protection strategies. However, the balance between foods may also significantly impact cardiometabolic health.

Here, we explore the balance and imbalance in long-term dietary food intake to quantify food pairing patterns, providing insights beyond conventional dietary indices and single-food intake frequencies. Using data from our Guangdong Gut Microbiome Project (GGMP; n = 6994) and the National Health and Nutrition Examination Survey (NHANES; n = 7350) cohorts, we observed that long-term food pairing patterns are independent of single food intake frequency and dietary indices. We identified 1759 and 306 cardiometabolic-related long-term food pairing patterns from two cohorts (false discovery rate (FDR) < 0.05), respectively. Notably, around 80% of these pairing foods not individually associated with cardiometabolic traits, and food pairing pattern associations with cardiometabolic traits at hyper food group level were consistent across Eastern and Western populations. Besides, mediation analysis revealed that 72.7% of long-term food pairing patterns affected cardiometabolic traits through 31 microbial genera, with *Clostridiumsensustricto1* playing a predominant role. Moreover, multi-step mediation analysis found that microbes mediated the impact of long-term food pairing patterns on cardiometabolic traits primarily through their metabolic pathways, such as pyruvate fermentation to propanoate and ergothioneine biosynthesis pathways.

Our data suggest that balance between dietary foods is broadly associated with cardiometabolic traits by modulating gut microbial functionalities, in contrast to the lower occurrence when considering individual foods in isolation. These results offer a novel perspective for designing personalized dietary strategies beyond present dietary indices to enhance cardiometabolic health.

Keywords: Population study; Dietary foods; Long-term food pairing patterns; Gut microbiome; Cardiometabolic health

Journal Pre-proofs

1. Introduction

Journal Pre-proofs

Nutrition is a well-established influencing factor for human cardiometabolic health [1,2]. It has been widely accepted that both the intake of specific foods and dietary pattern indices are associated with cardiometabolic health. For instance, cohort-based studies have shown that the intake of specific foods, such as red meat, can increase cardiovascular mortality risk [3–5] , while the dietary approaches to stop hypertension (DASH) dietary pattern index [6] and mediterranean diet (MED) index [7] are negatively associated with cardiovascular mortality risk. It is important to note that the former confirms the importance of specific foods in disease prevention, while the latter illustrates the cumulative effect of a group of foods.

Nevertheless, in addition to those two aspects, the daily diet of each individual encompasses a diverse array of foods that exhibit clear regional differences. For instance, Western settings exhibit a predominantly Western diet characterized by a higher intake of processed meat, red meat, butter, high-fat dairy products, and refined grains [8,9]. In contrast, Eastern settings are characterized by an Eastern diet, with higher intakes of whole grains, vegetables, and seafood [10]. Importantly, different foods are not metabolized independently but undergo complicated interactions that are still far from fully understood [11]. The balance and imbalance of different food intakes can regulate the composition of the gut microbiome and host metabolism, ultimately influencing human health. For example, maintaining a balance between vitamin-enriched fruits and fiber-enriched vegetables has been shown to reduce the risk of all-cause mortality [12]. In different food combinations, the characteristic gut microbiome and its metabolites may coordinate or mediate the occurrence and development of cardiovascular diseases, obesity, and other chronic diseases [13–18]. Thus, investigating food pairing patterns and their associations with individual health and disease may enhance our understanding of personalized nutrition from an ecological perspective. However, investigation of food pairing patterns and their impact on human cardiometabolic health remains limited.

In this study, we aim to elucidate the effects of balanced and imbalanced long-term food pairing patterns on human cardiometabolic phenotypes between populations with different dietary preferences (Fig. 1). Additionally, we further interpret how long-term food pairing patterns influence cardiometabolic health from the perspective of the gut microbiome and its metabolic functionalities. This study offers a unique angle on understanding the role of diet and ultimately assists in designing personalized diet to improve human health.

Journal Pre-proofs

Journal Pre-proofs

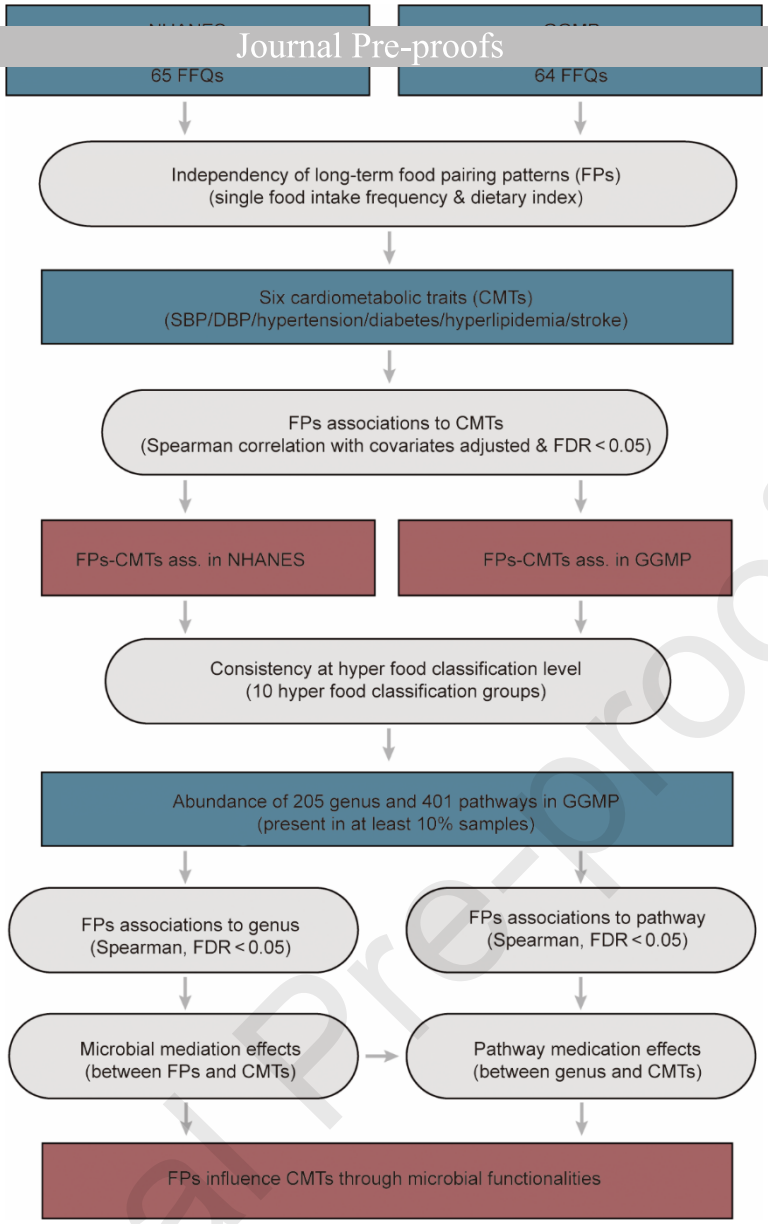


Fig. 1. Overview of the study design and analysis workflow. This study involves two large-scale prospective cohorts, including the NHANES (number (*n*) = 7350) and the GGMP (*n* = 6994). A total of 65 and 64 normalized unique monthly food intake frequencies were collected based on food frequency questionnaires (FFQs) from the NHANES and GGMP cohorts, respectively. Additionally, six cardiometabolic traits (CMTs) were assessed, including systolic blood pressure (SBP), diastolic blood pressure (DBP), and the prevalence of hypertension, diabetes, hyperlipidemia, and stroke. For the GGMP cohort, gut microbiome data were also available. ass., association.

2. Methods

2.1. Study cohorts

In this study, two large-scale population-based cohorts have been involved, including the NHANES (*n* = 7350) and the GGMP (*n* = 6994). NHANES was conducted by the National Center for Health Statistics (NCHS) to obtain data on the health and nutritional status of the non-institutionalized US population. NHANES used a complex, multi-stage, stratified, clustered sampling design to achieve a nationally representative sample. This nationwide survey included demographic and basic health data, standardized medical examinations, and laboratory tests. The NCHS Research Ethics Review Board approved all the data collection procedures. Detailed descriptions of NHANES are available elsewhere [19]. For this study, we involved 7350 out of 62 160 NHANES participants from 1999 through 2010 in the United States for whom detailed dietary habits were available, and 54 810 were not eligible because of missing data on complete FFQ. We further separated them into discovery set (*n* = 4070; NHANES data from 2005 to 2010) and replication set (*n* = 3280; NHANES data from 1999 to 2004) to verify the reproducibility of long-term food pairing patterns in Western population. The GGMP cohort (*n* = 7009) is a large community-based cross-sectional cohort conducted between 2015 and 2016 from Guangdong Province, China. Detailed information regarding the host metadata and sample collection have been reported previously [20]. The GGMP cohort was

55% female and 45% male, with a mean age of 53 years (standard deviation (SD) = 15). The mean body mass index (BMI) was [redacted] hom socio-demographic features, disease status, detailed dietary habit and stool microbiome information is available. The GGMP cohort study was approved by the Ethical Review Committee of the Chinese Center for Disease Control and Prevention under Approval Notice No. 201519-A. Written consent was also obtained from all participants.

Journal Pre-proofs

2.2. Dietary information from food frequency-based questionnaires

The participants from NHANES completed a 65-item FFQ to assess the frequencies of specified food items they had consumed over the past time without considering the portion size. 65 food items were classified into ten food categories: 14 grains, seven meats, 11 milks, one egg, ten vegetables, four fruits, ten drinks, two legumes, three oils, and three snacks (Table S1 in Appendix A). The participants from GGMP completed a 64-item FFQ to assess the frequencies of specified food items they had consumed over the past time without considering the portion size. 64 food items were classified into nine food categories: eight grains, 12 meats, seven milks, three eggs, eight vegetables, three fruits, 11 drinks, six legumes, and six snacks (Table S2 in Appendix A).

