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Propionylated high-amylose maize starch alleviates obesity by modulating gut microbiota in high-fat diet-fed mice

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ABSTRACT

Obesity threatens human health worldwide, and mounting findings have found that gut microbiota (GM) changes induced by diet intervention influence its development. This study aims to investigate the anti-obesity effects and GM changes of propionylated high-amylose maize starch (PS) in C57BL/6J mice fed with high-fat diet (HFD). In our results, PS decreased the body weight of HFD-fed mice after 8 weeks and regulated the glucose stability and insulin resistance. High-amylose maize starch (HAMS) and PS regulated the serum lipid levels and inflammatory response. Moreover, PS yielded more propionate relative to HAMS, proving that introduced propionyl groups could be released in the colon. 16S rRNA results showed that PS altered GM with the increase of bacteria (S24-7 and *Ruminococcus*) and decrease of harmful genera, which is linked to the anti-obesity effect. Our results provide a reference for the design of functional dietary fibers inducing high propionate production and GM modulation.

1. Introduction

Obesity threatens human health worldwide, and obese individuals are more likely to develop several chronic metabolic dysregulations, such as dyslipidemia and type 2 diabetes (Singla, Bardoloi, & Parkash, 2010). The role of gut microbiota (GM) in obesity was initially documented by the finding that, body fat content increased in germ-free mice after conventionalizing with microbiota from normal mice (Backhed et al., 2004). After that, mounting evidence has revealed that GM changes may influence host metabolism, including nutrient intake, energy balance, and lipid metabolism (Le Chatelier et al., 2013; Tremaroli & Bäckhed, 2012). Dietary fiber supplementation has been demonstrated with beneficial effects on the composition and structure of the microbial community, providing a potential target for obesity prevention and treatment (Shang et al., 2017; Shang et al., 2017).

Short-chain fatty acids (SCFAs) are the most important metabolites from fiber fermentation and have broad impacts on host physiology, such as intact gut barrier and intestinal homeostasis (Flint, Scott, Louis, & Duncan, 2012; Hooper, Littman, & Macpherson, 2012). Acetate, propionate, and butyrate are predominant SCFAs (90–95 %), and their ratios are approximately 60 %, 25 %, and 15 %, respectively (Macfarlane & Macfarlane, 2003). Propionate is mainly metabolized in the liver

Abbreviations: ASVs, non-singleton amplicon sequence variants; AUC, area under the curve; eWAT, epididymal white adipose tissue; F/B, Firmicutes to Bacteroidetes ratio; GLP-1, glucagon-like peptide-1; HAMS, high-amylose maize starch; HDL-C, high-density lipoprotein cholesterol; HE, hematoxylin and eosin; HFD, high-fat diet; IL-6, interleukin-6; ND, normal diet; NEFA, non-esterified fatty acid; OGTT, oral glucose tolerance test; PCoA, principal coordinate analysis; PERMANOVA, permutational multivariate analysis of variance; PS, propionylated high-amylose maize starch; PYY, peptide YY; RS, resistant starch; SCFAs, short-chain fatty acids; TC, total cholesterol; TG, total triglycerides; TNF-α, tumor necrosis factor-α.

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(Song et al., 2019) and is shown to trigger gut peptide production, which is related to appetite regulation, glucose, and insulin response (Gibson, 1999; Jiao, Yu, He, Yu, Zheng, Luo, & Wang, 2021). The high amount of propionate production during fermentation was also shown to reduce serum lipids, regulate energy homeostasis, and alleviate cardiovascular disease (Den Besten et al., 2015; Wong, De Souza, Kendall, Emam, & Jenkins, 2006). Rizzatti, Lopetuso, Gibiino, Binda, and Gasbarrini (2013) performed fecal transplants from human twin donors inconsistent with obesity in germ-free mice and found adiposity was related to caecal propionate concentration.

