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Different Isocaloric Meals and Adiposity Modify Energy Expenditure and Clinical and Metabolomic Biomarkers During Resting and Exercise States in a Randomized Crossover Acute Trial of Normal-Weight and Overweight/Obese Men

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ABSTRACT

Background: Few studies have assessed the integrative effects of diet, BMI, and exercise on postprandial changes in energy and circulating metabolic profiles.

Objectives: We aimed to assess the collective effects of 3 isocaloric meals high in carbohydrate (74.2% energy), fat (64.6% energy), or protein (39.5% energy) on energy expenditure and clinical and metabolomic biomarkers under resting and exercise conditions in normal-weight and overweight/obese men.

Methods: This crossover controlled acute trial included 20 normal-weight (BMI, 18.5 to <24 kg/m^2) and 20 overweight/obese (BMI ≥24 kg/m^2) men aged 18–45 years. Each of 3 test meals was provided for 2 continuous days: a resting day without exercise, followed by an exercise day with a bicycling exercise of 50% maximal oxygen consumption (postprandial 90–120 minutes). Energy expenditure (exploratory outcome of primary interest) was measured using indirect calorimetry. Fasting and postprandial 2-hour serum clinical and metabolomic biomarkers (secondary interest) were measured. Mixed models were used to examine the effects of meal, time, and/or BMI category.

Results: On the resting day, no significant between-meal differences were detected for energy expenditure. However, high-carbohydrate and high-fat meals induced the highest postprandial 2-hour increase in glucose (0.34 ± 0.15 mmol/L) and triglyceride (0.95 ± 0.09 mmol/L), respectively, while the high-protein meal reduced glucose (−0.48 ± 0.08 mmol/L) and total cholesterol (−0.01 ± 0.03 mmol/L; all P_{meal} values < 0.001). On the exercise day, a high-carbohydrate meal significantly promoted the carbohydrate oxidation rate but suppressed the fat oxidation rate (P_{meal} < 0.05), while its postprandial glucose response was attenuated by bicycling (−0.31 ± 0.03 mmol/L; $P_{exercise}$ < 0.001). We identified 69 metabolites as key features in discriminating between the 3 meals, and overweight/obese men had more varieties of metabolites than normal-weight men.

Conclusions: Three isocaloric meals induced unique postprandial changes in clinical and metabolomic biomarkers, while exercise prevented the hyperglycemia induced by a high-carbohydrate meal. Overweight/obese men were more responsive to the meal challenges than normal-weight men. This trial was registered at clinicaltrials.gov as NCT03231618. *J Nutr* 2022;152:1118–1129.

Keywords: macronutrients, energy expenditure, fat oxidation, untargeted metabolomics, amino acids

Introduction

As a major risk factor of cardiometabolic diseases and premature mortality, obesity has become a huge health burden, with a global prevalence of 12.0% among adults in 2015 (1, 2). Nutrition transitions from traditional, plant-based diets to more animal-based diets and sedentary lifestyles are key drivers of the obesity epidemic in many countries, particularly Asian countries

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like China, which has the largest obese population worldwide (3). Thus, understanding the integrative effects of diets and physical activity on energy balance and cardiometabolic health is urgently needed to establish more effective prevention strategies for obesity and related cardiometabolic diseases.

As primary energy sources, carbohydrate, fat, and protein are essential to regulate the energy metabolism (4). In a previous meta-analysis of acute intervention studies, meals containing high amounts of protein or carbohydrate generally induced higher total energy expenditure (TEE) and diet-induced thermogenesis (DIT) values than high-fat (HF) meals, although the results were inconsistent across studies (5). Moreover, a high-carbohydrate (HC) meal was reported to increase the mean and 3-hour postprandial glucose values more than an isocaloric HF meal (6), while a high-protein (HP) meal could significantly reduce the postprandial cumulative increment of glucose (7). In addition, body weight and physical activity are known to modulate the energy balance and glucose and lipid metabolisms. After consuming an HC meal, overweight men showed significantly lower fat oxidation but higher circulating fatty acids and triacylglycerol levels than lean men (8). In contrast, no significant differences in DIT and substrate oxidation were observed between lean and obese subjects after consuming an HC or HF meal in a crossover trial (9). Meanwhile, acute exercise was found to enhance fatty acid oxidation (10) and decrease the postprandial increment of glucose by 35.5% (11). However, evidence is scarce regarding to the collective impacts of diets, BMI, and postprandial exercise on energy expenditure and the metabolism.

With recent technology developments, untargeted metabolomics have been widely used to identify postprandial metabolomic responses (12, 13), food fingerprints (14, 15) and dietary patterns, such as the Mediterranean diet (16). A recent study demonstrated that acute exercise could trigger changes in metabolic and cardiovascular pathways (10). However, few studies have investigated metabolomic features following meals with different macronutrient compositions. To fill these knowledge gaps, we aimed to systematically evaluate the acute effects of 3 isocaloric meals with different macronutrient proportions on the energy expenditure and clinical and metabolomic biomarkers among normal-weight and overweight/obese Chinese men under resting and exercise conditions in a crossover controlled trial.

Methods

Participants

This randomized, crossover controlled acute trial was performed from September 2017 to February 2018 in Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences. All potential participants were recruited by an advertisement and screened with a short questionnaire and physical examination. Since most available studies with crossover designs generally have 15–25 participants (17, 18), a total of 40 men (20 overweight/obese and 20 normal-weight men) were included after accounting for dropouts. Due to different body compositions and hormonal effects, men and women have differences in postprandial thermogenesis in a resting state (19), as well as in substrate oxidation during exercise (20). Therefore, we only included men in the current study to avoid gender influences. Eligible participants were: *1*) men aged 18–45 years; and *2*) had a BMI of 18.5 to <24 kg/m^2 for normal-weight men or ≥24 kg/m^2 for overweight/obese men. The exclusion criteria were: *1*) being previously diagnosed with a chronic disease, such as severe cardiovascular, liver, or kidney disease; stroke; type 2 diabetes; cancer; a psychological disorder; thyroid dysfunction; or any alimentary tract disease that could affect the energy metabolism; *2*) heavy alcohol use [>14 standard drinks (~196 g ethanol)/week] or use of medications (Trimetazidine, Metoprolol, Levothyroxine, etc.) or nutritional supplements (Coenzyme Q10 and L-carnitine, etc.) that could affect the energy metabolism within the 3 months prior to the trial; and *3*) planning to change body weight, physical activity, or dietary habits within the 3 months prior to the trial.

In total, 56 potential participants attended the screening visit and 40 eligible participants (20 normal-weight and 20 overweight/obese men) were enrolled (**Supplemental Figure 1**). The study protocols were approved by the institutional review board of the Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences. All participants provided written informed consent before the trial.

Study protocols and test meals

Prior to starting the intervention, all eligible participants were invited to the metabolic research unit (MRU) located in our institute campus for a baseline survey. Three isocaloric meals—namely, HC, HF, and HP meals (recipes and major nutrients are presented in **Supplemental Table 1**)—were prepared in a metabolic kitchen, while uneaten foods were carefully weighed and recorded by dietitians.