Both FFQs of NHANES and GGMP included common foods, such as rice, beer, coffee, beef, yogurt, and wine. However, due to the differences between Western and Eastern dietary habits, there were also many unique foods in both questionnaires. For example, the NHANES cohort's FFQ contained hot cocoa, margarine, dark bread, and so forth, and the GGMP cohort's FFQ contained wood fungus, crab, thousand egg, and so forth. In the data processing of FFQs for both cohorts, we all normalized the intake frequency to convert the consumption frequency of daily, weekly, quarterly, and annual consumption into monthly consumption frequency for food-related analysis in context.

2.3. Construction of long-term food pairing patterns

We hypothesized that the balance and imbalance of food intake, as captured through long-term FFQs, could provide unique insights into the role of diet in cardiometabolic health. These aspects are not adequately reflected in traditional single-food intake frequency analyses or dietary indices. Specifically, we proposed two types of food pairing patterns: additive food pairing pattern (AFP), which assumes synergistic effects between two foods; and subtractive food pairing pattern (SFP), which assumes antagonistic effects between two foods. The AFP and SFP were calculated using the normalized monthly consumption frequency of individual foods according to the following formulas:

$$AFP_{food1\&food2} = Frequency_{food1} + Frequency_{food2}$$

$$SFP_{food1\&food2} = Frequency_{food1} - Frequency_{food2}$$

where $Frequency_{food1}$ and $Frequency_{food2}$ denote the monthly intake frequencies of the two foods being paired. Specifically, AFP reflects the combined consumption frequency of two foods, with higher AFP values indicating more frequent joint consumption. This suggests that the combined dietary impact of the two foods may be amplified. SFP, on the other hand, captures the difference in consumption frequencies between two foods. Positive or negative SFP values indicate imbalances in relative consumption, which may influence dietary effects in distinct ways. Balanced food pairings suggest that the combined consumption frequency of two foods significantly impacts phenotypes, whereas imbalanced pairings indicate that large differences in consumption frequencies between the two foods may uniquely affect phenotypic outcomes.

As we have 65 and 64 normalized food intake frequency traits for NHANES and GGMP, respectively. Based on the above formulas, we finally generated 2080 and 2016 long-term food pairing patterns (AFP and SFP) for the two cohorts separately, thus providing a comprehensive assessment of potential long-term food pairing patterns across cohorts.

2.4. Independency of long-term food pairing patterns on top of previous dietary indicators

To investigate the independency of long-term food pairing patterns proposed here, we applied Spearman correlation to assess the correlation between 2080 AFPs/SFPs and individual food intake frequency, as well as other dietary indices, including healthy eating index 2020 (HEI2020) [21], healthy eating index 2015 (HEI2015) [22], alternative healthy eating index (AHEI) [23], DASH [24], DASH Index in serving sizes from the DASH trial (DASHI) [25], alternate MED score (aMED) [26], MED index in serving sizes from the Prevention with Mediterranean Diet (PREDIMED) trial (MEDI) [27], and dietary inflammation index (DII) [28].

2.5. Long-term food pairing pattern associations to cardiometabolic traits

To assess associations between long-term food pairing patterns and cardiometabolic traits in the NHANES cohort, continuous AFPs and SFPs were inverse-rank transformed and corrected for age, sex, race, BMI, and smoking status. Spearman correlation was then applied to assess the correlation between 2080 AFPs/SFPs and six cardiometabolic traits (SBP, DBP, hypertension, diabetes, hyperlipidemia, and stroke) in the NHANES discovery and replication sets, respectively (Table S3 in Appendix A). In the GGMP cohort, 2016 AFPs/SFPs were associated with six cardiometabolic traits after adjusting for age, sex, BMI, and smoking status by using Spearman correlation (Table S4 in Appendix A). The false discovery rate (FDR) was calculated using the Benjamini-Hochberg procedure [29].

To further assess the robustness of our findings, a sensitivity analysis was conducted by including diet diversity as an addi[redacted] was calculated based on the FFQ data.

Journal Pre-proofs

2.6. Gut microbiome data processing

For the GGMP cohort study, participants received a stool sampler, ice bags, and instructions for proper sample collection and storage. After defecation, participants placed the fecal sample with the ice bag in the provided ice box and sent it to a nearby collection point within 24 hours. Collected samples were further transferred to the laboratory and stored in –80 °C freezers until further processing. Total bacterial DNA was extracted using a fecal DNA bead isolation kit (Bioeasy, Beijing) according to the manufacturer's instructions and the 16S ribosomal RNA (rRNA) gene V4 region was amplified and sequenced on an Illumina Hiseq 2500 (Beijing Genome Institute, China).

We further performed taxonomic classification using direct taxonomic binning instead of operational taxonomic unit (OTU) clustering methods by taking advantage of the recent MiBioGen consortium pipeline [30]. In brief, sequence quality-based read filtering was performed using Fastp (v0.23.2). Clean reads were randomly rarefied to 10 000 reads per sample and sequences from all samples were merged into one file. Read identification was performed by using an RDP Classifier (v2.12) based on the SILVA database (v128) and the read labels of each sequence for each sample were summarized. The microbial metabolic pathways were annotated by the PICRUSt2 pipeline [31], which predicts the function of gene sequences and infers the abundance of pathways based on the OTUs from QIIME2 [32]. Finally, 205 genera and 401 pathways with a prevalence of more than 10% were included for downstream analysis.

2.7. Microbial associations to long-term food pairing patterns and cardiometabolic traits

We hypothesized a potential connection between the gut microbiome, long-term food pairing pattern, and cardiometabolic health. To assess associations between long-term food pairing patterns and gut microbiome, Spearman correlation was applied to assess the correlation between 2016 AFPs/SFPs and the relative abundance of 205 gut microbial genera in the GGMP cohort, adjusted for age, sex, BMI, and smoking status (Table S5 in Appendix A). To further determine the association between specific foods and gut microbiome, we linked dietary frequency to gut microbial genera with Spearman correlation, adjusted for age, sex, BMI, and smoking status (Table S6 in Appendix A). The FDR was calculated using the Benjamini–Hochberg (BH) method [29].

Next, to test associations between the gut microbiome and six cardiometabolic traits, we conducted Spearman correlation analysis to identify the correlation between the relative abundance of 205 gut microbial genera (adjusted for age, sex, BMI, and smoking status) and cardiometabolic health (Table S7 in Appendix A) in GGMP cohort.

2.8. Mediation analysis

For microbial features associated with both long-term food pairing patterns (FDR < 0.05) and cardiometabolic traits (FDR < 0.05), we first checked whether the long-term food pairing patterns were associated with the six cardiometabolic traits using Spearman correlation (FDR < 0.05). Next, we carried out mediation analysis using the mediate function from mediation (v4.5.0) [33] R package to infer the mediation effect of the microbiome for long-term food pairing patterns impacting cardiometabolic traits (Table S8 in Appendix A).

2.9. Linking the microbial taxa to metabolic pathways

We initially verified the gut microbial genera mediators identified in the above mediation analysis and assessed their associations with gut microbial pathways. Next, we selected microbial metabolic pathways showing statistical significance (FDR < 0.05) to delve deeper into their associations with cardiometabolic traits using Spearman correlation analysis (FDR < 0.05). We then carried out mediation analysis using the mediate function from mediation (v4.5.0) [33] R package to infer the mediation effect of microbial pathwaysof mediator genera impacting on cardiometabolic traits (Table S9 in Appendix A).

3. Results

3.1. Long-term food pairing patterns show independence from both single food intake frequency and dietary indices

The impacts of diet on cardiometabolic health are primarily focused on the intake of individual foods and dietary patterns reflected by various dietary indices. However, understanding the balance and imbalance of different food intakes may provide additional explanations for cardiometabolic health. Here, we propose a long-term food pairing pattern, which employs addition and subtraction to establish configurable connections from one diet to another, including AFP and SFP (Section 2). Specifically, the higher the AFP, the more frequent the intake of either/both diets; and the higher the SFP, the greater the consumption frequency of one food compared to another.

In this manner, we have generated 2080 and 2016 long-term food pairing pattern traits based on 65 and 64 food frequencies from the NHANES [19] and our GGMP [20] cohorts, respectively. It is noteworthy that there were only six overlapping food

Journal Pre-proofs

frequency items (rice, yogurt, beef, beer, wine, and coffee) between the two cohorts (Tables S1 and S2 in Appendix A), sugg the distribution of 15 overlapping long-term food pairing patterns between populations, we observed that only the AFP between yogurt and beef intake frequency was comparable between populations ($P_{\text{Wilcoxon}} = 0.08$; Fig. 2(a)), while all the others were different (FDR < 0.05; Fig. S1 in Appendix A). For instance, pairings between rice and beer exhibited significant differences between populations ($P_{\text{Wilcoxon}} = 5.0 \times 10^{-324}$; Fig. 2(a)). This was evidenced by a lower total intake of these two foods (as indicated by a lower AFP between rice and beer) and a more balanced consumption frequency (SFP closer to 0) in the US compared to China (Fig. 2(a)).

Furthermore, we tested if the long-term food pairing patterns proposed here show independence from other diet-related indicators by assessing the association of food pairing patterns with single diets and dietary pattern indices. 2080 long-term food pairing patterns derived from 65 single food intake frequencies in the NHANES cohort were weakly correlated with both the frequency of a single food intake (Fig. 2(b)) and eight widely recognized diet indices (Fig. 2(c)), including HEI2020 [21], HEI2015 [22], AHEI [23], DASH [24], DASHI [25], aMED [26], MEDI [27], and DII [28]. Similar results were also observed in the GGMP cohort for associations to single food intake frequencies (Figs. S2 in Appendix A). Thus, it is essential to recognize that long-term food pairing patterns serve as independent traits and may play a unique role in cardiometabolic health beyond dietary indicators.

