



# The Association of Fried Meat Consumption With the Gut Microbiota and Fecal Metabolites and Its Impact on Glucose Homoeostasis, Intestinal Endotoxin Levels, and Systemic Inflammation: A Randomized Controlled-Feeding Trial

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## OBJECTIVE

This randomized controlled-feeding trial aimed to determine the impact of fried meat intake on the gut microbiota and fecal metabolites and whether such impacts influenced host glucose homoeostasis, intestinal endotoxin levels, and systemic inflammation.

## RESEARCH DESIGN AND METHODS

A total of 117 overweight adults were randomized into two groups. Fifty-nine participants were provided fried meat four times per week, and 58 participants were restricted from fried meat intake, while holding food group and nutrient compositions constant, for 4 weeks. The gut microbiota was analyzed by 16S rRNA sequencing. Glucose and insulin concentrations at 0, 30, 60, and 120 min of an oral glucose tolerance test, fecal microbiota-host metabolite levels, and intestinal endotoxin and inflammation serum biomarker levels were measured. The area under the curve (AUC) for insulin, insulinogenic index (IGI), and muscle insulin resistance index (MIRI) were calculated.

## RESULTS

The participants who consumed fried meat had lower IGI values than the control subjects, but they had higher MIRI and AUC values of insulin and lipopolysaccharide (LPS), TNF- $\alpha$ , IL-10, and IL-1 $\beta$  levels ( $P < 0.05$ ). Fried meat intake lowered microbial community richness and decreased *Lachnospiraceae* and *Flavonifractor* abundances while increasing *Dialister*, *Dorea*, and *Veillonella* abundances ( $P \text{ FDR} < 0.05$ ), provoking a significant shift in the fecal metabolite profile, with lower 3-indolepropionic acid, valeric acid, and butyric acid concentrations and higher carnitine and methylglutaric acid concentrations ( $P \text{ FDR} < 0.05$ ). Changes in these metabolite levels were significantly associated with changes in IGI and MIRI values and LPS, FGF21, TNF- $\alpha$ , IL-1 $\beta$ , and IL-10 levels ( $P < 0.05$ ).

## CONCLUSIONS

Fried meat intake impaired glucose homoeostasis and increased intestinal endotoxin and systemic inflammation levels by influencing the gut microbiota and microbial-host metabolites.

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See accompanying article, p. 1907.

A suboptimal diet is an important preventable risk factor for type 2 diabetes (1,2), and in recent years, interest in the association between fried food intake and the risk of type 2 diabetes has been increasing. A few prospective studies indicated that a higher frequency of fried food consumption was associated with a higher incidence of type 2 diabetes (3,4), which suggested that excessive energy intake and the production of hazardous material during frying probably mediated this association; in contrast, another prospective study from the Mediterranean region, in which foods were frequently consumed as part of a Mediterranean diet, did not support this association (5). Similarly, a dietary intervention study of 17 insulin-resistant women found that consumption of food fried in unsaturated fatty acids had beneficial effects on insulin sensitivity (6). These studies suggested that confounders, such as other nutrients and hazardous material production during frying, influenced the association between fried food and type 2 diabetes. It is still largely unknown whether and how fried food intake influences the development of type 2 diabetes if these confounders are rigorously controlled.

Furthermore, meat, as the major protein source, is commonly used for frying in daily life. Although protein in meat is largely digested in the upper intestine, ~10% may reach the large bowel and become available for fermentation by the gut microbiota (7). It has been documented that the rate of protein digestibility in meat is decreased during frying (8,9), and the lower digestibility probably results in more undigested protein available for the gut microbiota. Recent studies also found that higher fried meat intake correlated with lower diversity of the gut microbial community in humans (10) and appears to influence the composition and activity of the gut microbiota in animals (7,11). In this study, our research team examined the effects of fried meat and selected cometabolites in a randomized controlled-feeding trial conducted in healthy overweight young adults. We also included information from our research using gastric gavage to examine the effects of selected cometabolites in mice (Supplementary Material).

## RESEARCH DESIGN AND METHODS

### Participant Recruitment

Participants in this randomized controlled-feeding trial were recruited among healthy overweight adults in Harbin from January to February 2020, and the trial was registered at [chictr.org.cn](http://www.chictr.org.cn), no. ChiCTR1900028562. The inclusion criteria were age 18–35 years old, BMI > 24 kg/m<sup>2</sup>, and consumption of fried food more than one time per week. Exclusion criteria were as follows: individuals who 1) had taken antibiotics, probiotics, and prebiotics within 3 months; 2) suffered from diabetes, dyslipidemia, or gastrointestinal disease; 3) had received surgical treatment within 3 months; 4) undertook frequent strenuous exercise or protein supplementation; or 5) had smoking or drinking habits. The volunteers who met the inclusion criteria were subjected to a physical condition survey and an electronic dietary questionnaire survey for collection of baseline information before the intervention. The study design was approved by the ethics committee of Harbin Medical University. The nature and potential risks of the study were explained to the participants before written informed consent was obtained.

### Intervention Strategies

The participants were randomly assigned to the experimental group and control group through generation of random allocation sequences to ensure that there was no significant difference in the baseline characteristics of demographics, dietary intake, anthropometry, and biochemical indexes between the two groups. Detailed information on the method used to generate the random allocation sequence and allocation concealment mechanism is presented in Supplementary Material.