As one type of dietary fiber, resistant starch (RS) is defined as the starch fraction that cannot be digested in the small intestine and can reach the large colon for gut microbial utilization. Growing evidence has shown that acylated starch could be a targeted way to deliver more specific SCFA to the large colon accompanied by physiological effects, including the anti-diabetic (Li, Wang, Wang, Wang, Yao, Strappe, & Guo, 2022) and anxiety effects (Kimura-Todani, Hata, Miyata, Takakura, Yoshihara, & Zhang, 2020) of acetylated starch as well as alleviating abnormal ovarian morphology (He, Shi, Qi, Wang, Zhao, Zhang, & Chen, 2022) and DSS-induced colitis (Li, Cheng, Li, Li, Hong, & Gu, 2021) of butylated starch. RS fermentation is normally associated with lower propionate production in comparison with acetate and butyrate (Brouns, Kettlitz, & Arrigoni, 2002). In our previous studies, introducing propionyl groups to high-amylose maize starch is an effective way to slowly release a substantial amount of propionate assessed by an *in vitro* fermentation study (Xie et al., 2021), and certain GM members such as *Roseburia* and *Blautia* proliferated (Xie et al., 2019). The subsequent study proved the weight-reducing effects and GM modulation of high-amylose maize starch in the obese model (Hu, Zheng, Qiu, Chen, Zeng, Zhang, & Zheng, 2022). Song, Shen, Wang, Li, and Zheng (2019) confirmed that sodium propionate intervention could reduce the accumulation of white adipose and regulate GM dysbiosis. A clinical trial found that increasing propionate release through inulin-propionate ester could prevent weight gain and improve insulin sensitivity in overweight adults in comparison with the inulin group (Chambers et al., 2015), and the propionate release was positively linked to the decreased proinflammatory interleukin-8 levels and the increased abundance of *Fusicatenibacter saccharivorans* (Chambers et al., 2019).

In the present study, we aimed to elucidate the enhanced weight-reducing effect of propionylated high-amylose maize starch (PS) in high-fat diet (HFD)-fed mice. We hypothesized that PS supplementation *in vivo* can provide more propionate production compared to HAMS, and the high production of propionate from PS can alleviate obesity by regulating GM in HFD-fed mice. To explore the potential mechanism, body weight gain, glycemic response, blood biochemical indices, and inflammation levels were measured during an eight-week PS intervention. Furthermore, we determined the fecal SCFA concentration and the alteration of GM in response to PS supplementation. Our results provide feasible ideas for the design of functional dietary fibers inducing high propionate production in the colon with beneficial outcomes.

2. Materials and methods

2.1. Materials and diet information

High-amylose maize starch (HAMS, Hylon 7, amylose content of 69.8 ± 0.3 %) was obtained from Ingredion (Bridgewater, NJ, USA). The PS used in this study was prepared by adding 10 % of propionic anhydride according to the method reported elsewhere (Xie et al., 2019). A normal chow diet (ND, 10 % energy from fat) was set as one control diet, and HFD was set as the model diet which contains 60 % energy from fat (Wang et al., 2015). The other two diets (HAMS and PS diet) were maintained on the HFD which contains 10 % HAMS and PS (based on the dry weight). Compositions of the diets are provided in Table S1 of supplementary data.

2.2. Animals and treatments

The experimental design was approved by Institutional Animal Care and Use Committee (Approval NO. SHRM-IACUC-024), and all the experimental procedures followed the Animal Ethical and Welfare Approval. Briefly, thirty-six male C57BL/6J mice in four-weeks old were purchased from SLAC Laboratory Animal Co., Ltd. (Shanghai, China) and housed in an SPF-level feeding room. After arrival, all mice were provided ND for the first week, and four groups (9 mice per group) were set randomly. All mice were fed ad libitum with one of the diets during the intervention, and their body weight changes were recorded weekly. After the eight-week intervention, all mice were sacrificed under inhalation of isoflurane at a lethal dose. Then blood samples were collected immediately for the total cholesterol (TC), total triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), non-esterified fatty acid (NEFA), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and Lipopolysaccharide (LPS) determination using the corresponding commercial kits.