The 3 test meals generated 6 test meal sequences, which were randomly assigned to each participant via simple randomization. Each of 3 test meals was provided during 2 continuous days with a 10-day wash-out period between different meals, while the 2 continuous testing days consisted of a resting day and an exercise day. After overnight fasting (≥7 hours), participants arrived at the MRU between 08:00 and 08:30 on each test day. After a 30-minute rest on a bed at a temperature of 22°C to 25°C, the resting energy expenditure (REE) was measured, information on appetite and satiety was collected, and fasting venous blood samples were obtained. After consuming the test meal, the REE was measured and information on appetite and satiety was collected at postprandial 0, 1, 2, and 3 hours on the resting day, while the exercise energy expenditure (EEE) was measured during postprandial 90–120 minutes when performing a bicycling exercise with 50% maximal oxygen consumption (VO_2max) on the exercise day. Postprandial 2-hour venous blood samples were obtained on both days (**Supplemental Figure 2**).

Indirect calorimetry

Oxygen uptake (VO_2) and carbon dioxide production (VCO_2) were determined by indirect open circuit calorimetry (JEAGER Oxycon Pro; CareFusion UK Ltd) with breath-by-breath expired gases as calibration. The VO_2 and VCO_2 were measured for 15 minutes under the fasting condition and hourly until postprandial 3 hours on the resting day, or

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Supplemental Figures 1–6, Supplemental Tables 1–5, and Supplemental Methods are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/jn/.

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Abbreviations used: CHO, carbohydrate oxidation rate; DIT, diet-induced thermogenesis; EEE, exercise energy expenditure; FC, fold change; FDR, false discovery rate; FFM, fat-free mass; FO, fat oxidation rate; GGT, gamma-glutamyl transferase; HC, high-carbohydrate; Hcy, homocysteine; HF, high-fat; His, histidine; HP, high-protein; iAOC, incremental area over the curve; iAUC, incremental area under the curve; MRU, metabolic research unit; PLS-DA, partial least squares discrimination analysis; REE, resting energy expenditure; RQ, respiratory quotient; TEE, total energy expenditure; Trp, tryptophan; UA, uric acid; VIP, variable importance on projection; VCO_2, carbon dioxide production; VO_2, oxygen uptake; VO_2max, maximal oxygen consumption.

continuously for 30 minutes during bicycling from postprandial 90 to 120 minutes on the exercise day (**Supplemental Figure 2**). Participants breathed normally while wearing a mask connected to a gas analyzer. Data from the first 5 minutes of each 15-minute detection period were discarded to allow participants to enter a steady state. The TEE, EEE and respiratory quotient (RQ) were calculated from the VO_2 and VCO_2 using the formula provided by Weir (21). The DIT of each test meal was calculated as the fasting REE subtracted from the postprandial REE. The carbohydrate oxidation rate (CHO) and fat oxidation rate (FO) were calculated according to equations by Frayn (22) (**Supplemental Methods**).

Anthropometric and blood biochemical measurements

During the baseline survey, information on demographic characteristics, lifestyle factors, health status, and use of medications and nutritional supplements were collected by a trained dietician using standardized general questionnaires. Dietary intake was assessed using an FFQ modified from a validated questionnaire used in the 2002 National Nutrition and Health Survey in China (23), while physical activity levels were measured with the International Physical Activity Questionnaire (24). Visual analogue scales were applied to assess subjective satiety, hunger, appetite, and prospective consumption during fasting and postprandial 0, 1, 2, and 3 hours on the resting day.

Body weight, height, waist and hip circumferences, and blood pressure were measured at the baseline survey following a standardized protocol, and BMI was calculated as kg/m^2. A normal weight was defined as a BMI of 18.5 to <24 kg/m^2, while overweight/obesity was defined as a BMI ≥24 kg/m^2. Body composition, including fat mass, fat-free mass (FFM), and bone mineral density, was measured using DXA (QDR-4500, Hologic) with software (version 11.2.1) built into the scanner and accurate quality control measures.

Venous blood samples were collected after overnight fasting and at postprandial 2 hours on both the resting day and the exercise day. Serum samples were separated out and stored at −80°C before analysis. Serum glucose, insulin, triglycerides, total cholesterol, HDL cholesterol, LDL cholesterol, gamma-glutamyl transferase (GGT), and uric acid (UA) concentrations were assessed by an automatic biochemical analyzer (Cobas 8000 c702 Analyzer) with commercial reagents purchased from Roche Diagnostics Limited.

Untargeted metabolomics

The serum untargeted metabolomics profile was measured by LC-MS following the standard procedure (25). Briefly, a total of 468 serum samples from 39 participants were analyzed in 3 different batches. Serum samples from the same person (12 time points) were analyzed within the same batch and randomly injected for data acquisition. Blank (100% acetonitrile) and quality control samples were inserted every 10 samples. The LC-MS analysis was performed with an ultra-high-performance LC system (Nexera UHPLC LC-30A, SHIMADZU Technologies) coupled to a quadruple time-of-flight MS (AB 6600 TripleTOF, SCIEX) in positive and negative ion modes.

Raw data were processed using ProteoWizard with the R package XCMS (version 1.46). Metabolite identification was performed using online MetDNA (http://metdna.zhulab.cn/). Metabolites were excluded if they were missing in >20% of samples, had a CV >30%, or were unnamed. Among the remaining metabolites, missing or 0 values were imputed with half of the minimum positive values from the original data. A total of 657 metabolites were included in the final analysis after normalization with *MetNormalizer* (Supplemental Methods) (26).

Statistical analysis

This was an exploratory study, and the exploratory outcomes of primary interest were the between-group differences in postprandial changes of energy expenditure parameters (REE, RQ, DIT, CHO, and FO on the resting day; TEE, EEE, RQ, CHO, and FO on the exercise day). The exploratory outcomes of secondary interest were the between-group differences in postprandial changes of clinical biomarkers (glucose, insulin, triglyceride, total cholesterol, LDL cholesterol, HDL cholesterol, GGT, UA) and subjective appetite, as well as the between-group differences in metabolomic biomarkers.

Baseline descriptive statistics are presented as mean ± SD and n (%) for continuous and categorical variables, respectively. Baseline differences between overweight/obese and normal-weight men were evaluated using a Student's *t*-test for continuous variables and a Fisher's exact test for categorical variables. On the resting day, a mixed-model repeated-measure analysis (fixed effects = diet, time, and diet-by-time interaction; random effects = subjects) was performed for energy expenditure and subjective appetite data at multiple time points with a post hoc Tukey's honestly significant difference test used for between-group comparisons. On the exercise day, the effects of diets on energy expenditure were examined by linear mixed models (fixed effects = diet; random effects = subjects) with Tukey post hoc tests for multiple comparisons. Quantile-quantile plots and skewness were used to assess the normality of the distribution, and a skew distribution was defined if skewness was not between −1 and 1. Paired Student's *t*-tests (normal distribution) and paired Wilcoxon tests (skew distribution) were used to compare fasting and postprandial 2-hour serum biochemical data. The incremental area under the curve (iAUC) and incremental area over the curve (iAOC) were calculated by the accumulation of trapezoidal surfaces. The iAUC was used as postprandial 3-hour increment of DIT, FO, and satiety, while the iAOC was used as postprandial 3-hour decrement of CHO, hunger, appetite, and prospective consumption. A 2-sided P value < 0.05 was considered statistically significant, and all analyses were performed using SPSS (IBM) and R (R-Project). For clinical biomarkers, a false discovery rate (FDR) was used for multiple corrections.