All the meals in this trial were finished in the canteen of Harbin Medical University under supervision of the trained personnel. The formula for dietary intervention was mainly based on the Alternate Health Eating Index (AHEI)-2010 (12) (Supplementary Table 1). The amount of food groups and composition of nutrients that were provided in the two groups were constant, with an AHEI score >85 (Supplementary Table 2), but differed in meat cooking methods, which was frying at 150°C for <3 min in the experimental group and boiling, steaming,

or dressing with sauce at 100°C in the control group. Fried meat was provided four times per week in the experimental group. Before the intervention, a pre-experiment was performed to calculate the amount of oil and starch per gram of meat required for frying to ensure isocaloric feeding between the two groups (Supplementary Fig. 1). Trained personnel weighed and recorded the leftovers of each participant to calculate the actual dietary intake of each person, and the participants were not allowed to eat any food or beverages other than the uniform meal. The participants were asked to maintain their habitual daily physical activities, which were also monitored by collection of records of daily steps and a questionnaire of physical activities during the intervention.

### Sample Size and Power Analysis

Sample size calculation (assuming a 7% difference between the groups in the area under the curve [AUC] for insulin levels during an oral glucose tolerance test, with an SD for the insulin level AUC = 20 mU/L \* h) showed that a total sample size of 39 participants per group was necessary to reach statistical significance with a power level of  $1 - \beta = 0.8$  and a significance level of  $\alpha = 0.05$  (13). For the glucose level AUC, a sample size of 25 participants per group was required to detect a 1.20 mmol/L \* h significant difference, assuming that the SD was 1.5 mmol/L \* h (14). We therefore recruited 130 participants to the study (65 in each intervention group), allowing for a 10% dropout rate.

### Sample Collection

After the participants entered the trial, they had a 1-week balance period and then began a 4-week dietary intervention. Blood and morning urine samples were collected at 0, 2, and 4 weeks of the intervention, while fecal samples were collected at 0 and 4 weeks (Supplementary Fig. 2A). The participants underwent a 2-h 75-g oral glucose tolerance test after a 12-h overnight fast, and blood samples were collected at 0, 30, 60, and 120 min. The detailed procedure of blood and fecal sample collection can be found in Supplementary Material.

### Compliance Indicator Measurement

BMI was calculated as weight in kilograms divided by the square of the height in meters. The concentrations of 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx) and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) in urine samples, as biomarkers of heterocyclic amines, were measured by ultra-performance liquid chromatography–tandem mass spectrometry (UPLC-MS/MS) (ACQUITY UPLC-Xevo TQ-S, Waters Corp., Milford, MA). The nitrogen contents in food and feces were detected by the automatic Kjeldahl apparatus method, and the apparent protein digestibility was calculated. Serum advanced glycation end products (AGEs) were measured with an ELISA kit (Cusabio, College Park, MD). Targeted analysis of serum fatty acids was performed with the TRACE GC/PolarisQ-MS system (Thermo Finnigan, Austin, TX) and DB-WAX capillary column (J&W Scientific, Folsom, CA).

### Main Outcome Measurement

Serum glucose was quantified with an Automatic Biochemical Analyzer (Roche Diagnostics, Mannheim, Germany). Serum insulin was measured with an automatic microparticle chemiluminescence immunoassay system (Beckman Coulter, Brea, CA). HbA<sub>1c</sub> levels were measured with an automatic glycosylated hemoglobin analyzer (HA-8380; ARKRAY, Tokyo, Japan). Serum C-peptide levels were measured with an ELISA kit (Cusabio). Total AUCs for glucose and insulin levels were calculated with the trapezoidal method (15). The insulinogenic index (IGI) was calculated,  $\Delta\text{insulin (0–30 min, } \mu\text{U/mL})/\Delta\text{glucose (0–30 min, mg/mL)}$  (16), and the muscle insulin resistance index (MIRI) was calculated as follows: slope of the blood glucose concentration decrease (60–120 min)/mean insulin concentration (17).

### Secondary Outcome Measurement

Serum levels of lipopolysaccharide (LPS), LPS binding protein (LBP), soluble LPS receptor CD14 (sCD14), adiponectin, and fibroblast growth factor 21 (FGF21) were measured with ELISA kits (Cusabio). All serum inflammatory factors, including TNF- $\alpha$ , IFN- $\gamma$ , IL-1 $\beta$ , IL-2, IL-4, IL-6, IL-8, and IL-10, were quantified with use of Luminex assay technology (R&D Systems, Minneapolis, MN).

### 16S rRNA Sequencing

Detailed methods for DNA extraction, PCR, and amplification sequencing are provided in Supplementary Material. FASTQ files were demultiplexed, merge paired, and quality filtered using Quantitative Insights Into Microbial Ecology (QIIME) software (version 1.9.0). Sequences were clustered into operational taxonomic units (OTUs) with 97% similarity and annotated based on the SSU rRNA database of SILVA 132.

### Measurement of Cometabolite Levels

A total of 174 fecal microbial-host cometabolites were quantified by a UPLC-MS/MS system (Metabo-Profile, Shanghai, China). Detailed information about standards, procedures, conditions, and methods is provided in Supplementary Material.