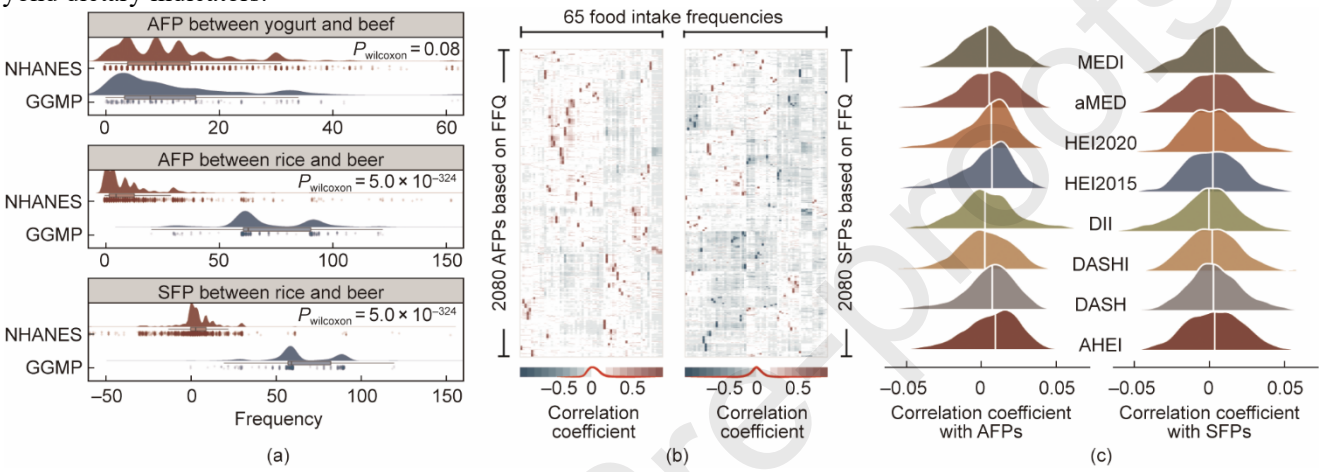


Fig. 2. Long-term food pairing patterns independent of both single food intake frequency and dietary pattern. (a) Distribution of specific long-term food pairing patterns across populations, presented as a density map (top), boxplot (middle), and jitter scatter plot (bottom). The boxplot displays the median, first quartile, and third quartile (25th and 75th percentiles) of the food pairing pattern levels. Whiskers extend to the smallest and largest values within 1.5 times the interquartile range (IQR). Each dot represents one sample. *P* values from the Wilcoxon test are shown accordingly. (b) Heatmap illustrating associations between 65 single food intake frequencies and 2080 food pairing patterns, estimated using Spearman correlation. The intensity of the color represents the correlation strength. (c) Distribution of associations between eight dietary indices and 2080 food pairing patterns, estimated using Spearman correlation.

3.2. Single foods exhibit limited associations with cardiometabolic traits but their pairings demonstrate pronounced effects

We then assessed the influence of long-term food pairing patterns on cardiometabolic health, including blood pressures (SBP and DBP) and the prevalence of hypertension, diabetes, hyperlipidemia, and stroke, using 65 food intake frequencies collected from 4070 participants in the NHANES study during 2005–2010 as the discovery set (Table S10 in Appendix A). Additionally, a replication set consisting of 3280 NHANES participants recruited from 1999 to 2004 was involved (Table S10). We used linear models to adjust for potential confounders (sex, age, race, BMI, and smoking status) and further applied Spearman correlation to establish associations between long-term food pairing patterns and cardiometabolic traits. In total, 1607/1502 significant associations with six cardiometabolic traits at FDR < 0.05 were observed for AFPs/SFPs, comprising 58.6%/54.4% of those associations that were further replicated in the replication set ($P < 0.05$, Fig. 3(a)).

In detail, the replicable associations including 143/136 SBP-related AFPs/SFPs (42 overlapped) derived from 45/49 single foods (41 overlapped), 94/75 DBP-related AFPs/SFPs (eight overlapped) derived from 39/38 single foods (32 overlapped), 210/174 hypertension-related AFPs/SFPs (74 overlapped) derived from 63/62 single foods (61 overlapped), 449/406 diabetes-related AFPs/SFPs (282 overlapped) derived from 65/65 single foods (65 overlapped), 13/7 hyperlipidemia-related AFPs/SFPs (zero overlapped) derived from 11/9 single foods (four overlapped), and 33/19 stroke-related AFPs/SFPs (one overlapped) derived from 16/20 single foods (seven overlapped). Notably, each single food interacted with at least one other food to form a cardiometabolic-related long-term food pairing pattern (Fig. 3(b)), suggesting the broad impact of long-term food pairing patterns on human cardiometabolic health. The results of the sensitivity analysis indicated that adjustments for diet diversity had minimal impact on the findings (Figs. S3 and S4 in Appendix A).

To ascertain whether the intake frequency of single foods is associated with those cardiometabolic traits without constructing food pairing patterns, we adjusted for the same confounding factors as mentioned above. Consequently, we

identified 19 foods related to SBP, 12 to DBP, 12 to hypertension, 11 to diabetes, one to hyperlipidemia, and four to stroke (disJournal Pre-proofs the cardiometabolic association results with single foods and long-term food pairing patterns, we found that 80.8%/82.5% of cardiometabolic-related AFPs/SFPs were formed by single foods that were not individually associated with cardiometabolic traits (Fig. 3(c)). For instance, the intake frequencies of both flour tortillas and hard liquor were not individually associated with the risk of diabetes (Fig. 3(d)). However, the AFP between the two foods showed a significantly negative association with the risk of diabetes (Fig. 3(d)). Besides, the intake frequencies of both sweet potato and chocolate candy were not individually associated with the risk of hypertension (Fig. 3(e)). However, the SFP between the two foods showed a significantly positive association with the risk of hypertension (Fig. 3(e)).

Additionally, we observed that consistent associations between single foods and cardiometabolic traits may change or even reverse when their pairing patterns are considered. For instance, higher intake frequencies of hot cocoa and diet cola/soda were both associated with an increased risk of diabetes (Fig. S5 in Appendix A). However, the SFP between them showed negative associations with the risk of diabetes (Fig. S5). Therefore, the proposed long-term food pairing patterns provide an additional layer of information that is independent of single food intake in relation to human cardiometabolic health.

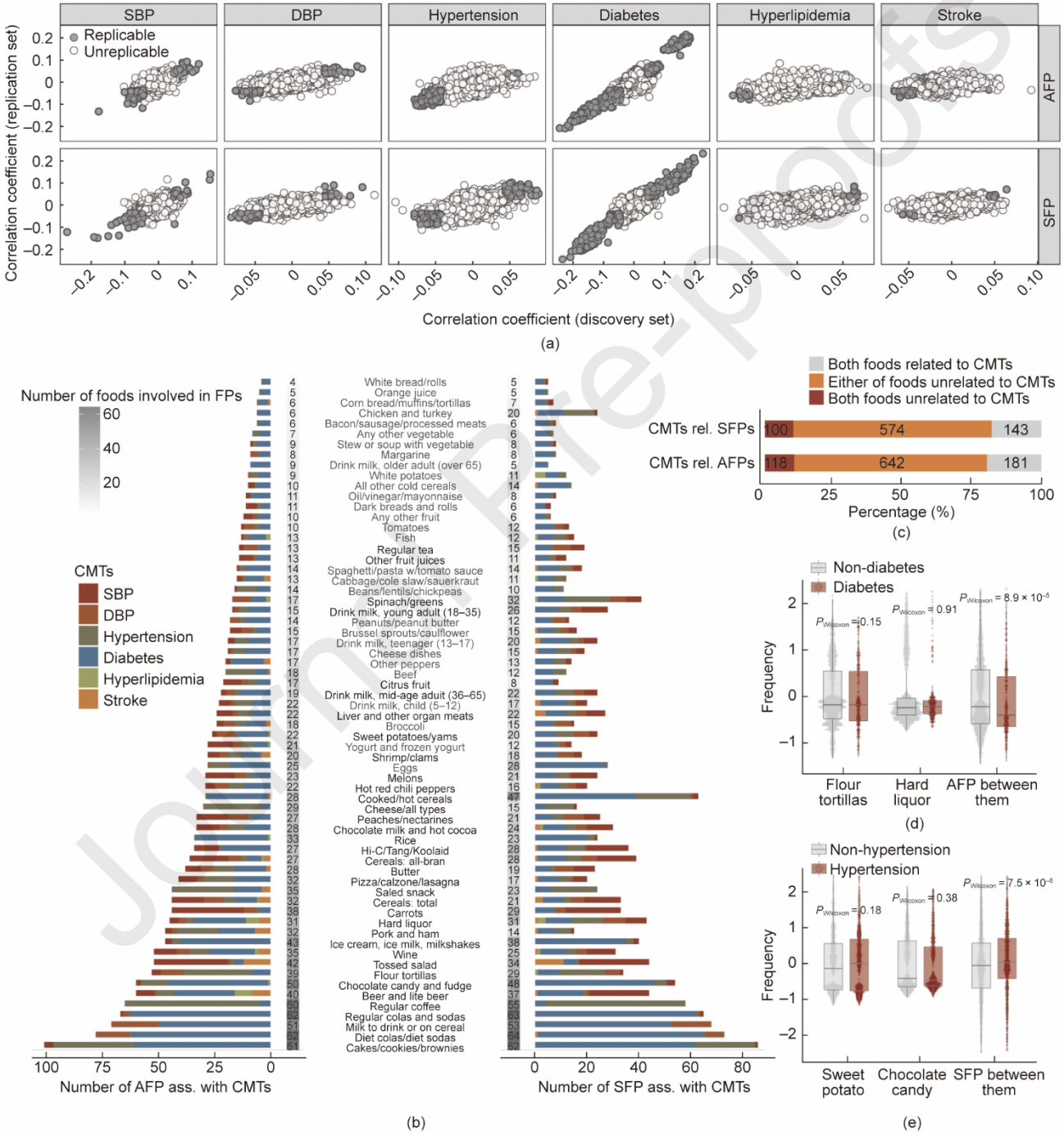


Fig. 3. Long-term food pairing pattern associations to cardiometabolic traits. (a) Long-term food pairing pattern associations with the six cardiometabolic traits in the NHANES discovery and replication sets. The *X*-axis indicates the Spearman correlation coefficient from the discovery set, and the *Y*-axis indicates the Spearman correlation coefficient from the replication set. Each dot represents one FP. Dark grey dots show replicable associations (discovery set at FDR < 0.05, replication set at $P < 0.05$), while light grey dots show non-replicable associations. (b)