For untargeted metabolomic data, univariate and multivariate analyses were performed with all log-transformed metabolite concentrations. Mixed-effect models that included time, test meals, and the interaction between time and test meals were built using the R package-lme4 (v.3.1.1). Fold changes (FCs) of metabolites were calculated as the ratio of postprandial 2-hour concentrations to fasting concentrations. The FDR was used for multiple corrections and significance was set at a corrected P value < 0.05. To identify the between-meal differences in postprandial metabolomic changes, a partial least squares discrimination analysis (PLS-DA) was performed with the FCs of the metabolites as predicted variables and different meals as categorical variables. Variable importance on projection (VIP) values were used to assess the importance of each metabolite in the PLS-DA model. To identify corresponding metabolic pathways that were significantly altered by different test meals, metabolite pathway enrichment analyses were performed using *MetaboAnalystR*, with metabolites having an FDR-adjusted P value < 0.05 in the mixed-effect model and a VIP >1.0 in PLS-DA (27).

Results

Of the 40 admitted participants, 39 participants completed the trial, as 1 overweight/obese man dropped out due to a time conflict (Supplemental Figure 1). All 3 isocaloric meals were well accepted, and more than 98% of the test meals were completely consumed. No adverse effects were reported.

Baseline characteristics

As shown in **Table 1**, the mean BMIs and body fat percentages were 21.5 kg/m^2 (SD, 1.5 kg/m^2) and 17.9% (SD, 5.5%), respectively, in normal-weight men and 28.4 kg/m^2 (SD, 2.9 kg/m^2) and 27.9% (SD, 4.9%), respectively, in overweight/obese men. Compared with normal-weight men, overweight/obese men showed significantly higher values for systolic blood pressure, weight, waist circumference, FFM, and aerobic capacity indices (absolute values of VO_2max and maximal fat oxidation rate), but a lower education level (all P values < 0.05). However, there was no significant difference among aerobic capacity indices after adjusting for FFM.

TABLE 1 Baseline participant characteristics[1]

Characteristic	Total ($N = 39$)	Normal weight ($n = 20$)	Overweight/obesity ($n = 19$)	P^2
Questionnaire information				
Age, years	26.0 ± 2.6	26.1 ± 2.1	25.8 ± 3.1	0.712
Education level, years	17.8 ± 2.0	18.7 ± 1.8	17.0 ± 1.9	0.007
Physical activity level,[3] *n*	—	—	—	0.546
Low	6 (15.4)	3 (15.0)	3 (15.8)	
Moderate	22 (56.4)	13 (65.0)	9 (47.4)	
Heavy	11 (28.2)	4 (20.0)	7 (36.8)	
Total energy intake, kcal/d	2510 ± 871	2520 ± 993	2510 ± 757	0.954
Carbohydrate, % energy	68.4 ± 11.6	70.1 ± 11.4	66.8 ± 11.8	0.377
Fat, % energy	33.7 ± 18.2	33.6 ± 19.9	33.8 ± 16.8	0.982
Protein, % energy	23.1 ± 8.8	23.4 ± 9.0	22.8 ± 9.0	0.841
Anthropometric measurements				
SBP, mmHg	123.1 ± 10.6	119.7 ± 7.9	126.7 ± 12.1	0.037
DBP, mmHg	81.0 ± 8.8	78.7 ± 6.5	83.4 ± 10.3	0.094
Weight, kg	73.4 ± 13.4	63.6 ± 5.9	83.8 ± 11.0	<0.001
Waist circumference, cm	85.8 ± 11.2	77.5 ± 6.4	94.6 ± 7.9	<0.001
BMI, kg/m^2	24.9 ± 4.2	21.5 ± 1.5	28.4 ± 2.9	<0.001
Body fat percentage, %	22.8 ± 7.3	17.9 ± 5.5	27.9 ± 4.9	<0.001
FFM, kg	54.9 ± 6.7	51.0 ± 4.5	58.9 ± 6.3	<0.001
BMD, g/cm^2	0.98 ± 0.10	0.96 ± 0.09	1.00 ± 0.10	0.217
Aerobic capacity				
MFO				
mg/min	244.9 ± 102.2	211.3 ± 94.0	280.2 ± 100.6	0.033
mg/(min·kg weight)	3.31 ± 1.22	3.25 ± 1.29	3.37 ± 1.18	0.761
mg/(min·kg FFM)	4.42 ± 1.70	4.08 ± 1.67	4.78 ± 1.69	0.201
VO_2max				
mL/min	2150 ± 367	2000 ± 283	2310 ± 382	0.006
mL/(min·kg weight)	29.7 ± 4.3	31.4 ± 3.3	27.8 ± 4.5	0.006
mL/(min·kg FFM)	39.2 ± 4.4	39.1 ± 3.5	39.3 ± 5.3	0.919

[1] Values are presented as the mean ± SD and n (%) for continuous variables with a normal distribution and categorical variables, respectively. Abbreviations: BMD, bone mineral density; DBP, diastolic blood pressure; FFM, fat-free mass; MFO, maximal fat oxidation rate; SBP, systolic blood pressure; VO_2max, maximal oxygen uptake rate.
[2] Between-group differences were compared using a Student's *t*-test and Fisher exact test for continuous variables and categorical variables, respectively.
[3] Physical activity was categorized as 3 levels (high, moderate, and low) based on the International Physical Activity Questionnaire.

Energy expenditure

On the resting day, the REE and FO increased significantly after all 3 meals, while the RQ and CHO decreased significantly with time (all P_{time} values < 0.01 except for CHO in normal-weight men after the HC meal; **Table 2**). There were no significant between-meal differences in postprandial changes of REE, RQ, and DIT (all P_{meal} values > 0.05). Compared with normal-weight men, overweight/obese men showed a lower RQ and CHO, but a higher FO at all postprandial times after the HP meal (all P values < 0.05; Table 2). On the exercise day, compared with the HF and HP meals, the HC meal significantly promoted CHO but suppressed FO in both normal-weight and overweight/obese men during exercise (**Table 3**; all P values < 0.05). Compared with normal-weight men, overweight/obese men showed higher TEE values across 3 meals, and higher net EEE values after consuming the HF and HP meals (Table 3; all P_{weight} values < 0.05).