2.3. Oral glucose tolerance test

After being fed for seven weeks, the oral glucose tolerance test (OGTT) was carried out according to the details published previously (Anhe et al., 2015). Mice were administered by gavage with glucose (2.0 g/kg body weight) after fasting for 8 h, and plasma glucose concentrations were tested with a blood glucose meter (Accu-Check; Roche Diagnostics, Mannheim, Germany) at 30, 60, 90, and 120 min after the administration by taking blood from the tail. Insulin level in the serum was measured with the corresponding kits. The area under the curve (AUC) of each group was calculated following the equation. A, B, C, and D represent blood glucose levels at 30, 60, 90, and 120 min.

$$AUC\ \left(h{\cdot}mmol{\cdot}L^{-1}\right) = 0.25 \times A + 0.5 \times B + 0.75 \times C + 0.5 \times D \quad (1)$$

2.4. Hematoxylin and eosin staining

Samples of epididymal white adipose tissues (eWAT) were embedded in paraffin after being fixed with a 10 % neutral formalin solution. Then the tissues were sectioned to a thickness of 5–6 μm after being stained with hematoxylin and eosin (H&E) dye. The analysis of pathological changes was performed by using a light microscope.

2.5. Fecal short-chain fatty acids measurement

The measurement of fecal SCFA concentrations was determined by GC–MS using Agilent 7890A/5975C instrument as previously reported (Liu, Li, et al., 2017). In brief, 100 mg of feces were mixed with NaOH and 2-methyl-butyric acid and incubated at 4 °C for 2 h, after which the mix was centrifuged (13,000 rmp, 30 min). The supernatant (500 μL) was collected for derivatization by mixing with distilled water, platelet cytotoxic factor solution, and isopropanol/pyridine solution, and extracted with *n*-hexane. The mixture (1 μL) with a split ratio of 10:1 was injected into the GC–MS equipped with Agilent HP-5 capillary column (30 m × 0.25 mm × 0.25 μm). The program of the oven runs as follows: the initial temperature was maintained at 60 °C for 5 min before increasing to 250 °C with the speed of 10 °C/min, and the temperature of the oven was held at 250 °C for another 5 min. Helium was set at 1 mL/min as the carrier gas, and the temperature of the front inlet, transfer line, and electron impact ion source was 280, 250, and 230 °C, respectively.

2.6. Fecal DNA extraction, library preparation, and Illumina sequencing

Feces (100 mg) from animals were extracted with the OMEGA Soil DNA Kit (D5625-01, Omega Bio-Tek, Norcross, GA, USA). The purity and concentration of the extracted DNA were measured before library

preparation. The genomic DNA was subjected to amplification of the V3–V4 hypervariable region of the 16S rRNA gene with forward primer 338F (5′-ACT CCT ACG GGA GGC AGC A-3′) and reverse primer 806R (5′-GGA CTA CHV GGG TWT CTA AT-3′), and agarose gel electrophoresis was used to detect the quality of the amplification. Sample-specific 7-bp barcodes were incorporated into each sample in the second round of PCR reaction, and final PCR products were purified by using Agencourt AM Pure Beads (Beckman Coulter, Indianapolis, IN). Finally, equimolar amplicons were pooled and subjected to pair-end 2 × 300 bp sequencing (Illumina NovaSeq 6000 SP Reagent Kit-500 cycles).