Overview of long-term food pairing pattern associations with cardiometabolic traits per single food. The *Y*-axis lists all 65 different foods from FFQ [redacted] whose single intake frequencies are significantly associated with at least one of the six cardiometabolic traits (Spearman correlation, FDR < 0.05). The *X*-axis indicates the number of significant food pairing patterns associations with cardiometabolic traits derived from a certain food. Numbers in the squares represent the number of single foods involved in cardiometabolic-related long-term food pairing patterns. (c) Summary of long-term food pairing patterns with significant associations to cardiometabolic traits. Gray represents the intake frequencies of two foods within a long-term food pairing pattern both individually associated with cardiometabolic traits, while red represents interactions where neither of the two foods is individually associated with cardiometabolic traits. Orange represents pairings where either of the two foods within a long-term food pairing pattern is individually associated with cardiometabolic traits. Numbers in the bar plot represent significant AFP/SFP associations to cardiometabolic traits. rel., related. (d) The AFP between flour tortillas and hard liquor showed a difference in diabetes prevalence, while neither the intake frequencies of the two foods showed a difference between diabetic and non-diabetic participants. The box plots show the median, first quartile, and third quartile (25th and 75th percentiles) of normalized residuals of food pairing patterns levels. The upper and lower whiskers extend to the largest and smallest values no further than 1.5 × IQR, respectively. Each dot represents one sample. The *P* value from the Wilcoxon test is shown accordingly. (e) The SFP between sweet potato and chocolate candy showed a difference in hypertension prevalence, while neither the intake frequencies of the two foods showed a difference between hypertensive and non-hypertensive participants. The box plots show the median, first quartile, and third quartile (25th and 75th percentiles) of normalized residuals of food pairing patterns levels. The upper and lower whiskers extend to the largest and smallest values no further than 1.5 × IQR, respectively. Each dot represents one sample. The *P* value from the Wilcoxon test is shown accordingly.

Journal Pre-proofs

3.3. Long-term food pairing pattern associations to cardiometabolic traits at the hyper food classification group level exhibit consistency across populations

We further examined whether the observed cardiometabolic-related long-term food pairing patterns in the westernized NHANES cohort showed consistency in our GGMP cohort with eastern dietary preferences. Using the same strategy, we established 104/89 SBP-related AFPs/SFPs, 36/30 DBP-related AFPs/SFPs, 24/20 hypertension-related AFPs/SFPs, 2/1 diabetes-related AFPs/SFPs, one hyperlipidemia-related SFPs, and one stroke-related SFPs (FDR < 0.05, Fig. 4(a)). By comparing the number of identified cardiometabolic-related long-term food pairing patterns, we observed that the top long-term food pairing pattern associations were mainly related to hypertension-related traits, including SBP, DBP, and the prevalence of hypertension between the two cohorts (Fig. 4(a)).

Given the fact that only a fewer number of dietary habits were completely consistent between NHANES and GGMP cohorts (Tables S1 and S2), we broadened our perspective and analyzed the similarity of cardiometabolic-related long-term food pairing patterns between the two cohorts by categorizing long-term food pairing patterns into ten hyper food groups, including milk, grain, meat, fruit, and others (Fig. 4(b)). In doing so, we observed that 16, 9, 13, and 2 pairing patterns between hyper food classification group derived from long-term food pairing patterns, associated with SBP, DBP and hypertension, respectively, were shared in both cohorts (Fig. 4(b)). Of note, we also observed many non-overlapping cardiometabolic-related long-term food pairing patterns at the hyper food classification group level (Fig. 4(b)) between the two cohorts, indicating potential regional-specific long-term food pairing patterns on human cardiometabolic health. For instance, the AFP of all-bran cereals and diet colas/sodas was positively associated with the risk of diabetes in the NHANES cohort ($r_{\text{Spearman}} = 0.18$, $P = 3.2 \times 10^{-32}$; Fig. 4(c)), while the AFP of tofu shreds and wood fungus was negatively associated with SBP in the GGMP cohort ($r_{\text{Spearman}} = -0.17$, $P = 6.8 \times 10^{-4}$; Fig. 4(d)).

When plotting the effect size of each cardiometabolic-related long-term food pairing pattern at the hyper food classification group level (Fig. 4(e)), we observed that many foods from the fruit category, pairing with categories such as vegetables, drinks, grains, and milks, exhibited consistent negative associations with SBP between populations (Fig. 4(e), marked in red). Besides, we observed that despite some long-term food pairing patterns showing large variations between cohorts, they exhibited consistent associations with cardiometabolic traits. For example, the AFP of rice and beer showed substantial variation between populations (Fig. 2(a)), but demonstrated consistent positive associations with SBP in both the NHANES cohort and GGMP cohort (Fig. 4(f)). This underscores the consistent effects of long-term food pairing patterns on cardiometabolic traits among populations with diverse eating habits at hyper food classification group level. It provides valuable data for designing personalized dietary strategies that consider long-term food pairing patterns at the hyper food classification group level to enhance cardiometabolic health.