### Statistical Analysis

Baseline characteristics are presented as the mean (SD) for continuous variables and number (percentage) for categorical variables. A generalized linear mixed model was established to analyze the effect of the intervention on the main outcomes, in which participants were random effects and the intervention models were fixed effects. General linear models and  $\chi^2$  tests were used to compare baseline characteristics, the differences in the compliance indicators, and the secondary outcomes before and after intervention or between groups. A subgroup analysis was also performed, and its aim and detailed procedure are provided in Supplementary Material.

The Abundance-based Coverage Estimator (ACE) and Chao1 estimator and Shannon index were used to analyze the richness and diversity of the microbial community (18). Principal coordinate analysis based on Bray-Curtis distance was performed to compare the global microbiota composition between groups at the OTU levels. Linear discriminant analysis (LDA) and effect size were used to emphasize statistical significance and biological relevance and find biomarkers with significant differences between groups. The set value of the LDA score was 2 (19). Orthogonal partial least squares discriminant analysis (OPLS-DA) was performed to examine the overall distribution of fecal metabolites between groups, and the cross-validation permutation test

was conducted to validate the fitting situation of this model. Metabolites with variable importance in projection  $>1$  were identified to be analyzed in univariate analysis.

In the univariate analysis, the Student *t* test or Mann-Whitney *U* test was used to examine the significant differences in the levels of individual genera, microbiota predicted pathways, and fecal metabolites before and after intervention or between groups, and the *P* values were adjusted using the Benjamini-Hochberg false discovery rate (*P* FDR). Partial Spearman rank correlation analyses between changes in abundant bacterial genera and fecal metabolites and altered outcome variables were used with adjustment for differences in BMI before and after intervention.

OPLS-DA was performed with SIMCA-P software (14.0), and other statistical analyses were performed in R 3.6.1. A two-sided *P* value  $<0.05$  was considered statistically significant for the generalized linear mixed models or general linear models, and a *P* FDR  $<0.05$  was considered statistically significant for Student *t* test or Mann-Whitney *U* test in the univariate analysis of individual genera and fecal metabolites.

## RESULTS

### Feeding Trial Process and Baseline Characteristics

Of the 150 participants assessed for eligibility, 130 were randomized. During the intervention stage, 7 dropped out from the control group, 6 dropped out from the fried meat group, and 117 participants completed the study (Supplementary Fig. 2B). The baseline characteristics regarding demographics, dietary intake, anthropometry, and biochemical indexes were similar between the two groups (all *P*  $>0.05$ ) (Table 1).

### Compliance Assessment

During the intervention, the energy intake was reduced, whereas the AHEI score was increased, and the daily physical activity was similar between the two groups (Supplementary Fig. 3A–C). The levels of BMI, saturated fatty acids, n-3 fatty acids, n-6 fatty acids, MeIQx, PhIP, and AGEs and the digestibility of protein in the two groups were similar at baseline (Supplementary Fig. 3D–K). After the intervention, the participants

**Table 1—Baseline characteristics of the participants (n = 117)**

Characteristics	Control group (n = 58)	Fried meat group (n = 59)	P
Age, years	21.73 (2.97)	21.13 (2.66)	0.110
Female, n (%)	31 (53.45)	33 (55.93)	0.332
BMI, kg/m <sup>2</sup>	26.39 (2.80)	26.06 (2.38)	0.498
Body fat rate, %	26.11 (6.33)	26.93 (6.05)	0.773
Waist-to-hip ratio, %	0.87 (0.16)	0.86 (0.15)	0.420
Laboratory measurements			
HbA <sub>1c</sub> , %	5.37 (0.20)	5.36 (0.22)	0.848
mmol/mol	35.19 (2.20)	35.09 (2.40)	0.803
Fasting glucose, mmol/L	4.68 (0.41)	4.64 (0.39)	0.232
Fasting insulin, $\mu$ U/mL	4.42 (2.10)	4.68 (1.89)	0.214
IGI, $\mu$ U/mmol $\times 10^3$	20.93 (13.09)	22.53 (13.87)	0.526
MIRI, mmol/min $\cdot \mu$ U $\times 10^{-5}$	1.64 (2.38)	1.56 (2.40)	0.848
AUC of glucose, mmol $\times$ min/L	679.13 (93.40)	662.92 (90.11)	0.389
AUC of insulin, $\mu$ U $\times$ min/mL	3,605.75 (1,574.29)	4,036.86 (1,907.20)	0.225
Dietary intake			
Energy, kcal/day	2,669 (586.52)	2,634 (605.48)	0.341
Total fat, %TE	26.62 (7.39)	26.28 (6.98)	0.724
Carbohydrate, %TE	61.73 (10.39)	61.41 (11.39)	0.981
Protein, %TE	11.65 (3.70)	12.31 (3.74)	0.480
Component of AHEI-2010 score			
Vegetables	5.32 (2.94)	5.03 (3.04)	0.725
Fruit	4.88 (2.80)	5.40 (3.14)	0.524
Whole grains	4.84 (2.91)	5.43 (3.05)	0.466
Polyunsaturated fatty acids	5.36 (2.96)	5.00 (3.03)	0.659
Nuts and legumes	5.36 (3.01)	5.00 (2.98)	0.659
Long-chain n-3 fats	5.08 (3.03)	5.23 (2.98)	0.851
Red and processed meat	4.8 (2.92)	5.47 (3.04)	0.413
Sugar-sweetened drinks	5.32 (2.84)	5.03 (3.13)	0.725
Sodium	4.64 (2.93)	5.60 (2.99)	0.234
Alcohol	10.00	10.00	1
Total score	55.60 (11.50)	57.20 (10.84)	0.598
Physical exercise habits, n (%)	39 (67.24)	40 (67.80)	0.896
Frequency of consumption of fried foods, n (%)			0.814
1–3 times per week	31 (53.45)	34 (57.62)	0.653
4–6 times per week	25 (43.10)	22 (37.29)	0.525
$\geq 7$ times per week	2 (3.45)	3 (5.08)	0.665