2.7. Bioinformatics

The raw sequence data were demultiplexed by using the demux plugin followed by primers cutting with cutadapt plugin (Martin, 2011). DADA2 was used to filter, denoise and merge quality and remove chimera (Callahan et al., 2016). Mafft was aligned with non-singleton amplicon sequence variants (ASVs) for constructing a phylogeny with fasttree2 (Katoh, Misawa, Kuma, & Miyata, 2002; Price, Dehal, & Arkin, 2009). QIIME2 and R packages (4.1.1) were used to conduct sequence data analyses. For beta diversity, principal coordinate analysis (PCoA) with unweighted uniFrac distance matrix was adopted to measure the structural alteration of microbial communities, and pairwise permutational multivariate analysis of variance (PERMANOVA) tests were applied to find changes between two different mice groups. Based on the prevalence of ASVs across groups, the R package "Venn Diagram" was used to present the common and unique ASVs among four mice groups (Zaura, Keijser, Huse, & Crielaard, 2009). Heatmaps were constructed to depict changes in relative abundances at the genus level, and compositional changes in the bacterial community were calculated by using differential abundance analysis 2 (DESeq2) (Love, Huber, & Anders, 2014).

2.8. Statistical analysis

Body weight, glucose level, serum parameters, and SCFAs levels have at least three repeated values and the results were shown as mean ± standard deviation (SD). SPSS 22.0 statistical product and service solutions (SPSS, Inc. Chicago, IL, USA) were applied to calculate the difference for each parameter among mice groups with Duncan's multiple-range test ($p < 0.05$ was set as a statistically significant difference in this study). A nonparametric test (Kruskal Wallis test followed by pairwise Wilcox test) was employed if data were not normally distributed and the variances were not homogeneous. Two-way ANOVA with repeated measures (weeks/times) followed by Duncan's post hoc test was performed for body weight and glucose tolerance changes. The results were finally visualized by GraphPad Prism software package version 9.0 (GraphPad Software, Inc., CA, USA). Association between microbial changes and different indexes (body and eWAT weight, blood indexes, and SCFA production) were found by corr.test in R, and the results were plotted with the package "pheatmap".

3. Results and discussion

3.1. Body weight and eWAT changes

Fig. 1 presents the body weight and eWAT changes of the tested mice in different groups. There were no significant differences in initial body weight among the four mice groups (Fig. 1A), whereas mice fed with HFD showed a remarkable increase in body weight after 8 weeks of feeding in comparison with the ND group. Mice in the HAMS group suppressed body weight increase ($p < 0.05$) relative to the model group (HFD, Fig. 1B), and PS supplementation significantly attenuated body weight gain in comparison with the HAMS group, indicating that PS has an anti-obesity effect in mice fed with HFD. These results are in line with published findings indicating that the administration of sodium propionate or inulin-propionate ester could effectively decrease weight growth (Chambers et al., 2015; Song et al., 2019). Belobrajdic, King, Christophersen, and Bird (2012) reported that adiposity and body weight gain were controlled in mice when 8 % RS was added to diets, along with the increase in the serum level of gut hormones. Moreover, eWAT from the tested mice was isolated and weighted in the 8th week of the study. Mice in the HFD group exhibited a higher eWAT weight compared to the ND group, whereas PS treatment suppressed this weight gain induced by HFD (Fig. 1C, $p < 0.05$), which is in line with the results from body weight gain (Fig. 1B). The combination results implied that eWAT might be related to the weight-reducing effects of PS in HFD-fed mice.

To further confirm whether the reduction in body weight gain was correlated with adipocyte size, histopathological morphologies of eWAT were selected and observed via H&E staining. As shown in Fig. 1D, the average adipocyte size was remarkably larger in the mice fed with HFD than that in the ND group, which is consistent with the changes in eWAT weight (Fig. 1C). HAMS alleviated the adipocyte hypertrophy established by HFD, in particular, PS reduced the mean adipocyte area compared to HAMS, indicating that PS supplementation could suppress