Journal Pre-proofs

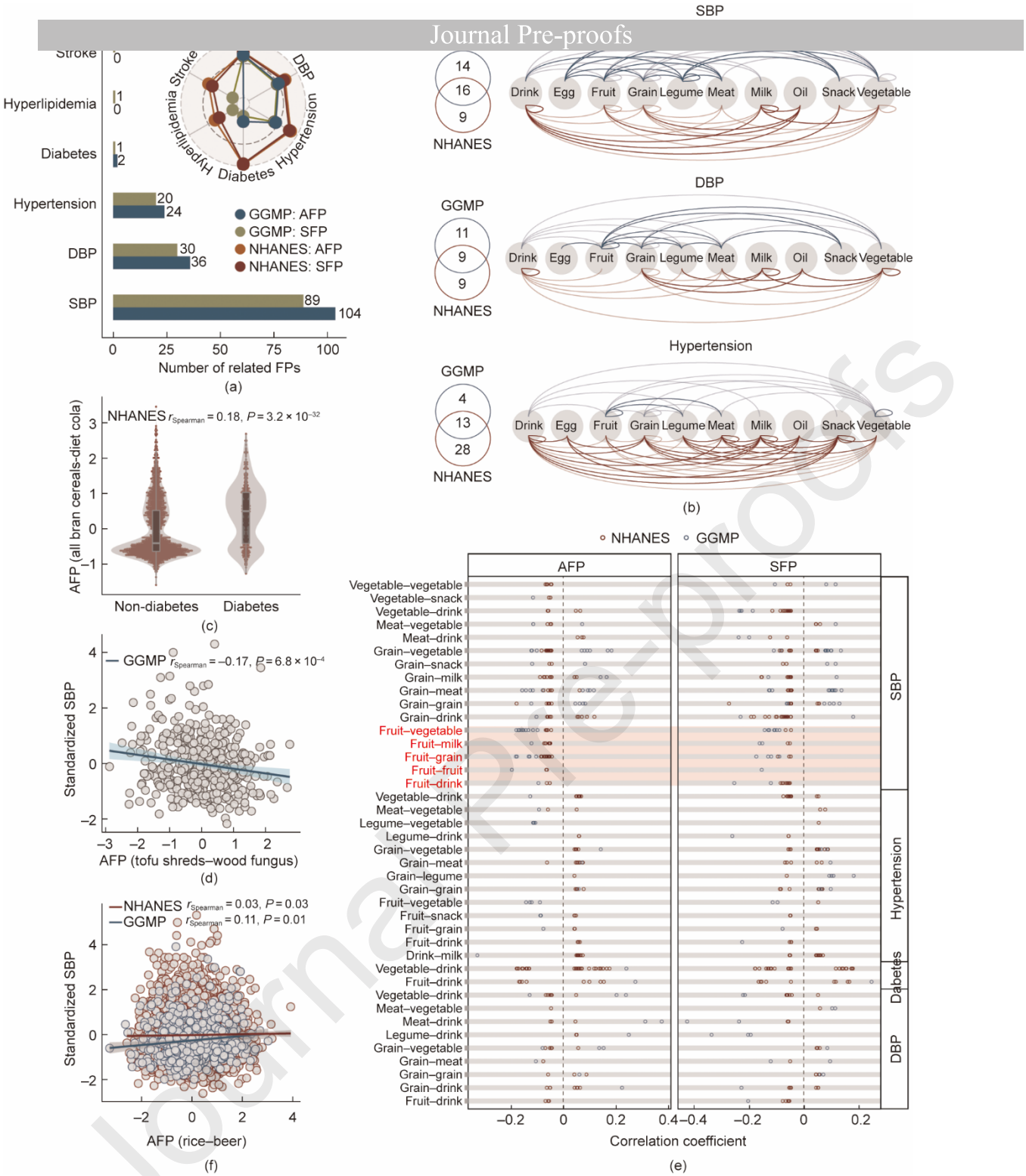


Fig. 4. Cardiometabolic trait-related long-term food pairing patterns show consistency at the hyper food classification group level between populations. (a) Summary of long-term food pairing pattern associations with cardiometabolic traits between populations. The bar plot shows the number of long-term food pairing pattern associations with cardiometabolic traits in the GGMP cohort. The radar plot compares the number of significant long-term food pairing pattern associations with cardiometabolic traits between the NHANES and GGMP cohorts. (b) Comparison of long-term food pairing pattern associations with cardiometabolic traits at hyper food classification group level between populations. The Venn diagram summarizes the overlap of significant long-term food pairing pattern associations with a specific cardiometabolic trait at hyper food classification group level between populations. The arc diagram on the left illustrates the detailed food pairing patterns at hyper food classification group level between populations. Darker arcs indicate non-overlapping associations, while lighter arcs represent shared associations. (c) The AFP between all-bran cereals and diet colas shows a difference in diabetes prevalence. The box plots display the median, first quartile, and third quartile (25th and 75th percentiles) of normalized residuals of AFP levels. The upper and lower whiskers extend to the largest and smallest values within 1.5 × IQR, respectively. Each dot represents one sample. The *P* value from the Spearman correlation is shown accordingly. (d) The SFP between tofu shreds and wood fungus is negatively associated with SBP. The *X* and *Y* axes indicate the normalized residuals of SBP and the SFP between tofu shreds and wood fungus, respectively. Each dot represents one sample. The line shows the linear regression line, with gray shading representing the 95% confidence interval (CI). The Spearman correlation coefficient and *P* value are shown accordingly. (e) Individual long-term food pairing pattern associations with cardiometabolic traits at hyper food classification group level between populations. Each dot represents an

individual long-term food pairing pattern association with a specific cardiometabolic trait, grouped at hyper food classification level. The left *Y*-axis [redacted], and the right *Y*-axis indicates cardiometabolic traits with significant associations with long-term food pairing patterns. Long-term food pairing patterns at hyper food classification group level marked in red show the same association directions with cardiometabolic traits between populations. The *X*-axis indicates the Spearman correlation coefficient of long-term food pairing pattern associations with cardiometabolic traits. (f) The SFP between rice and beer is positively associated with SBP in both populations. The *X*-axis indicates normalized residuals of long-term food pairing pattern, and the *Y*-axis indicates normalized SBP. Each dot represents one sample.

Journal Pre-proofs

3.4. The impact of long-term food pairing patterns on gut microbiome composition

We recently demonstrated that the gut microbiome significantly contributes to host cardiac metabolism, as evidenced by its impact on inter-individual variations in various categories of plasma metabolites [9,34]. Thus, characterizing the influence of long-term food pairing patterns on the composition of the gut microbiome may provide mechanistic insights into the etiologies of human cardiometabolic disorders. By associating long-term food pairing patterns with 205 microbial genera with a prevalence rate of more than 10% in the GGMP cohort (n = 6994), while adjusting for gender, age, BMI, and smoking status, we identified 3575 associations between 734 AFPs and 179 genera, and 2728 associations between 585 SFPs and 196 genera, respectively (FDR < 0.05, Table S5). In the meantime, we also examined the association between single foods and gut microbiomes without constructing long-term food pairing patterns. In total, we identified 854 associations between 196 gut microbial genera and 56 foods (FDR < 0.05, Table S6).

Interestingly, we observed that long-term food pairing patterns exhibited broad associations with genera from *Clostridiales*, *Burkholderiales*, *Bacteroidales*, and the *Erysipelotrichales* order (Fig. 5(a)), as reflected by distinct clusters in the association heatmaps (Figs. S6 and S7 in Appendix A). However, their associations with single foods were rather sparse (Figs. S6 and S7), indicating that the gut microbiome may have a propensity for specific long-term food pairing patterns independent of individual foods. For instance, microbial associations with long-term food pairing patterns derived from fresh fruit encompassed not only all 33 fresh fruit-related genera but also numerous genera unrelated to fresh fruit (Fig. 5(b)). Furthermore, specific foods paired with others to be widely associated with gut microbiome composition, but their individual associations with microbial genera were limited (Fig. 5(c)). For example, long-term food pairing patterns formed by mutton and 26 other foods were linked to 45 genera, however, mutton itself was not directly linked to any genus (Fig. 5(c)). Additionally, the number of genera associated with long-term food pairing patterns derived from tofu exceeded eight times the number of genera specifically related to tofu (Fig. 5(c)). Moreover, the proposed AFPs and SFPs also have independent associations with gut microbial composition, as reflected by the non-overlapping associations (Fig. 5(d)), indicating that these proposed two types of long-term food pairing patterns have unique effects on the gut microbiome composition. For instance, AFP between rice and other cereals was linked to 36 different genera, which were not associated with their SFP. Conversely, the SFP between rice and other fried foods was linked to 42 different genera but not associated with their AFP (Fig. 5(d)).

Journal Pre-proofs

Journal Pre-proofs

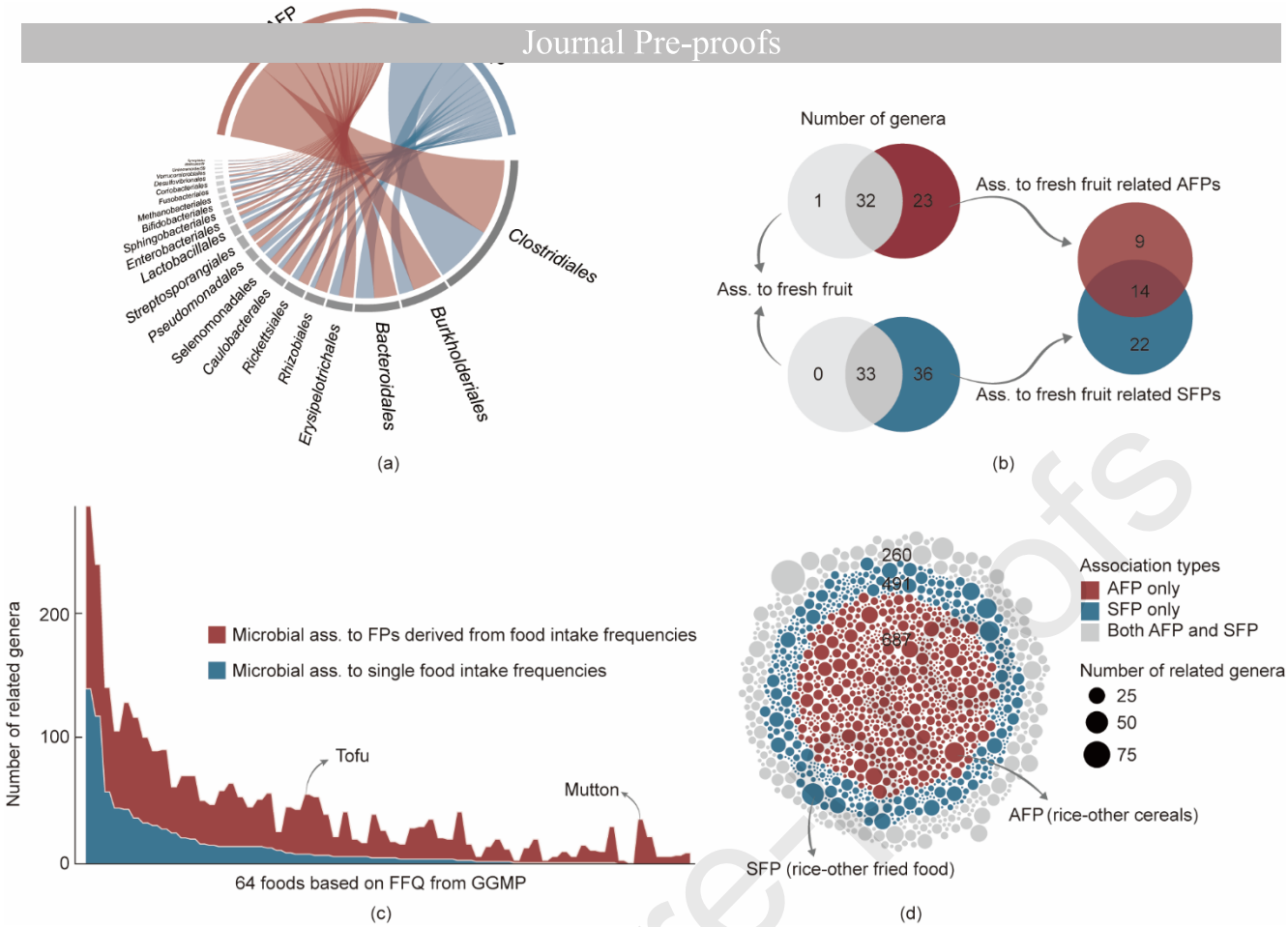


Fig. 5. The influence of long-term food pairing patterns on gut microbiome composition. (a) Summary of long-term food pairing pattern associations with the composition of gut microbial genera. The associated AFPs are colored red, and the associated SFPs are colored blue. Each line indicates a significant association between a food pairing pattern and gut microbial genera within the same order (Spearman correlation, FDR < 0.05). (b) Comparison of microbial genera associated with fresh fruit intake frequency and long-term food pairing patterns derived from fresh fruit. The Venn diagram on the left indicates the overlap of microbial genera associated with fresh fruit intake frequency and long-term food pairing patterns derived from fresh fruit. The diagram on the right shows the overlap of microbial genera associated only with additive or SFPs. (c) Comparison of microbial associations with intake frequency of single foods and long-term food pairing patterns derived per single food. The *X*-axis lists all 64 different foods from FFQs in the GGMP cohort. The *Y*-axis indicates the number of microbial genera with significant associations with single food intake frequencies and food pairing patterns derived from per single food. (d) Overlap of long-term food pairing pattern associations with microbial genera between additive and SFPs. Microbial associations with both additive and SFPs are marked in grey, while those only associated with additive or SFPs are marked in red and blue, respectively. Each dot represents one food pairing pattern, with the dot size indicating the number of related genera.