Continuous variables are presented as mean (SD), and categorical variables are presented as n (%). %TE, percentage of total energy.

in the control group had lower BMI and AGE levels, whereas they had higher levels of n-3 fatty acids and protein digestibility than the participants in the experimental group (all  $P < 0.05$ ).

### Changes in the Main and Secondary Outcomes

During the intervention, the rates of change in HbA<sub>1c</sub> and C-peptide levels and the AUC of glucose levels in the two groups did not differ significantly (Fig. 1A, B, and E). The IGI value significantly increased in both groups; however, it was significantly higher in the control group than in the experimental group after the intervention (29.75 vs. 24.75  $\mu$ U/mmol  $\times$

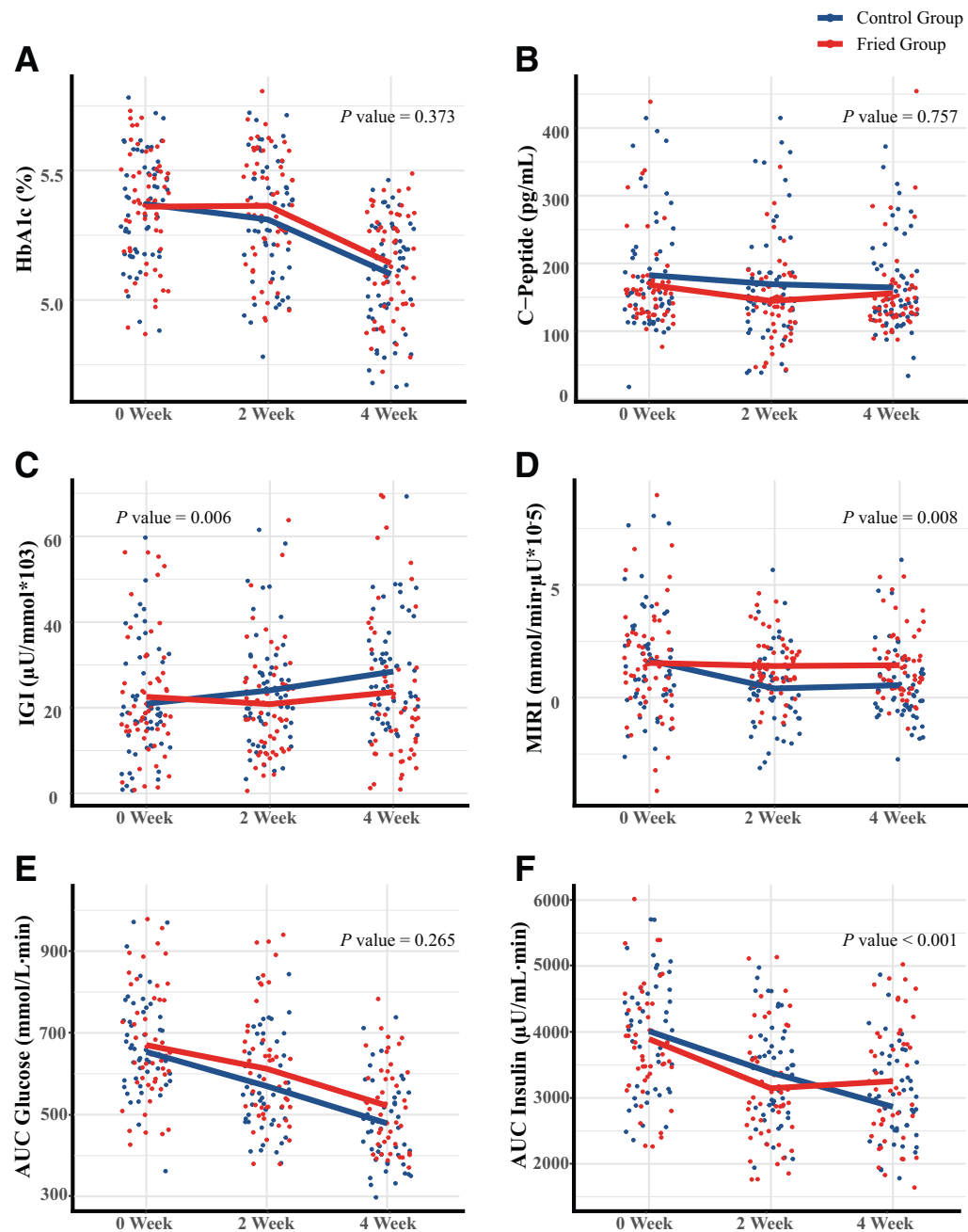
$10^3$ ,  $P = 0.006$ ) (Fig. 1C). Meanwhile, the MIRI and insulin level AUC values significantly decreased in both groups, and they were significantly lower in the control group than in the experimental group (0.82 vs. 1.67 mmol/min  $\cdot \mu$ U  $\times 10^{-5}$ ,  $P = 0.008$  for MIRI; 3,310.5 vs. 2796.0  $\mu$ U/mL  $\cdot$  min,  $P < 0.001$  for insulin levels) (Fig. 1D and F).