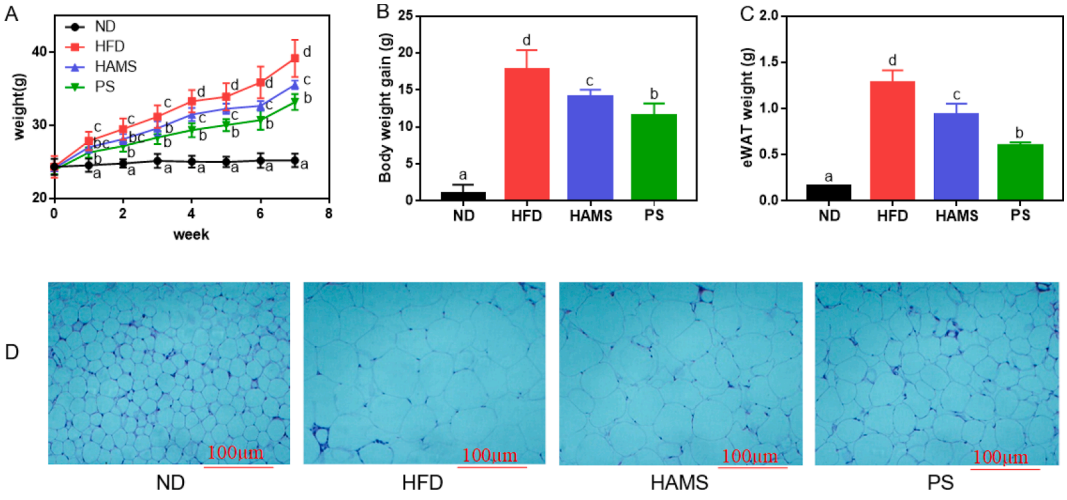

Fig. 1. Effects of PS on body weight (A), body weight gain (B), eWAT weight (C) and the H&E staining of eWAT adipocytes (D) of HFD-fed mice. Values marked with different letters indicate significant differences ($p < 0.05$) within each section. (ND, normal diet; HFD, high-fat diet; HAMS, high-amylose maize starch; PS, propionylated high-amylose maize starch diet).

the HFD-induced fat growth by decreasing fat accumulation and adipocyte size.

3.2. Supplementation with PS changed glucose tolerance and insulin resistance

Fasting glucose, OGTT, fasting insulin, and AUC value under glucose were used to represent the effect of PS on glucose tolerance in mice fed with HFD in the 7th week of the study. As present in Fig. 2A, hyperglycemia was induced in the HFD group with a great increase in the fasting glucose level in comparison with the ND group. However, the fasting blood glucose concentration in mice treated with PS was remarkably reduced by 21.7 % in comparison with the HFD group. After oral administration of glucose, mice fed with HFD showed a higher level of blood glucose from 0 to 120 min when compared to the ND group, and the glucose concentration decreased after HAMS and PS supplementation (Fig. 2B). According to the previous report, PS showed a high RS level due to the "*exo*-pitting" digestion pattern of HAMS (Xie et al., 2019), and thus the glucose release in the digestive tract could be delayed compared to the HFD group (Fig. 2B). It was also well documented that insulin resistance can be reflected through a reduction in the capacity to perform insulin-mediated transport of glucose to the liver, and thus blood insulin concentration has been used to indicate insulin sensitivity in the host (Ren, Hu, Luo, & Yang, 2015; Vogeser et al., 2007). Consistently, the HFD group displayed the highest fasting insulin concentration, whereas treatment with HAMS decreased the insulin value compared to the HFD group, and PS presented a greater insulin-reducing effect in the corresponding mice (Fig. 2C). In addition, the highest AUC value ($p < 0.05$) was measured in mice fed with HFD, which was significantly reduced by 15.43 % with PS supplementation (Fig. 2D). These results indicate that PS improved glucose tolerance induced by HFD.