Clinical markers and subjective satiety levels

Among all participants, the HC meal induced the highest postprandial 2-hour increments of glucose and insulin on the resting day (FDR-corrected $P_{meal} < 0.05$), while both increments were significantly attenuated on the exercise day (FDR-corrected $P_{exercise} < 0.05$). The HF meal induced the highest postprandial increments of triglyceride on both the resting day and the exercise day (FDR-corrected $P_{meal} < 0.05$). The HP meal induced the lowest postprandial increments of glucose, total cholesterol, and GGT, but the highest postprandial increments of UA on the resting day (all FDR-corrected P_{meal} values < 0.05). These significant associations persisted for all clinical markers except for GGT on the exercise day (**Figure 1**; **Supplemental Table 2**). Compared with normal-weight men, overweight/obese men showed greater increases in insulin and triglycerides following the HC meal (**Supplemental Table 3**; all FDR-corrected P_{weight} values < 0.05). After consuming any test meal, the subjective level of satiety increased, while levels of hunger, appetite, and prospective consumption significantly decreased (all P_{time} values < 0.001), although no significant between-meal differences were observed (**Supplemental Figure 3**).

Untargeted metabolomics

Among all participants, 25, 17, and 49 out of the total 657 metabolites were significantly changed after the HC, HF, and HP meals, respectively, on the resting day (**Supplemental Figure 4**A–C), while 18, 33, and 48 metabolites, respectively, were significantly changed on the exercise day (Supplemental Figure 4D–F). In the PLS-DA, the 3 test meals were clearly

TABLE 2 Fasting and postprandial parameters of energy expenditure on the resting day among normal-weight and overweight/obese participants[1]

		Time after test meal, hours				
	Fasting	0	1	2	3	P_{time}[2]
Resting energy expenditure, kJ/h						
HC meal						
NW	313 ± 12.4[b]	371 ± 11.1[a]	370 ± 12.5[a]	364 ± 11.2[a]	372 ± 12.8[a]	0.002
OB	314 ± 15.0[b]	375 ± 15.2[ab]	375 ± 16.9[a]	374 ± 14.3[a]	373 ± 13.8[a]	<0.001
P_{weight}[3]	0.976	0.396	0.806	0.593	0.952	
HF meal						
NW	304 ± 11.0[b]	372 ± 14.9[a]	378 ± 13.4[a]	373 ± 14.1[a]	386 ± 14.6[a]	<0.001
OB	312 ± 13.1[b]	375 ± 14.8[a]	385 ± 14.3[a]	391 ± 14.5[a]	387 ± 10.6[a]	<0.001
P_{weight}[3]	0.589	0.206	0.252	0.546	0.539	
HP meal						
NW	311 ± 14.8[b]	382 ± 14.4[a]	368 ± 12.7[a]	384 ± 13.2[a]	371 ± 13.0[a]	<0.001
OB	312 ± 9.6[b]	389 ± 12.5[a]	385 ± 10.8[a]	386 ± 10.1[a]	372 ± 12.4[a]	<0.001
P_{weight}[3]	0.964	0.512	0.881	0.643	0.977	
P_{meal}[4]						
NW	0.865	0.821	0.853	0.577	0.640	
OB	0.508	0.485	0.842	0.668	0.293	
Respiratory quotient						
HC meal						
NW	0.84 ± 0.02[a]	0.82 ± 0.02[a]	0.75 ± 0.01[b]	0.78 ± 0.02[ab]	0.75 ± 0.02[b]	<0.001
OB	0.84 ± 0.02[a]	0.78 ± 0.02[b]	0.73 ± 0.01[bc]	0.76 ± 0.02[bc]	0.72 ± 0.01[c]	<0.001
P_{weight}[3]	0.850	0.182	0.228	0.237	0.278	
HF meal						
NW	0.83 ± 0.02[a]	0.83 ± 0.02[a]	0.76 ± 0.01[ab]	0.78 ± 0.02[ab]	0.75 ± 0.02[b]	<0.001
OB	0.83 ± 0.02[a]	0.77 ± 0.02[b]	0.71 ± 0.02[b]	0.74 ± 0.02[b]	0.71 ± 0.02[b]	<0.001
P_{weight}[3]	0.790	0.089	0.030	0.107	0.134	
HP meal						
NW	0.85 ± 0.01[a]	0.84 ± 0.02[a]	0.76 ± 0.01[b]	0.81 ± 0.02[a]	0.75 ± 0.02[b]	<0.001
OB	0.82 ± 0.02[a]	0.76 ± 0.02[ab]	0.71 ± 0.01[bc]	0.74 ± 0.02[bc]	0.70 ± 0.01[c]	<0.001
P_{weight}[3]	0.073	0.002	0.009	0.002	0.013	
P_{meal}[4]						
NW	0.666	0.793	0.915	0.459	0.980	
OB	0.208	0.582	0.572	0.604	0.530	
Diet-induced thermogenesis, kJ/h						
HC meal						
NW	—	58.4 ± 6.78	57.3 ± 7.42	51.5 ± 5.88	58.8 ± 8.73	0.674
OB	—	41.8 ± 11.34	51.6 ± 10.16	60.7 ± 9.18	57.1 ± 11.17	0.120
P_{weight}[3]	—	0.211	0.648	0.402	0.903	
HF meal						
NW	—	67.9 ± 11.54	74.1 ± 9.29	69.3 ± 9.11	82.5 ± 9.13	0.463
OB	—	50.2 ± 7.29	60.7 ± 7.32	66.3 ± 10.02	52.8 ± 9.03	0.315
P_{weight}[3]	—	0.207	0.267	0.822	0.027	
HP meal						
NW	—	70.6 ± 10.6	57.0 ± 8.43	72.8 ± 7.71	60.3 ± 12.3	0.093
OB	—	57.2 ± 9.32	53.6 ± 8.49	64.2 ± 8.90	60.0 ± 10.8	0.702
P_{weight}[3]	—	0.347	0.782	0.467	0.986	
P_{meal}[4]						
NW	—	0.658	0.270	0.124	0.195	
OB	—	0.524	0.743	0.913	0.887	
Carbohydrate oxidation rate, g/h						
HC meal						
NW	7.06 ± 1.04[a]	7.69 ± 1.54[a]	3.34 ± 1.09[a]	3.34 ± 1.45[a]	2.32 ± 1.15[a]	0.055
OB	6.21 ± 1.49[a]	5.96 ± 1.75[ab]	2.36 ± 1.61[b]	3.09 ± 1.51[b]	0.13 ± 1.83[b]	<0.001
P_{weight}[3]	0.312	0.336	0.301	0.409	0.838	
HF meal						
NW	7.00 ± 1.29[ab]	7.86 ± 1.90[a]	3.07 ± 1.11[b]	4.88 ± 1.47[ab]	2.13 ± 1.25[b]	<0.001
OB	6.72 ± 1.53[a]	3.81 ± 1.83[ab]	1.85 ± 1.29[b]	0.80 ± 1.61[b]	0.77 ± 1.43[b]	<0.001
P_{weight}[3]	0.718	0.064	0.026	0.069	0.134	

(*Continued*)

TABLE 2 (*Continued*)