The biomarkers of intestinal endotoxin levels and systemic inflammation were similar between the two groups at baseline (Supplementary Fig. 4). After the intervention, the LPS, LBP/sCD14, TNF- $\alpha$ , IL-1 $\beta$ , and IL-10 levels in the control group were significantly lower than those in the experimental group, whereas

FGF21 levels were significantly greater in the control group than in the experimental group (all  $P < 0.05$ ). The IL-2, IL-4, and IL-8 levels were all significantly decreased, and they did not differ significantly between the two groups.

### Subgroup Analysis

A total of 45 participants who habitually consumed fried food infrequently before they participated in the trial were identified. The baseline characteristics were similar between the two groups (Supplementary Table 3). The results in the subsample were consistent with those of the total sample (Supplementary Fig. 5), which further supported that the



**Figure 1**—The differences for the main outcomes in terms of HbA<sub>1c</sub> (A), C-peptide (B), IGI (C), MIRI (D), and AUC for glucose (E) and insulin (F).  $P$  values refer to the differences for the changing rate of biomarkers being different from 0.

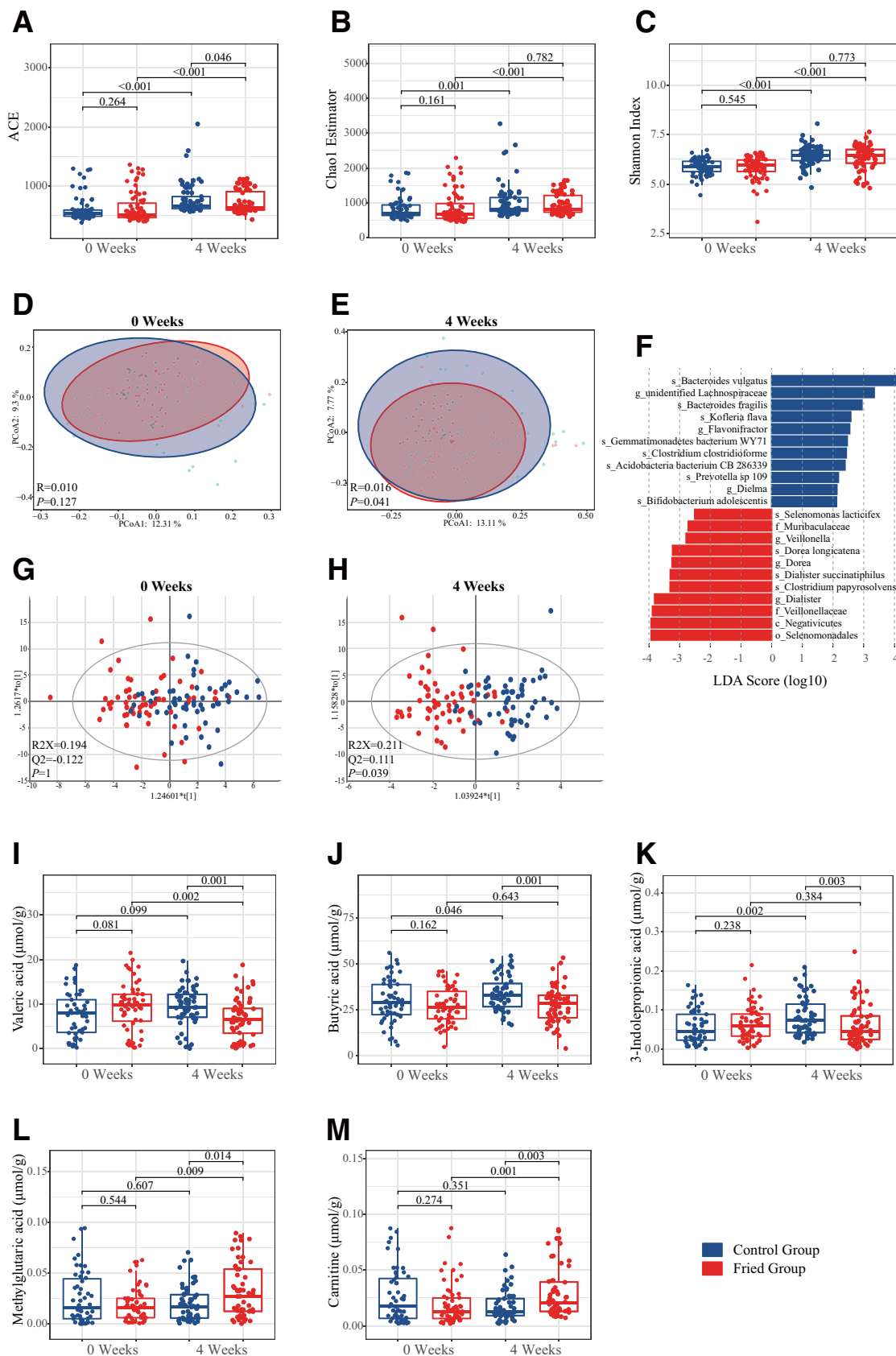
differences for the main and secondary outcomes were due to the effect of fried meat intake rather than from the restriction of fried food.