3.3. PS alleviated dyslipidemia and inflammation response

Obese individuals are more likely to be linked with higher risks of dyslipidemia and cardiovascular disease (Zhang et al., 2004). To further understand the effect of supplementation with PS on lipid metabolism, we next measured the serum concentrations (TC, TG, LDL-C, HDL-C, and NEFA) in different groups after intervention. It has been reported that abnormal metabolism is generally reflected in the increase of serum lipid concentrations, including TC, TG, and LDL-C, which are positively linked with obesity and related metabolic disorders (Shang et al., 2017; Shang et al., 2017). Consistent with previous studies (Liu, Li, et al., 2017; Liu, Qiao, et al., 2017; Sonnenburg & Backhed, 2016), TC in mice fed with HFD showed higher circulating concentrations as compared to the ND group. It was noteworthy that the lipid changes were regulated in mice supplemented with PS, as the elevated lipids TC in mice fed with HFD were regulated. Previous reports regarding the HFD-induced obese rat models indicated that types 2 and 4 RS can effectively decrease serum TG and total TC, which might be linked with a reduction in increased fatty acid and oxidative stress (Shimotoyodome, Suzuki, Fukuoka, Tokimitsu, & Hase, 2010; Si, Zhou, Strappe, & Blanchard, 2017). HFD exhibited a lower HDL-C concentration compared to the ND group. However, HAMS supplementation significantly increased HDL-C levels, and the highest concentration was observed in the PS group (Fig. 2H). Accordingly, increasing concentrations of HDL-C could be a therapeutic way for the suppression of cardiovascular disease (Barter et al., 2007). Moreover, feeding with HFD led to a remarkable increase in the serum NEFA level relative to the ND-fed mice, and PS supplementation decreased NEFA content significantly (Fig. 2I). Under normal conditions, the rest of NEFA goes into the blood circulation after being partly eliminated through β-oxidation (Koutsari, 2006). NEFA accumulation could lead to lipo-toxicity and impair cellular function, which is associated with a decline in insulin sensitivity (Qin et al., 2019). This result might be related to the insulin result observed in Fig. 2C.

Previous studies have suggested that obesity was also characterized by low-grade systemic and adipose tissue inflammation, and it's well accepted that TNF-α, IL-6, and LPS levels are promoted in obesity (Cani et al., 2007; Harte, Silva, Creely, Mcgee, Billyard, Youssefelabd, & Sharada, 2010). In our present study, the serum contents of cytokines (TNF-α, IL-6, and LPS) were measured by an ELISA assay. As shown in Fig. 2J–L, the concentration of TNF-α, IL-6, and LPS in the serum showed an increasing trend in the HFD group in comparison with the values in mice fed with ND. It was well reported that TNF-α is a critical pro-inflammatory cytokine, whereas the endotoxemia LPS influences the