3.5. The gut microbiome mediates associations between long-term food pairing patterns and cardiometabolic traits

Of the 220 unique long-term food pairing patterns associated with cardiometabolic traits and 1319 associated with microbes, 192 food pairing patterns were associated with both (Fig. 6(a)). To evaluate whether microbes can mediate the long-term food pairing patterns impact on cardiometabolic traits, we associated 205 gut microbial genera with the six cardiometabolic traits and established 668 significant associations with 190 genera (FDR < 0.05, Fig. 6(b), Table S7), including 138 SBP-related genera, 116 DBP-related genera, 127 hypertension-related genera, 113 diabetes-related genera, 126 hyperlipidemia-related genera, and 48 stroke-related genera.

We then employed mediation analysis to explore the pathways through which long-term food pairing patterns influence host cardiometabolic traits via gut microbe. Our analysis identified 755 mediation linkages, with 160 long-term food pairing pattern features (comprising 86 AFPs and 74 SFPs) significantly affecting host cardiometabolic traits through 31 gut microbial genera (FDR < 0.05, Fig. 6(c)). Notably, we found that out of the 220 unique long-term food pairing patterns linked to cardiometabolic traits, 72.7% demonstrated a noteworthy influence on these traits via the gut microbiome (Table S8). This underscores the critical role of the gut microbiome in mediating the impact of long-term food pairing patterns on cardiometabolic health.

Besides, a substantial portion (26.6%) of these mediated linkages were associated with long-term food pairing pattern impact on blood pressure via the *Clostridiumsensustricto1* genus. Clinical studies have implicated *Clostridiumsensustricto1*

in amino acid and central carbohydrate metabolism [35], as well as its potential to raise host blood pressure [36]. Here, we obseJournal Pre-proofs the abundance of *Clostridiumsensustricto1* (SBP: 44%, $P_{mediation} < 0.001$; DBP: 31%, $P_{mediation} < 0.001$; Fig. 6(d)). Our findings thus suggest that *Clostridiumsensustricto1* may play a role in mediating the effect of long-term food pairing patterns on cardiometabolic health.

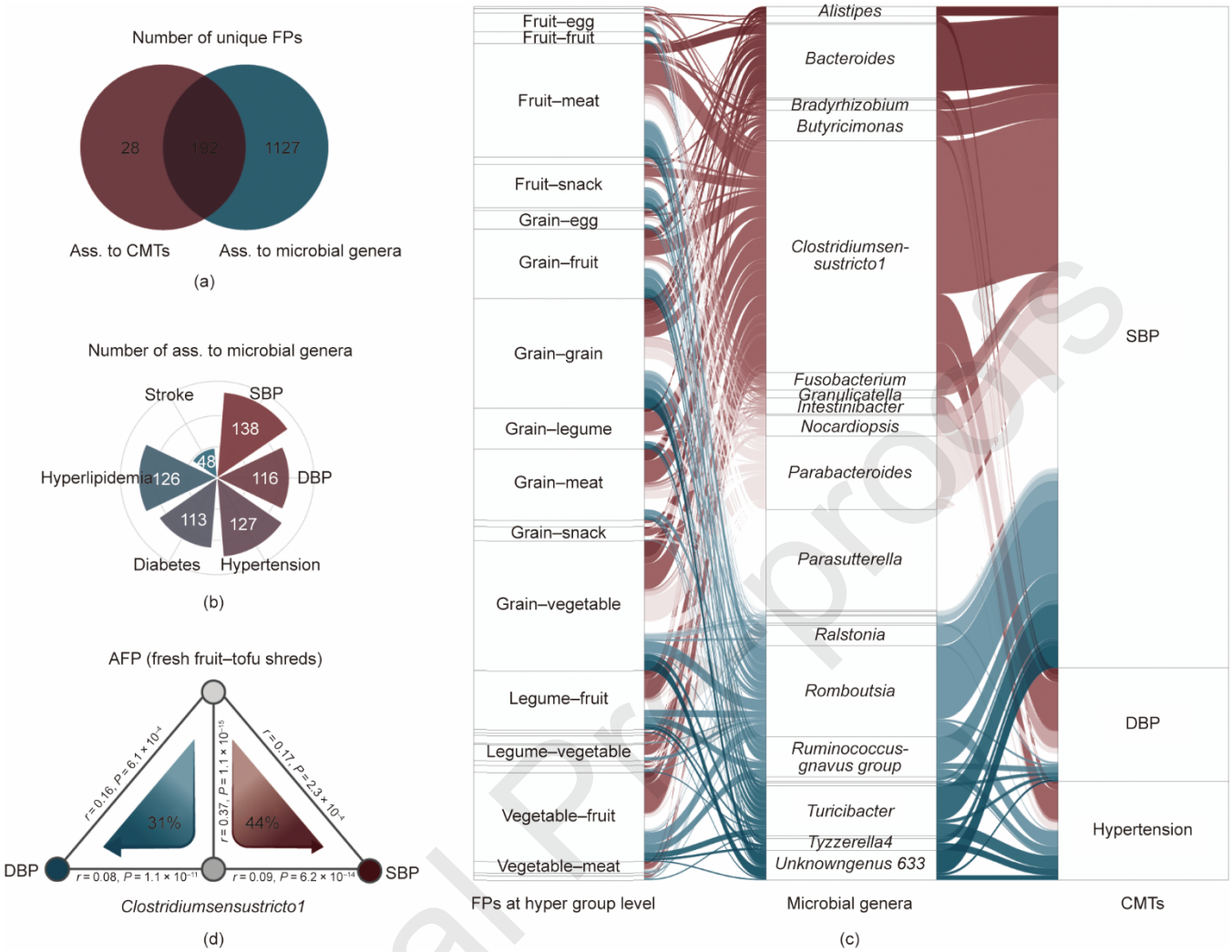


Fig. 6. The gut microbiome mediates associations between long-term food pairing patterns and cardiometabolic traits. (a) Overlap of long-term food pairing patterns with significant associations to cardiometabolic traits and gut microbial genera. (b) Summary of microbial associations with different cardiometabolic traits. The number indicates significant associations at FDR < 0.05. (c) Gut microbial genera mediate long-term food pairing pattern associations with cardiometabolic traits. The Sankey plot illustrates significant mediation effects of the gut microbiome at FDR < 0.05. Shown are long-term food pairing patterns at hyper food classification group level (left), microbial genera (middle), and cardiometabolic traits (right). Curved lines connecting the panels denote significant mediation linkages, with colors representing different gut microbes acting as mediators. (d) *Clostridiumsensustricto1* mediates the AFP between fresh fruit and tofu shreds in association with both SBP and DBP. The gray lines indicate the associations between the two factors, with corresponding Spearman coefficients and *P* values. The mediation effects are shown with arrows.

3.6. The mediation effects of gut microbiome rely on metabolic pathways

To delve into the functional role of gut microbes in mediating the impact of long-term food pairing patterns on cardiometabolic traits, we examined how these microbes influence such traits through their metabolic pathways. Among the 31 genera identified as mediators of long-term food pairing patterns on cardiometabolic traits (FDR < 0.05, Table S8), we characterized 237 metabolic pathways associated with both cardiometabolic traits and microbial genera with mediator roles (FDR < 0.05, Table S9).

Further utilizing mediation analysis, we identified 4019 significant mediation linkages involving 212 pathways that mediate the impact of microbes on cardiometabolic traits (FDR < 0.05, Table S9). Notably, we observed that microbes mediate the impact of long-term food pairing patterns on cardiometabolic traits through pathways involved in well-known short-chain fatty acid and amino acid biosynthesis. For instance, propionic acid, a short-chain fatty acid produced from dietary fiber by the gut microbiome, is known to play a crucial role in cardiometabolic health [14,37]. Our results indicated that the genera

Butyricimonas contributed to mediating the impact of long-term food pairing patterns derived from fiber-enriched vegetables and

Journal Pre-proofs

Additionally, previous studies have shown that gut microbiomes may promote the synthesis of trimethylamine (TMA) by utilizing ergothioneine from the host's daily diets [38], potentially leading to elevated blood pressure [39]. Here, we observed that 22 distinct gut microbial genera mediated the impact of long-term food pairing patterns on cardiometabolic traits through the ergothioneine biosynthesis I pathway (Table S9). Human beings primarily obtain ergothioneine from mushrooms [40]. Interestingly, we found that long-term food pairing patterns derived from ergothioneine-rich mushrooms influenced host blood pressure by affecting certain potentially pathogenic microbiomes capable of absorbing and metabolizing ergothioneine. Specifically, a higher host SFP of fresh mushrooms and fresh fruits (intaking more mushrooms and less fruits) elevated host SBP by increasing the abundance of certain potentially pathogenic genera belonging to the *Clostridiales* order, including *Clostridiumsensustricto1*, *Romboutsia*, and *Tyzzerella4* (Table S8). These genera acted through the ergothioneine biosynthesis I pathway to enhance their colonization and TMA synthesis, thereby contributing to the observed increase in host blood pressure (Figs. 7(b) and (c), Table S9). Taken together, these findings provide valuable insights into the functional role of gut microbes in mediating the impact of long-term food pairing patterns on cardiometabolic traits through metabolic pathways.