		Time after test meal, hours				
	Fasting	0	1	2	3	P_{time}[2]
HP meal						
NW	7.35 ± 1.05[a]	10.3 ± 1.80[a]	3.47 ± 1.21[b]	7.52 ± 1.35[a]	2.65 ± 1.42[b]	<0.001
OB	6.01 ± 1.08[a]	3.14 ± 1.69[ab]	1.89 ± 0.94[bc]	1.37 ± 1.26[abc]	1.25 ± 0.85[c]	<0.001
P_{weight}[3]	0.033	0.006	0.008	0.002	0.011	
P_{meal}[4]						
NW	0.311	0.010	0.059	0.001	0.092	
OB	0.239	0.471	0.125	0.469	0.562	
Fat oxidation rate, g/h						
HC meal						
NW	4.74 ± 0.48[b]	6.19 ± 0.65[ab]	7.57 ± 0.59[a]	7.31 ± 0.65[a]	7.69 ± 0.61[a]	<0.001
OB	5.37 ± 0.51[c]	6.48 ± 0.67[b]	8.16 ± 0.55[ab]	8.01 ± 0.59[ab]	8.44 ± 0.76[a]	<0.001
P_{weight}[3]	0.212	0.448	0.608	0.732	0.446	
HF meal						
NW	3.78 ± 0.57[c]	4.60 ± 0.75[bc]	6.62 ± 0.53[ab]	6.18 ± 0.68[ab]	7.02 ± 0.56[a]	<0.001
OB	3.96 ± 0.64[b]	6.42 ± 0.85[a]	8.46 ± 0.71[a]	8.14 ± 0.92[a]	8.32 ± 0.68[a]	<0.001
P_{weight}[3]	0.915	0.115	0.045	0.093	0.146	
HP meal						
NW	2.97 ± 0.40[c]	3.79 ± 0.60[bc]	6.43 ± 0.49[a]	4.80 ± 0.43[b]	6.92 ± 0.57[a]	<0.001
OB	4.66 ± 0.52[b]	6.91 ± 0.69[a]	8.53 ± 0.53[a]	7.60 ± 0.57[a]	9.01 ± 0.51[a]	<0.001
P_{weight}[3]	0.114	0.002	0.006	< 0.001	0.010	
P_{meal}[4]						
NW	0.239	0.025	0.227	0.005	0.468	
OB	0.213	0.388	0.198	0.523	0.696	

[1] Values are means ± SEMs. Labeled means without a common letter differ at a P value < 0.05. Abbreviations: HC, high-carbohydrate meal; HF, high-fat meal; HP, high-protein meal; NW, normal-weight participants; OB, overweight/obese participants.
[2] The effects of time on variables were examined by repeated-measurement mixed models with Tukey post hoc tests for multiple comparisons. For those with a significant P_{time} value, values with the same letter are not significantly different.
[3] The effects of BMI category on variables were examined by linear mixed models with post hoc multiple comparisons.
[4] The effects of diet on variables were examined by repeated-measurement mixed models.

separated by the global changes in metabolome on both the resting day and the exercise day (**Figures 2**A and 3A). In total, 112 and 111 metabolites (VIP1 >1; VIP2 >1) were identified as key metabolites that could significantly discriminate the features of 3 test meals on the resting day and the exercise day, respectively (Figures 2B and 3B). Of these, 69 metabolites were included on both days. A pathway enrichment analysis determined that these identified key metabolites were significantly enriched in 10 metabolic pathways, including the arginine biosynthesis, cysteine, and methionine metabolism and the glycine, serine, and threonine metabolism (Figures 2C and 3C). The 20 metabolites with the highest VIP in the PLS-DA analysis are listed in **Supplemental Table 4**.

Compared with normal-weight men, overweight/obese men had more altered metabolites after 3 test meals (overweight/obese men: 30 increased and 17 decreased; normal-weight men: 11 increased and 8 decreased; **Supplemental Table 5**). A pathway enrichment analysis showed similar results between normal-weight and overweight/obese participants (**Supplemental Figures 5** and **6**).

Discussion

In this crossover controlled acute trial, 3 isocaloric meals with different macronutrient compositions showed different postprandial metabolic profiles, whereas postprandial exercise specifically altered substrate oxidation and prevented hyperglycemia induced by an HC meal. Further, overweight/obese men were more vulnerable to meal challenges than normal-weight men.

To our knowledge, this is the first acute trial that systematically assessed the collective effects of diet, BMI category, and exercise on energy expenditure and the cardiometabolic-related metabolism. Consistent with previous findings in British (28) and Danish (17) participants, we did not detect significant between-meal differences in postprandial changes of REE, DIT, and substrate oxidation among Chinese men. This result suggests that total energy, rather than the macronutrient composition in an isocaloric HC, HF, or HP meal, may be the major determinant of postprandial energy expenditure. However, it remains to be clarified whether a longer study duration is needed, since most studies, including ours, were within postprandial 4 hours (5). In addition, the HC meal induced the highest postprandial 2-hour increment of glucose and insulin in our study, while the HP meal reduced the postprandial 2-hour increment of glucose and the HF meal resulted in the highest increment of triglyceride. The hyperglycemic response after the HC meal could be attributed to the composition of the meal, which included a high proportion of high-glycemic-index foods and a large glycemic load (29, 30). The high level of postprandial triglycerides after the HF meal could reflect increases in energy sources other than glucose. Meanwhile, the reduced glucose and total cholesterol levels after the HP meal may be related to the slower digestion of protein, while protein catabolism and gluconeogenesis both highly demanded adenosine triphosphate preferentially generated via glucose and fat metabolisms (31).

TABLE 3 Fasting and postprandial parameters of energy expenditure on the exercise day[1]