#### Changes in the Gut Microbiota and Fecal Metabolomic Profiles

The microbial community richness indicated by ACE and the Chao1 estimator was significantly increased in both groups, and ACE value in the control group was greater than that in the

experimental group (Fig. 2A and B). Additionally, the community diversity estimated by the Shannon index was significantly increased in both groups, and it did not differ significantly between the two groups (Fig. 2C). Moreover, there were significant differences in the overall composition of the gut microbiota at the OTU level between the two groups at the end point ( $R^2 = 0.016$ ,  $P = 0.041$ ) (Fig. 2E). The microbiota predicted pathways showed that the LPS

biosynthesis protein (ko01005), insulin resistance (ko04931), adipocytokine signaling (ko04920), and AMPK signaling (ko04152) pathways were significantly different between the two groups (Supplementary Fig. 6A). At the phylum level, the ratio of *Firmicutes* and *Bacteroidetes* was decreased in both groups, and it was significantly lower in the control group than in the experimental group ( $2.87 \pm 2.31$  vs.  $4.29 \pm 3.89$ ,  $P = 0.020$ ) (Supplementary Fig. 6B). At the





genus level, five differentially abundant genera of bacteria were identified (Fig. 2F). Compared with the control group, the experimental group had a greater abundance of *Dialister*, *Dorea*, and *Veillonella* but a lower abundance of *Lachnospiraceae* and *Flavonifractor* (all  $P$  FDR  $< 0.05$ ) (Supplementary Fig. 7).

Differences in the overall composition of fecal metabolites between the two groups were also observed at the end point ( $P = 0.039$ ) (Fig. 2K). Further stratification by metabolite categories showed that the metabolites of amino acids and short-chain fatty acids (SCFAs) were significantly different between the two groups (both  $P < 0.001$ ) (Supplementary Fig. 8A and F). Individual metabolic analysis identified carnitine, 3-indolepropionic acid (IPA), valeric acid, butyric acid, and methylglutaric acid (MGA). Compared with those in the control group, carnitine and MGA were enriched, whereas valeric acid, butyric acid, and IPA were significantly depleted in the fried meat group (all  $P$  FDR  $< 0.05$ ) (Fig. 2I–M).

### Correlation Analysis

The correlations among the changes in the gut microbiota, fecal cometabolite levels, and altered outcome variables are presented in Fig. 3. The changes in the abundance of *Flavonifractor* were positively correlated with changes in fecal valeric acid levels ( $r = 0.226$ ), whereas the changes in the abundance of *Dorea* were negatively correlated with it ( $r = -0.336$ ). Additionally, the changes in the abundances of *Dialister*, *Dorea*, and *Veillonella* were positively correlated with changes in fecal carnitine levels ( $r = 0.218$  for *Dialister*,  $r = 0.395$  for *Dorea*,  $r = 0.314$  for *Veillonella*) (all  $P < 0.05$ ).

The changes in fecal levels of valeric acid, butyric acid, and IPA were negatively correlated with changes in AUC for insulin levels ( $r = -0.266$  for valeric acid,  $r = -0.282$  for butyric acid), MIRI values ( $r = -0.243$  for butyric acid,  $r = -0.286$  for IPA), LPS levels ( $r = -0.198$  for butyric acid,  $r = -0.243$  for IPA) and TNF- $\alpha$  levels

( $r = -0.204$  for valeric acid,  $r = -0.352$  for butyric acid,  $r = -0.436$ ). The changes in fecal MGA levels were negatively correlated with IGI values ( $r = -0.285$ ), whereas they were positively correlated with TNF- $\alpha$  levels ( $r = 0.310$ ). The changes in fecal carnitine levels were positively correlated with MIRI values ( $r = 0.241$ ) and LPS ( $r = 0.214$ ) and LBP/sCD14 ( $r = 0.238$ ) levels (all  $P < 0.05$ ).