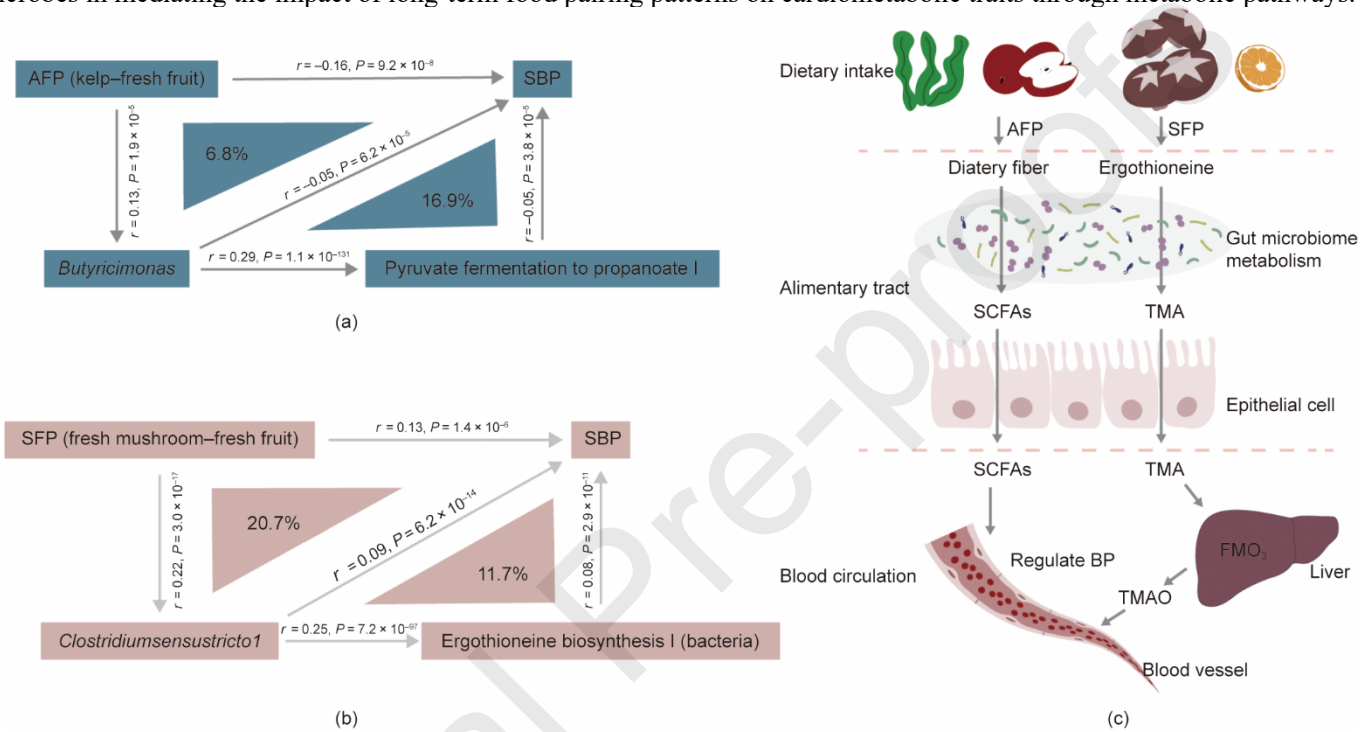


Fig. 7. The medication effects of gut microbiome in long-term food pairing pattern associations to cardiometabolic traits rely on microbial metabolic pathways. (a) The AFP between kelp and fresh fruit is associated with SBP through propanoate biosynthesis by *Butyricimonas*. The gray lines indicate the associations between the two factors, with corresponding Spearman coefficients and *P* values. The mediation effects of the specific gut microbiome and its metabolic pathway are shown with arrows. (b) The SFP between fresh mushrooms and fresh fruit is associated with SBP through ergothioneine biosynthesis by *Clostridiumsensustricto1*. The gray lines indicate the associations between the two factors, with corresponding Spearman coefficients and *P* values. The mediation effects of the specific gut microbiome and its metabolic pathway are shown with arrows. (c) Graphical illustration of the two examples mentioned above.

4. Discussion

It is widely acknowledged that both the consumption of certain foods such as red meat [4,41] and dietary patterns like DASH [6] and MED [7] are associated with cardiometabolic health. In contrast to previous dietary indicators, the concept of long-term food pairing patterns proposed here aims to elucidate the effects of balanced and imbalanced combinations of different foods on human cardiometabolic phenotypes, including AFPs and SFPs. Individual long-term food pairing pattern shows a weak correlation with classical dietary indicators such as dietary intake frequency or dietary pattern indices, it operates independently of these indicators in principle.

Using two large-scale population-based cohorts, we investigated associations between long-term food pairing patterns and cardiometabolic traits, observing consistent results across populations with vastly different dietary preferences. Specifically, we identified 942/817 and 166/142 cardiometabolic-related AFPs/SFPs in the NHANES cohort and our GGMP cohort after adjusting for potential confounding factors. Importantly, the consistency of pairings between hyper food classification groups, to which the aforementioned cardiometabolic-related long-term food pairing patterns belong, was evident across two cohorts characterized by entirely disparate dietary habits. For instance, both cohorts supported the negative association between the fruit category and drinks, grains, milks, and vegetables with SBP, indicating that increased pairing of fruits with these food

groups (higher AFPs) benefits host blood pressure. Moreover, the effects are enhanced when the consumption frequency of the [redacted] long-term food pairing patterns, emphasizing the importance of considering the intricate interplay among daily diets in understanding their impact on human cardiometabolic health.

Importantly, our observations highlight that individual foods forming long-term food pairing patterns consistently engage with other diets linked to cardiometabolic traits, contrasting with a lower occurrence when considering individual foods alone. Additionally, correlations between foods and cardiometabolic traits may change or reverse upon constructing long-term food pairing patterns. For instance, the positive association between diabetes and hot cocoa, as well as diet cola/soda, can reverse when these foods participate in constructing SFP. This underscores the potential oversight of prior studies focusing solely on single food-health relationships, neglecting the broader impact of diet-to-diet pairings. Further investigation is needed to determine if these findings can inform nutritional guidance in dietary practices, potentially through targeted interventions, longitudinal studies, or controlled trials assessing the practical implications of observed long-term food pairing patterns on cardiometabolic health.

To gain mechanistic insights into the impact of long-term food pairing patterns on human cardiometabolic traits, we examined the mediating role of gut microbes and their metabolic pathways. We found that certain foods paired with others, exhibited broad associations with microbial genera, surpassing their individual associations with gut microbiomes. This highlights the comprehensive nature of long-term food pairing patterns in relation to diet-associated gut microbiomes. Our mediation analysis identified 755 linkages, with 160 long-term food pairing patterns affecting host cardiometabolic traits through 31 gut microbiomes, indicating the moderating role of gut microbiome-mediated long-term food pairing patterns on host blood pressure. Furthermore, analysis of gut microbial metabolic pathways revealed that *Butyricimonas* may mediate the effects of long-term food pairing patterns from fiber-enriched vegetables and fruits on SBP, possibly through pyruvate fermentation to propanoate I pathways [14,37]. Additionally, long-term food pairing patterns involving ergothioneine-rich mushrooms [40] influenced host blood pressure by metabolizing ergothioneine to TMA biosynthesis [38], leading to increased blood pressure [39]. These findings elucidate potential mechanisms underlying the impact of long-term food pairing patterns on human cardiometabolic health.

We acknowledge several limitations in our study. Firstly, our study employs an observational design, inherently susceptible to unknown factors, despite adjusting for existing confounding variables to minimize interference. Independent replications in other cohorts with diverse settings could further bolster the robustness of the conclusions [42–44]. Secondly, long-term food pairing pattern calculations rely on FFQs, which have inherent limitations. FFQs are subject to recall bias and measurement error, potentially leading to misclassification of food frequencies. A common issue is under-reporting, where participants may underestimate their intake due to social desirability bias or memory limitations. Finally, while we have demonstrated a link between gut microbiota and associations between long-term food pairing patterns and cardiometabolic health, the underlying causal relationship remains unclear. Despite these limitations, our study provides a novel perspective on the potential role of long-term food pairing patterns in human cardiometabolic health and its underlying microbial insights. The long-term food pairing patterns reported here offer a comprehensive resource that can guide follow-up studies aimed at designing preventive and therapeutic precision nutritional strategies for human cardiometabolic health.

5. Conclusions

Our data suggest that the balance between long-term food pairing patterns is broadly associated with cardiometabolic traits by modulating gut microbial functionalities, in contrast to the lower occurrence when considering individual foods in isolation. These findings offer a novel perspective for designing personalized dietary strategies that extend beyond current dietary indicators to enhance cardiometabolic health.

Acknowledgments

We thank the participants and staff of the GGMP and NHANES for their collaboration. This project was funded by the National Natural Science Foundation of China (NSFC; 32394052 and 32270077); the Jiangsu Shuangchuang Project (Medical Innovation Team, Medical Expert and JSSCBS20221815); the Natural Science Foundation of Jiangsu (BK20220709); the Nanjing Medical University and Jiangsu Province Hospital (CZ0121002010039, TS202403, YNRCZN0301, CMC2024PY01, and GSKY20210105); the Suzhou Science and Technology Development Plan Foundation (SKY2021010); the Gusu Health Talent Program (GSWS202206); the Development of Jiangsu Higher Education Institutions Priority Academic Program (PAPD). The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Data and code availability

The National Health and Nutrition Examination Survey (NHANES) datasets are available in the NHANES repository. The gut microbiome sequencing data of the Guangdong Gut Microbiome Project is deposited at the European Nucleotide Archive with accession number PRJEB18535. The analysis codes are available via: https://github.com/MicrobiomeCardioMetaLab/FoodInteractions_project.