	HC			HF			HP			P_{meal}[3]
	Fasting	Exercise	Δ[2]	Fasting	Exercise	Δ[2]	Fasting	Exercise	Δ[2]	Δ
Total energy expenditure, kJ/h										
Total	312.4 ± 8.6	1310 ± 45	1000 ± 40.6	313.8 ± 8.9	1330 ± 43.9	1010 ± 39.1	313.1 ± 8.1	1320 ± 44.3	1010 ± 40	0.979
NW	283.3 ± 9.1	1210 ± 48.2	930 ± 44.3	289.4 ± 8.4	1220 ± 44.1	933 ± 41.3	284.5 ± 9.4	1230 ± 41.5	941 ± 37.3	0.975
OB	343 ± 11.2	1420 ± 70.2	1080 ± 65.8	339.5 ± 13.8	1440 ± 70	1100 ± 63.2	343.2 ± 9.2	1430 ± 73.9	1080 ± 69.5	0.981
P_{weight}[4]	<0.001	0.018	0.064	0.003	0.013	0.036	<0.001	0.022	0.078	
Respiratory quotient										
Total	0.87 ± 0.01	0.88 ± 0.01	0.01 ± 0.01 [a]	0.86 ± 0.02	0.82 ± 0.01	−0.04 ± 0.02 [b]	0.83 ± 0.01	0.82 ± 0.01	−0.02 ± 0.01 [b]	0.019
NW	0.87 ± 0.02	0.89 ± 0.02	0.02 ± 0.02	0.85 ± 0.02	0.83 ± 0.02	−0.03 ± 0.02	0.83 ± 0.01	0.83 ± 0.01	0 ± 0.01	0.105
OB	0.86 ± 0.01	0.87 ± 0.02	0 ± 0.02	0.86 ± 0.03	0.81 ± 0.01	−0.06 ± 0.03	0.83 ± 0.02	0.8 ± 0.01	−0.03 ± 0.02	0.158
P_{weight}[4]	0.897	0.335	0.431	0.759	0.339	0.289	0.817	0.067	0.278	
Carbohydrate oxidation rate, g/h										
Total	9.94 ± 0.76	49 ± 3.61	39.1 ± 3.45[a]	9.82 ± 1.56	32.0 ± 3.25	22.2 ± 2.77[b]	7.56 ± 0.83	31.0 ± 2.35	23.5 ± 2.46[b]	<0.001
NW	9.14 ± 1.17	48.7 ± 4.48	39.5 ± 3.91[a]	8.55 ± 1.11	32.3 ± 4.52	23.7 ± 3.97[b]	7.12 ± 0.73	33.4 ± 3.04	26.3 ± 3[b]	0.007
OB	10.8 ± 0.96	49.3 ± 5.85	38.6 ± 5.88[a]	11.1 ± 2.99	31.8 ± 4.81	20.7 ± 3.94[b]	8.02 ± 1.53	28.5 ± 3.6	20.4 ± 3.89[b]	0.010
P_{weight}[4]	0.286	0.929	0.889	0.413	0.947	0.591	0.589	0.296	0.236	
Fat oxidation rate, g/h										
Total	2.66 ± 0.3	12.7 ± 1.47	10.1 ± 1.38[b]	2.73 ± 0.55	19.7 ± 1.45	16.9 ± 1.28[a]	3.6 ± 0.34	20 ± 1.4	16.4 ± 1.27[a]	<0.001
NW	2.36 ± 0.44	10.4 ± 1.69	8.02 ± 1.53[b]	2.72 ± 0.4	17.1 ± 1.96	14.4 ± 1.81[a]	3.18 ± 0.3	16.7 ± 1.5	13.5 ± 1.48[a]	0.014
OB	2.97 ± 0.4	15.2 ± 2.35	12.2 ± 2.26[b]	2.75 ± 1.06	22.4 ± 2.01	19.7 ± 1.62[a]	4.05 ± 0.62	23.5 ± 2.18	19.4 ± 1.89[a]	0.013
P_{weight}[4]	0.316	0.103	0.130	0.976	0.065	0.036	0.205	0.014	0.019	

[1] Values are presented as means ± SEMs. Abbreviations: HC, high-carbohydrate meal; HF, high-fat meal; HP, high-protein meal; NW, normal-weight participants; OB, overweight/obese participants.
[2] The net exercise energy expenditure (Δ) was computed by subtracting energy expenditure during fasting from energy expenditure during exercise. Labeled means without a common letter differ at a P value < 0.05.
[3] The effects of diet on the net exercise energy expenditure (Δ) were examined by linear mixed models (fixed effects = diet; random effects = subjects) with Tukey post hoc tests for multiple comparisons.
[4] The effects of weight on energy expenditure parameters were evaluated with a Student's t-test.

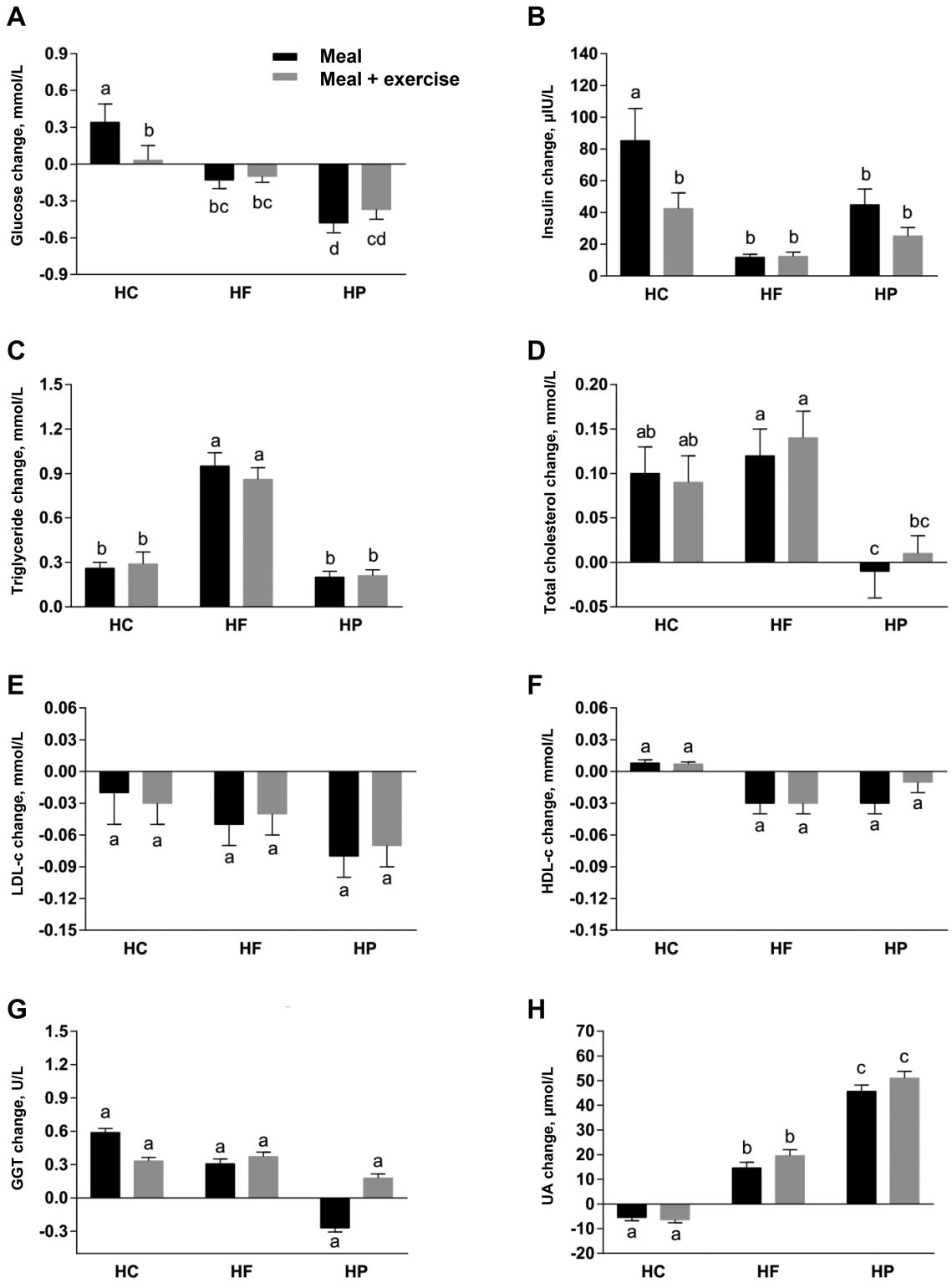

FIGURE 1 Postprandial 2-hour changes of serum clinical markers among all participants. Postprandial 2-hour changes were calculated as postprandial 2-hour absolute values minus fasting absolute values. The black columns represent data on the resting day and the gray columns represent data on the exercise day. (A) glucose, (B) insulin, (C) triglyceride, (D) total cholesterol, (E) LDL cholesterol, (F) HDL cholesterol, (G) GGT, and (H) UA. Labeled means without a common letter differ at a P value < 0.05. Abbreviations: GGT, gamma-glutamyl transferase; HC, high-carbohydrate meal; HF, high-fat meal; HP, high-protein meal; UA, uric acid.