### CONCLUSIONS

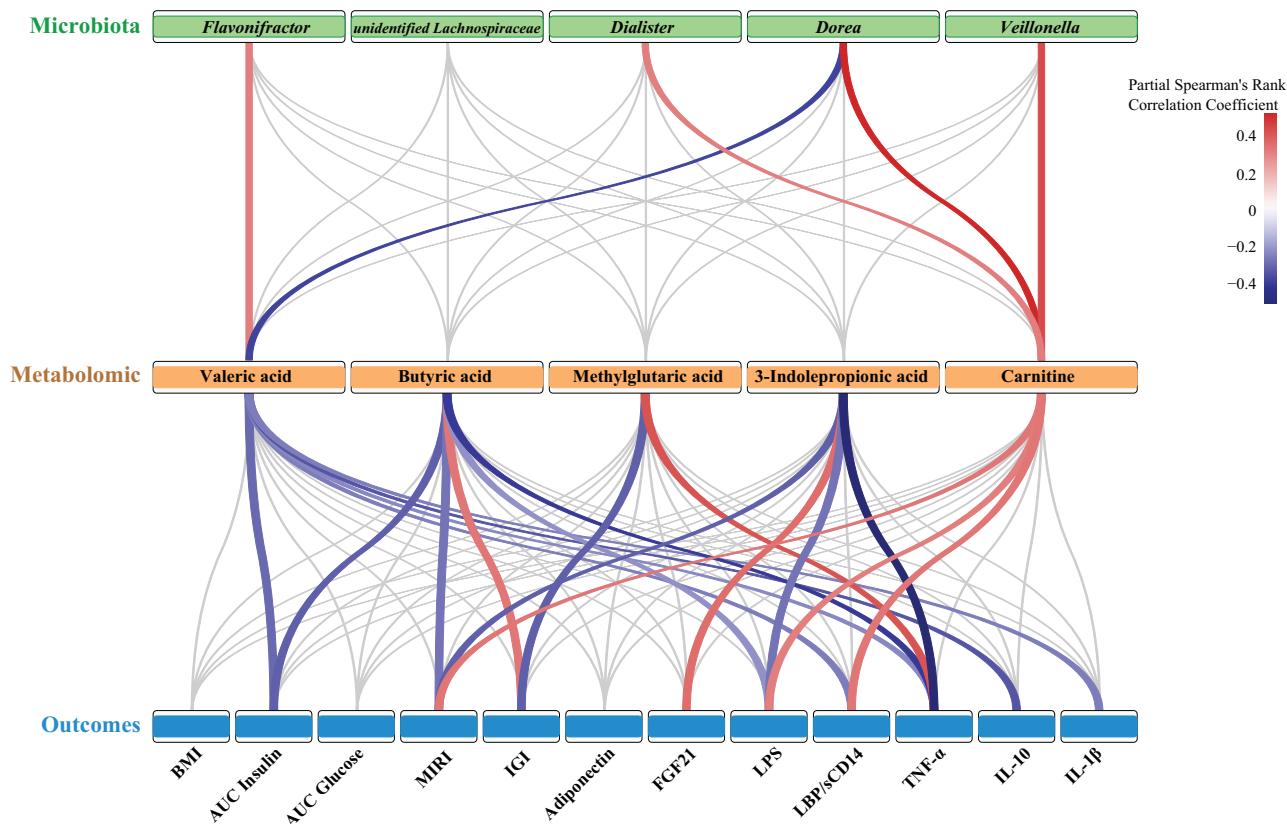
In this study, we found that fried meat consumption influenced the composition and function of the gut microbiota and microbial-host cometabolites, which were associated with impaired glucose homoeostasis and increased the levels of intestinal endotoxin and systemic inflammation in overweight young adults. Additionally, the effect of the identified cometabolites that were related to the fried meat intake on glucose homoeostasis was further demonstrated in the mice by giving these cometabolites through gastric gavage.

Although previous studies have investigated the association between fried food intake and the risk of type 2 diabetes, uncontrolled nutritional confounders made these findings inconsistent (3–6). In this study, we provided the participants with an isocaloric diet, for which the AHEI score was  $> 85$ , and the amount of food groups and composition of nutrients were held constant. However, we still found that participants who consumed fried meat had impaired glucose homoeostasis. It has been reported that high-heat cooking is a potent promoter of advanced glycation, and high levels of AGEs are therefore found in many fried foods (20). Previous studies showed that a diet with high levels of AGEs was associated with insulin resistance (21). Consistent with these studies, we also found that the participants in the fried group had higher levels of AGEs, although the frying temperature was rigorously controlled, and aromatic hydrocarbons were barely produced.

Moreover, we also observed that the participants in the experimental group had lower protein digestion and absorption rates. It has been reported that the protein digestion and absorption rate could influence the gut microbiota (22). In accordance, we also found that the participants in the experimental group had lower gut microbiota richness and that the overall structure of the gut microbiota at the OTU level was also different from that of the participants in the control group, suggesting that fried meat intake had a selective effect on the human gut microbiota. The microbiota predicted pathways also showed that those relating to glucose homoeostasis were also significantly changed during the intervention. Additionally, the ratio of *Firmicutes* and *Bacteroidetes*, the classical biomarker of type 2 diabetes (23), was significantly higher in the experimental group than in the control group, which further supported the effect of fried meat intake on glucose homoeostasis. At the genus level, fried meat intake decreased the abundances of *Lachnospiraceae* and *Flavonifractor*. *Lachnospiraceae* plays an important role in intestinal homeostasis (24), and it can prevent obesity and insulin resistance (25). *Flavonifractor* is an important species for intestinal health, and its abundance is negatively correlated with obesity (26). Moreover, the abundances of *Dialister*, *Dorea*, and *Veillonella* were increased in the experimental group. *Dialister* is a pathogenic bacterium, and its increased abundance is related to weight gain (27). *Dorea* abundance is positively correlated with obesity, and its increased abundance is found in patients with prediabetes (28).

Consistent with the gut microbiota results, fried meat intake also provoked significant shifts in fecal cometabolites, especially SCFA and amino acids metabolites. Fried meat intake significantly decreased the levels of some beneficial fecal cometabolites, including butyric acid, valeric acid, and IPA, whereas it

**Figure 2**—Changes in gut microbiota and fecal metabolomics profiles. The microbial community richness was indicated by ACE (A) and the Chao1 estimator (B). The community diversity of gut microbiota was estimated by the Shannon index (C). D and E: The overall composition of the gut microbiota at OTU levels before (D) and after intervention (E). F: The differential gut genera identified by LDA effect size. G and H: The overall composition of the cometabolites between host and gut microbiota before (G) and after (H) intervention. I–M: The differences for the fecal valeric acid (I), butyric acid (J), IPA (K), carnitine (L), and MGA (M) between the two groups.  $P$  values for the differences of the identified cometabolites were adjusted using the Benjamini-Hochberg FDR. R2X, the percentage of matrix information that the model can explain; Q2, the predictive power of the model calculated by cross validation;  $P$ , validation of the OPLS-DA from ANOVA of the cross-validated predictive residuals.