References

Journal Pre-proofs

[1] Gardner CD, Vadiveloo MK, Petersen KS, Anderson CAM, Springfield S, Van Horn L, et al. Popular dietary patterns: alignment with American heart association 2021 dietary guidance: a scientific statement from the American heart association. Circulation 2023;147(22):1715–30.
[2] Valles-Colomer M, Menni C, Berry SE, Valdes AM, Spector TD, Segata N. Cardiometabolic health, diet and the gut microbiome: a meta-omics perspective. Nat Med 2023;29(3):551–61.
[3] Zheng Y, Li Y, Satija A, Pan A, Sotos-Prieto M, Rimm E, et al. Association of changes in red meat consumption with total and cause specific mortality among US women and men: two prospective cohort studies. BMJ 2019;365:l2110.
[4] Al-Shaar L, Satija A, Wang DD, Rimm EB, Smith-Warner SA, Stampfer MJ, et al. Red meat intake and risk of coronary heart disease among US men: prospective cohort study. BMJ 2020;371:m4141.
[5] Ding M, Li J, Qi L, Ellervik C, Zhang X, Manson JE, et al. Associations of dairy intake with risk of mortality in women and men: three prospective cohort studies. BMJ 2019;367:l6204.
[6] Karam G, Agarwal A, Sadeghirad B, Jalink M, Hitchcock CL, Ge L, et al. Comparison of seven popular structured dietary programmes and risk of mortality and major cardiovascular events in patients at increased cardiovascular risk: systematic review and network meta-analysis. BMJ 2023;380:e072003.
[7] Wang P, Song M, Eliassen AH, Wang M, Fung TT, Clinton SK, et al. Optimal dietary patterns for prevention of chronic disease. Nat Med 2023;29(3):719–28.
[8] García-Bailo B, Brenner DR, Nielsen D, Lee HJ, Domanski D, Kuzyk M, et al. Dietary patterns and ethnicity are associated with distinct plasma proteomic groups. Am J Clin Nutr 2012;95(2):352–61.
[9] Chen L, Zhernakova DV, Kurilshikov A, Andreu-Sánchez S, Wang D, Augustijn HE, et al. Influence of the microbiome, diet and genetics on inter-individual variation in the human plasma metabolome. Nat Med 2022;28(11):2333–43.
[10] Shi W, Huang X, Schooling CM, Zhao JV. Red meat consumption, cardiovascular diseases, and diabetes: a systematic review and meta-analysis. Eur Heart J 2023;44(28):2626–35.
[11] Vernocchi P, Del Chierico F, Putignani L. Gut microbiota metabolism and interaction with food components. Int J Mol Sci 2020;21(10):3688.
[12] Wang DD, Li Y, Bhupathiraju SN, Rosner BA, Sun Q, Giovannucci EL, et al. Fruit and vegetable intake and mortality. Circulation 2021;143(17):1642–54.
[13] Chen J, Xiao Y, Li D, Zhang S, Wu Y, Zhang Q, et al. New insights into the mechanisms of high-fat diet mediated gut microbiota in chronic diseases. iMeta 2023;2(1):e69.
[14] Wang Y, Dong Q, Hu S, Zou H, Wu T, Shi J, et al. Decoding microbial genomes to understand their functional roles in human complex diseases. iMeta 2022;1(2):e14.
[15] Dong Q, Hua D, Wang X, Jiao Y, Liu L, Deng Q, et al. Temporal colonization and metabolic regulation of the gut microbiome in neonatal oxen at single nucleotide resolution. ISME J 2024;18:wrad022.
[16] Huang P, Dong Q, Wang Y, Tian Y, Wang S, Zhang C, et al. Gut microbial genomes with paired isolates from China illustrate probiotic and cardiometabolic effects. Cell Genomics 2024;4(6):100559.
[17] Shen Y, Li Y, Wu T, Dong Q, Deng Q, Liu L, et al. Early microbial intervention reshapes phenotypes of newborn Bos taurus through metabolic regulations. GigaScience 2024;13:giad118.
[18] Liu L, Zhou Q, Xu T, Deng Q, Sun Y, Fu J, et al. Non-differential gut microbes contribute to hypertension and its severity through co-abundances: a multi-regional prospective cohort study. iMeta 2025;4(1):e268.
[19] National Center for Health Statistics. National health and nutrition examination survey [Internet]. Atlanta: US Centers For Disease Control and Prevention; undated [cited 2024 Jan 5]. Available from: https://www.cdc.gov/nchs/nhanes/index.html.
[20] He Y, Wu W, Zheng HM, Li P, McDonald D, Sheng HF, et al. Regional variation limits applications of healthy gut microbiome reference ranges and disease models. Nat Med 2018;24(10):1532–5.
[21] Shams-White MM, Pannucci TE, Lerman JL, Herrick KA, Zimmer M, Meyers Mathieu K, et al. Healthy eating index-2020: review and update process to reflect the dietary guidelines for Americans, 2020–2025. J Acad Nutr Diet 2023;123(9):1280–8.
[22] Krebs-Smith SM, Pannucci TE, Subar AF, Kirkpatrick SI, Lerman JL, Tooze JA, et al. Update of the healthy eating index: HEI-2015. J Acad Nutr Diet 2018;118(9):1591–602.
[23] Chiuve SE, Fung TT, Rimm EB, Hu FB, McCullough ML, Wang M, et al. Alternative dietary indices both strongly predict risk of chronic disease. J Nutr 2012;142(6):1009–18.
[24] Fung TT. Adherence to a DASH-Style diet and risk of coronary heart disease and stroke in women. Arch Intern Med 2008;168(7):713.
[25] Appel LJ, Moore TJ, Obarzanek E, Vollmer WM, Svetkey LP, Sacks FM, et al. A clinical trial of the effects of dietary patterns on blood pressure. N Engl J Med 1997;336(16):1117–24.
[26] Fung TT, Rexrode KM, Mantzoros CS, Manson JE, Willett WC, Hu FB. Mediterranean diet and incidence of and mortality from coronary heart disease and stroke in women. Circulation 2009;119(8):1093–100.
[27] Estruch R, Ros E, Salas-Salvadó J, Covas MI, Corella D, Arós F, et al. Primary prevention of cardiovascular disease with a Mediterranean diet supplemented with extra-virgin olive oil or nuts. N Engl J Med 2018;378(25):e34.
[28] Shivappa N, Steck SE, Hurley TG, Hussey JR, Hébert JR. Designing and developing a literature-derived, population-based dietary inflammatory index. Public Health Nutr 2014;17(8):1689–96.
[29] Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc B 1995;57(1):289–300.
[30] Kurilshikov A, Medina-Gomez C, Bacigalupe R, Radjabzadeh D, Wang J, Demirkan A, et al. Large-scale association analyses identify host factors influencing human gut microbiome composition. Nat Genet 2021;53(2):156–65.
[31] Douglas GM, Maffei VJ, Zaneveld JR, Yurgel SN, Brown JR, Taylor CM, et al. PICRUSt2 for prediction of metagenome functions. Nat Biotechnol 2020;38(6):685–8.
[32] Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol 2019;37(8):852–7.
[33] Tingley D, Yamamoto T, Hirose K, Keele L, Imai K. mediation: R package for causal mediation analysis. J Stat Softw 2014;59(5):1–38.
[34] Kurilshikov A, van den Munckhof IC, Chen L, Bonder MJ, Schraa K, Rutten JH, et al. Gut microbial associations to plasma metabolites linked to cardiovascular phenotypes and risk: a cross-sectional study. Circ Res 2019;124(12):1808–20.
[35] Thomann AK, Wüstenberg T, Wirbel J, Knoedler LL, Thomann PA, Zeller G, et al. Depression and fatigue in active IBD from a microbiome perspective-a Bayesian approach to faecal metagenomics. BMC Med 2022;20(1):366.
[36] Huart J, Leenders J, Taminiau B, Descy J, Saint-Remy A, Daube G, et al. Gut microbiota and fecal levels of short-chain fatty acids differ upon 24-hour blood pressure levels in men. Hypertension 2019;74(4):1005–13.
[37] Cummings JH, Pomare EW, Branch WJ, Naylor CP, Macfarlane GT. Short chain fatty acids in human large intestine, portal, hepatic and venous blood. Gut 1987;28(10):1221–7.
[38] Dumitrescu DG, Gordon EM, Kovalyova Y, Seminara AB, Duncan-Lowey B, Forster ER, et al. A microbial transporter of the dietary antioxidant

ergothioneine. Cell 2022;185(24):4526–40.
[39] [redacted] luced hypertension. Redox Biol 2021;46:102115.
[40] Halliwell B, Cheah IK, Tang RMY. Ergothioneine—a diet-derived antioxidant with therapeutic potential. FEBS Lett 2018;592(20):3357–66.
[41] Zhong VW, Van Horn L, Greenland P, Carnethon MR, Ning H, Wilkins JT, et al. Associations of processed meat, unprocessed red meat, poultry, or fish intake with incident cardiovascular disease and all-cause mortality. JAMA Intern Med 2020;180(4):503–12.
[42] Sun W, Wang Y, Zhang H, Sheng Y, Fan J, Gu M, et al. eHypertension: a prospective longitudinal multi-omics essential hypertension cohort. iMeta 2022;1:e22.
[43] Ren HY, Lv Y, Ma BN, Gao C, Yuan HM, Meng HH, et al. Cohort profile: TRacing etiology of non-communicable diseases (TREND): rationale, progress and perspective. Phenomics 2024:1–8.
[44] Jiao Y, Xue J, Chen S, Sun W, Kong XQ, Chen L, et al. PBS: a prospective longitudinal multi-omics bariatric surgery cohort. Precis Clin Med 2023;6(4):pbad032.

Journal Pre-proofs

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The author is an Editorial Board Member/Editor-in-Chief/Associate Editor/Guest Editor for *[Journal name]* and was not involved in the editorial review or the decision to publish this article.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

The authors declare no competing interests.

Journal Pre-proofs