It was noteworthy that 30 minutes of bicycling not only significantly lowered FO and increased CHO values but also attenuated postprandial 2-hour hyperglycemia after the HC meal, which was in line with an earlier study showing that 45 minutes of bicycling reduced blood glucose by 35.5% (11). Acute exercise has previously been shown to inhibit lipolysis and fat oxidation, which could downregulate mitochondrial long-chain FO in healthy men after consuming carbohydrates

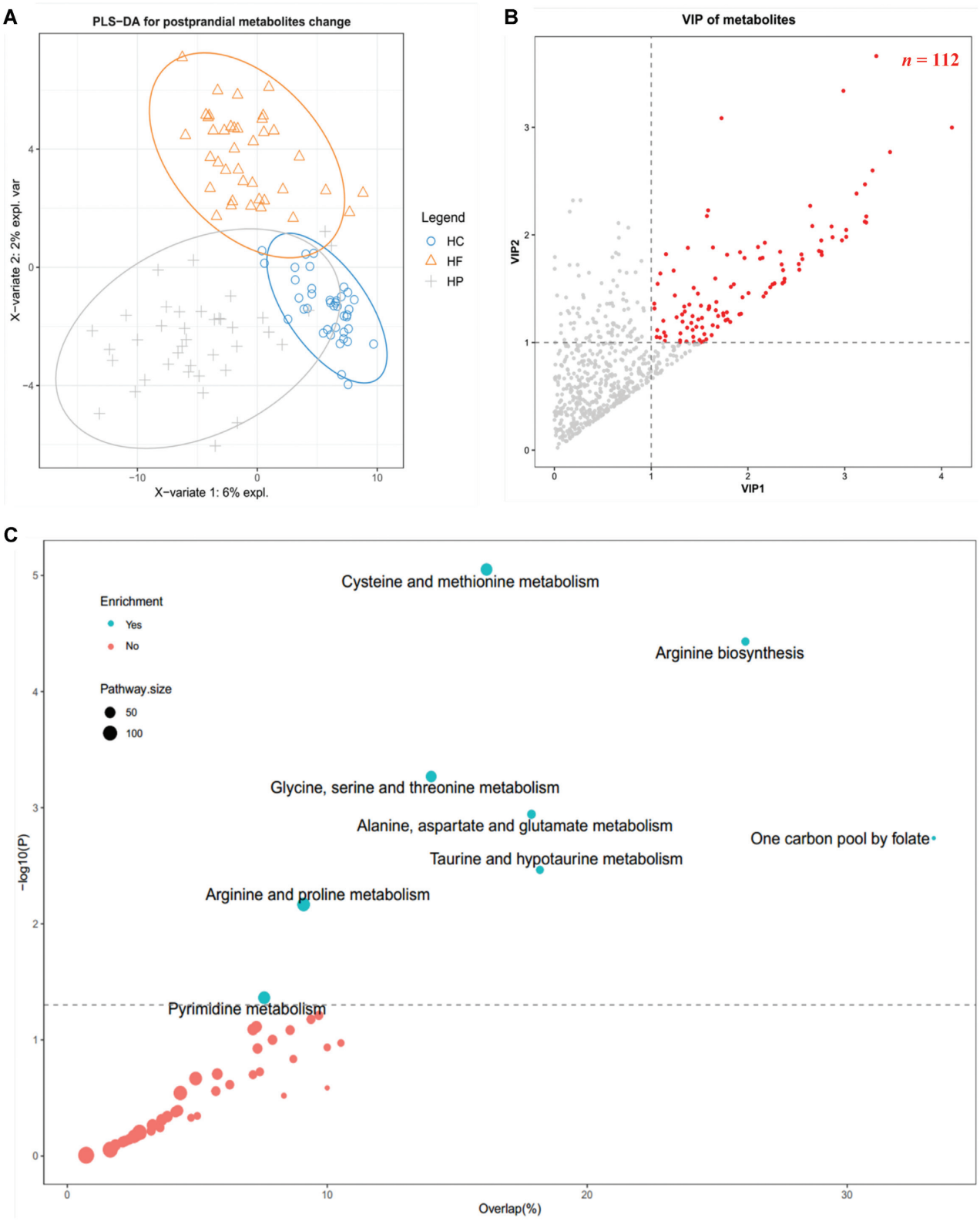

FIGURE 2 PLS-DA and pathway enrichment analysis of postprandial changes of metabolites on the resting day. Postprandial changes of metabolites were calculated as ratios of postprandial 2-hour values to fasting values. A (A) PLS-DA loading plot, (B) VIP plot, and (C) pathway enrichment analysis are presented. Abbreviations: HC, high-carbohydrate meal; HF, high-fat meal; HP, high-protein meal; PLS-DA, partial least squares discrimination analysis; VIP, variable importance in projection.

(10). Moreover, acute moderate-intensity exercise may also improve insulin sensitivity, while boosting glucose and fatty acid uptake in skeletal muscle during a hyperinsulinemic euglycemic clamp (32, 33). In addition, exercise could promote glucose utilization to produce sufficient ATP, thus flattening the postprandial glycemic peak (34). High postprandial blood glucose is a well-known risk factor for cardiometabolic diseases, such as arteriosclerosis and stroke (35, 36). In our study, the HC