**Figure 3**—Correlation analyses among gut microbiota, cometabolites, and biomarkers of glucose homeostasis, gut health, and inflammations. Red and blue lines, respectively, refer to significant positive and negative correlation, and gray lines refer to nonsignificant correlation; the color of the lines from light to dark reflected the magnitude of the correlation coefficients. FGF21, fibroblast growth factor 21; LBP, lipopolysaccharide binding protein; sCD14, soluble lipopolysaccharide receptor CD14.

also increased the levels of some deleterious cometabolites, including carnitine and MGA. A negative or positive association of these beneficial or deleterious cometabolites with the levels of biomarkers of glucose homeostasis was also observed. Butyric acid is an SCFA, and its beneficial effect on glucose homeostasis has already been demonstrated in previous studies (29,30). Therefore, we conducted the animal experiments to examine the effects of other identified cometabolites on glucose homeostasis, and the beneficial effect of valeric acid and IPA, and deleterious effects of carnitine on glucose homeostasis were further demonstrated, which were supported by previous studies. Valeric acid, as an SCFA, inhibits oxidative stress and neuroinflammation and modulates autophagy pathways (31). IPA can reduce plasma endotoxin levels, and consumption of an IPA-enriched diet can significantly lower fasting blood glucose levels and improve insulin resistance (32,33). Carnitine can be oxidized to

trimethylamine N-oxide (TMAO), and high levels of plasma TMAO are associated with cardiovascular disease and diabetes (34).

We also identified several important bacterial genera as sensitive to fried meat intake based on the correlation between changes in abundance of the gut microbiota constituents and cometabolites. The results indicated that fried meat intake could decrease the abundance of *Flavonifractor* and increase the abundance of *Dialister*, *Dorea*, and *Veillonella*, which resulted in downregulated valeric acid levels and upregulated carnitine levels in feces. These results are supported by previous studies. It has been reported that *Flavonifractor* can produce SCFAs, *Flavonifractor* participates in the metabolism of valeric acid (35), and high abundance of *Dorea* could decrease the abundance of SCFA-producing bacteria, resulting in the reduction in the levels of fecal SCFAs, including valeric acid (36). Moreover, *Dialister* and *Veillonella* are involved in host amino acid metabolism, which

could influence host TMAO levels through mediating carnitine production and metabolism (37,38).

In this randomized controlled-feeding trial, we applied 16S rRNA amplicon profiling coupled with quantitatively targeted bacterial metabolomics, which could provide additional knowledge for understanding the cross talk of host-gut microbiota metabolism in response to fried meat intake. However, we also recognize that our study had certain limitations. First, this study included only healthy overweight young adults because fried food is popular in this population (39), which could ensure compliance with this trial, and it has been documented that overweight during early adulthood is associated with a higher incidence of type 2 diabetes in late adulthood (40). Therefore, it is important to clarify the association between fried food intake and glucose metabolism in this population, which aids in establishing dietary intervention strategies for the early prevention of type 2 diabetes. However, these findings



might not apply to other populations. Second, fecal sampling was conducted only at baseline and at the end of the trial. Future studies with more frequent sampling may provide a more complete picture of changes in the gut microbiota and metabolites. Third, for maintenance of the AHEI score at >85, this study provided only fish and chicken for the participants, and the temperature and duration during frying were rigorously controlled to limit the production of hazardous materials. With these approaches, the possibility that the type of meat and cooking method would influence our results cannot be excluded, and the gut microbiota could also be influenced by some local environment. Fourth, although it has been reported that IGI and MIRI correlate with the *M* value well, the euglycemic-hyperinsulinemic clamp, the gold standard, was not used to measure insulin sensitivity. Therefore, future studies examining other types of fried meat with different frying conditions in other regions and measurement of insulin sensitivity using the euglycemic-hyperinsulinemic clamp are still needed to provide additional evidence regarding the health impacts of fried food.

In summary, fried meat intake impaired glucose homeostasis and increased the levels of intestinal endotoxin and systemic inflammation by influencing the gut microbiota and microbial-host metabolites.

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**Author Contributions.** Y.L., C.S., and T.H. conceived the study design. J.G., K.H., W.W., H.S., and H.W. participated in the execution of the study. J.G., X.G., and S.L. performed the statistical analysis. R.L., W.W., and Y.Z. repeated and validated the statistical analysis. K.H., X.L., W.W., and W.G. measured biomarker levels in biological samples. J.G., X.L., W.J., and W.W. performed mice tests and metabolites interventions. J.G., X.G., and T.H. wrote the manuscript. All authors provided critical revisions of the draft and approved the submitted draft. C.S. affirmed that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have

been omitted; and that any discrepancies from the study as originally planned (and, if relevant, registered) have been explained. C.S. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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