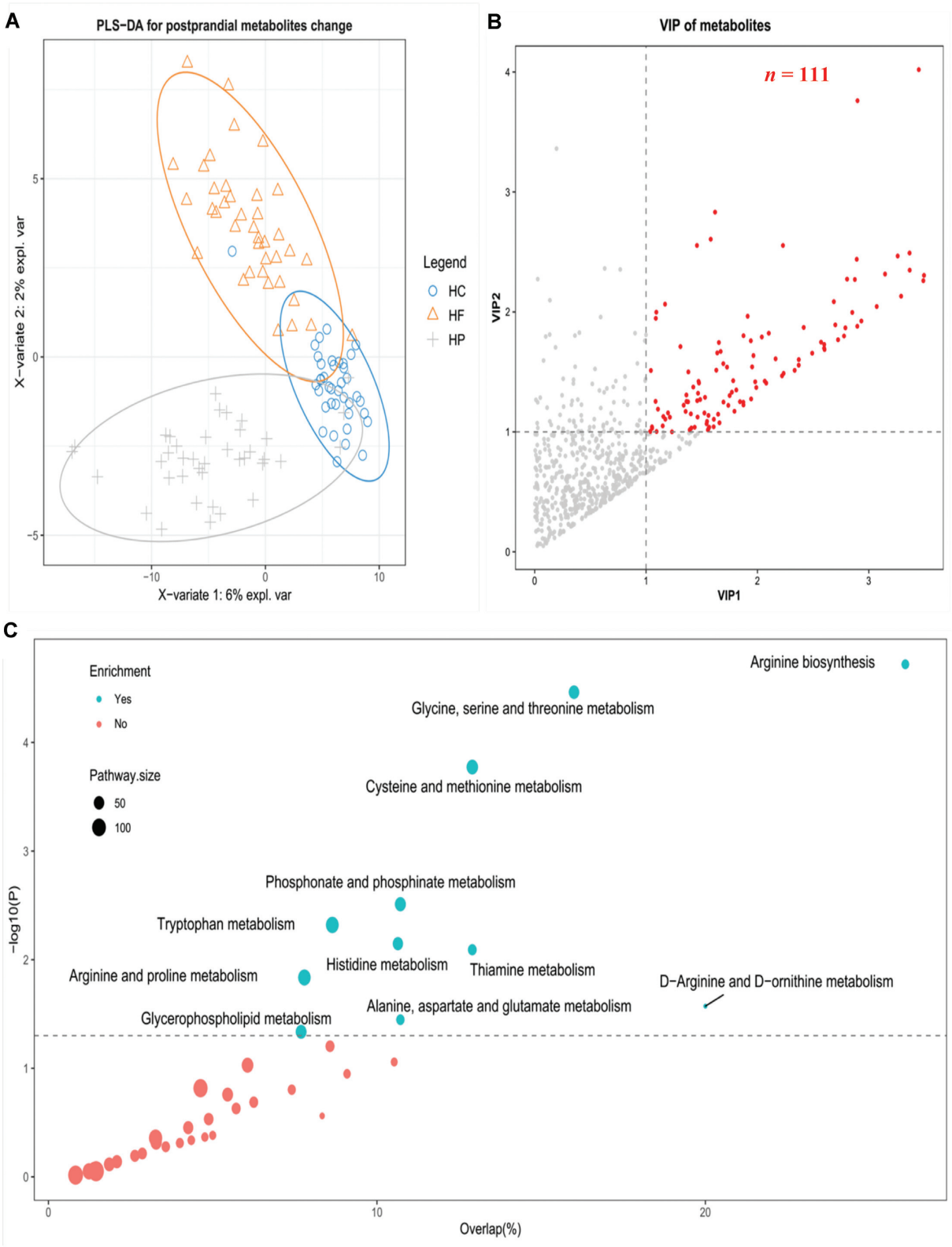

FIGURE 3 PLS-DA and pathway enrichment analysis of postprandial changes of metabolites on the exercise day. Postprandial changes of metabolites were calculated as ratios of postprandial 2-hour values to fasting values. A (A) PLS-DA loading plot, (B) VIP plot, and (C) pathway enrichment analysis are presented. Abbreviations: HC, high-carbohydrate meal; HF, high-fat meal; HP, high-protein meal; PLS-DA, partial least squares discrimination analysis; VIP, variable importance in projection.

intake provoked a higher postprandial glycemic response that could be effectively counteracted by 30 minutes of bicycling. These findings may be valuable for Chinese people, who are known to have habitually HC diets (37).

Interestingly, our study also found that the HP meal led to higher FO and lower CHO values only in overweight/obese men. HP meal challenges were previously reported to promote fat oxidation and energy expenditure in Western populations

with higher body fat masses (38, 39). Although the underlying mechanism is not fully understood, studies in animal models demonstrated that excessive β-oxidation and impaired carbohydrate utilization are important features of obesity-related insulin resistance (40, 41). HP intake could remarkably enhance postprandial lipid utilization, which may amplify the effects of obesity per se, since the lipolysis rate of adipose tissue is thought to regulate fatty acid uptake and oxidation (42, 43). Compared with normal-weight men, overweight/obese men in our study also showed significantly higher postprandial insulin and triglyceride levels after 3 meal challenges, and a more profound postprandial glucose response following the HC meal. It is recognized that obesity often coexists with chronic inflammation of the liver and skeletal muscle (44), as well as metabolic dysfunction and insulin resistance (45). Our findings suggest that overweight/obese individuals may be more vulnerable to exogenous stresses, such as dietary perturbations, and consequently exhibit impaired metabolic homeostasis (46).

With untargeted metabolomics, 69 metabolites were identified as the key features in distinguishing 3 isocaloric meals in PLS-DA. Most of these 69 metabolites were amino acids and their derivatives, of which homocysteine (Hcy), histidine (His), and tryptophan (Trp) increased the most after the 3 meal challenges. As an exclusive precursor of Hcy and an essential amino acid, methionine originates from dietary intake. In agreement with others (47), we found that the HP meal induced a much larger postprandial increase in Hcy than the HF and HC meals, suggesting that a postprandial change of Hcy could be a key indicator of protein intake. We also observed that the HP meal induced the largest increase in UA, which might partially result from activated Hcy and cystine metabolic pathways via amplified oxidative stress (48, 49). In addition, the HP meal challenge also provoked a larger postprandial response in His and Trp, which could suppress appetite by conversion to neuronal histamine (50) and participating serotonin synthesis (51), respectively. However, the roles of appetite-suppressing amino acids in regulating the energy balance after the HP meal challenge remain unclear. Additionally, overweight/obese men also showed more varieties of altered metabolites than normal-weight men (47 compared with 19 metabolites, respectively; Supplemental Table 5).

The strengths of our study included: *1*) use of 3 isocaloric meals that were carefully designed and prepared based on typical Chinese menus, thereby better simulating the daily eating behavior and digestion reaction; *2*) the crossover study design, which minimized inter-individual variations; and *3*) the comprehensive study design, which enhanced our current knowledge about the integrative effects of different meals, BMI categories, and exercise on energy and other metabolic responses. There were also several limitations. First, blood samples were collected only at the fasting and postprandial 2-hour time points; therefore, some important clinical and metabolomic biomarkers could have been missed. Second, we could not measure urinary nitrogen excretion and estimate protein oxidation rates due to the lack of 24-hour urine samples. Third, as a complicated exploratory study, the sample size of our study was based on previous studies with similar designs. Fourth, our study was only conducted in men and the results could not be extrapolated to women. Fifth, as an exploratory study, there could be an increased risk of a type I error in the analyses of energy expenditure and subjective appetite without multiple corrections. Finally, the effects of the meals on the endogenous metabolism should be interpreted with caution, since some metabolites identified in our study may be derived directly from specific food contents.

In conclusion, 3 isocaloric diets varying in macronutrients showed distinctive postprandial metabolic profiles, while exercise altered substrate oxidation and inhibited the hyperglycemia induced by the HC meal. Overweight/obese men appeared to be more vulnerable after the meal challenges and had worse metabolic profiles, especially when consuming the HP meal. Our findings need to be confirmed by additional studies with longer postprandial durations and larger sample sizes.

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The authors' responsibilities were as follows—XL and LS: designed the research, edited the paper, jointly directed the work, and had primary responsibility for the final content; QX, LS, YL, HYun, XS, HYin, and XC: conducted the research; QX: analyzed data and wrote the initial paper; and all authors: read and approved the final manuscript.

Data Availability

Data described in the manuscript, code book, and analytic code will be made available upon request pending application and approval by the corresponding authors.

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