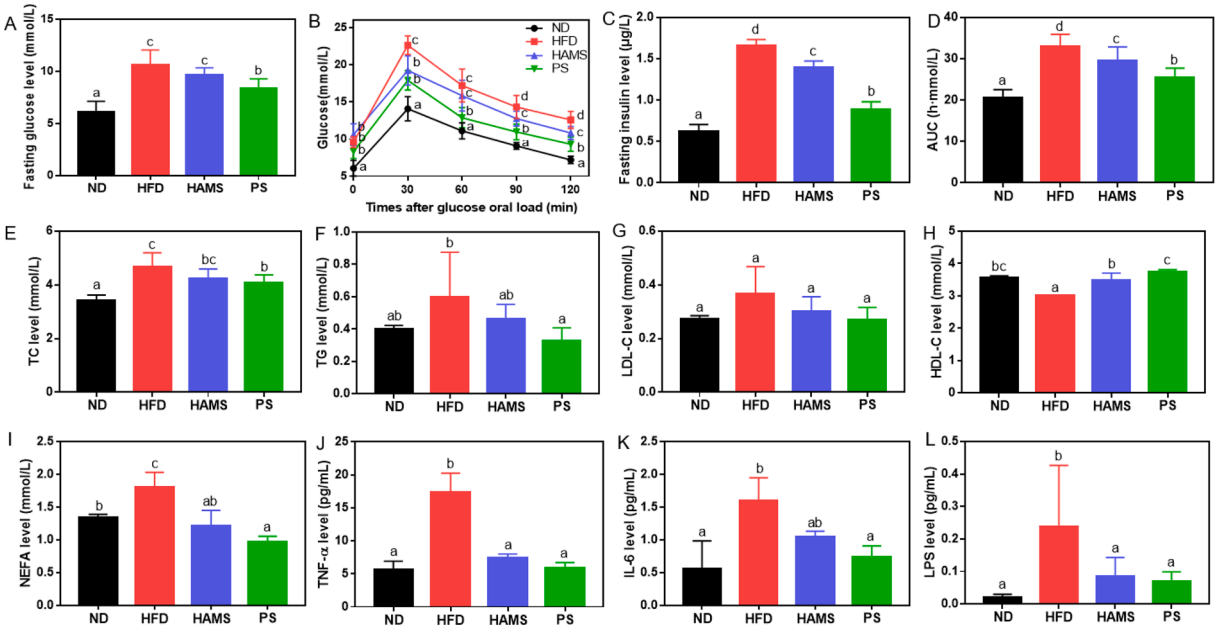

Fig. 2. Effects of PS on fasting glucose level (A), results of glucose tolerance tests (B), fasting insulin level (C), AUC in the oral glucose tolerance test (D), TC (E), TG (F), LDL-C (G), HDL-C (H), NEFA (I), TNF-α (J), IL-6 (K) and LPS (L) content of HFD-fed mice. Values marked with different letters indicate significant differences ($p < 0.05$) within each section. (ND, normal diet; HFD, high-fat diet; HAMS, high-amylose maize starch; PS, propionylated high-amylose maize starch).

generation of proinflammatory cytokines and results in chronic inflammation when fed with HFD in a long term. Notably, supplementation with HAMS and PS presented an obvious preventive effect on the upregulation of inflammatory cytokines, indicating that RS could regulate inflammation response by modulating the inflammatory cytokines in obese mice. Hotamisligil (2006) found that inflammatory cytokines are closely relevant to glucose resistance and obesity, which agrees with the improvement of glucose tolerance (Fig. 2A and B).

3.4. Changes in short-chain fatty acids metabolic profiling in feces

Acetate, propionate, and butyrate are the most important metabolites generated by GM through the fermentation of indigestible dietary substrates, providing an energy substrate for colonocytes, mitigating inflammation, and regulating satiety for its host (Koh, De Vadder, Kovatchevadatchary, & Backhed, 2016). To further examine whether the introduced propionyl groups could be released after PS supplementation in the HFD-feed mice, the SCFA concentration in feces of all mice groups before and after intervention were detected and the results were shown in Fig. 3. There was a low concentration in the SCFA levels of feces among all mice before the intervention, and both ND and HFD groups remained at low levels of acetate, propionate, and butyrate in the 8th week of the study. It has been recorded that SCFAs influence lipid metabolism, and deficiency in SCFAs especially propionate and butyrate production has been linked with metabolic diseases, including obesity and type 2 diabetes (Cheng & Lai, 2000; Qin et al., 2012). As shown in Fig. 3 A and C, the acetate and butyrate concentration in mice fed with HAMS showed a higher value than that in the HFD group, which is in agreement with the previous results that RS is a good butyrogenic fiber substrate (Fuenteszaragoza et al., 2011; Ze, Duncan, Louis, & Flint, 2012). It was reported that RS could exhibit anti-inflammatory functions with SCFAs production especially butyrate (Liu et al., 2018).

Notably, PS supplementation increased the concentration of propionate significantly in comparison with the HAMS group (Fig. 3B), indicating that the ester bond of PS was cleaved by bacterial enzymes in the colon (Clarke et al., 2011). Propionate is an efficient hepatic gluconeogenic substrate for cholesterol synthesis (Nguyen, Prykhodko, Hallenius, & Nyman, 2017), which could be mechanistically linked to the observed regulation of glucose tolerance and the improvement of dyslipidemia (Fig. 2). Moreover, propionate could significantly increase postprandial GLP-1 and PYY and reduce calorie intake, resulting in a remarkable reduction in weight gain as previously reported (Chambers et al., 2015, 2019). Fig. 3D shows the total SCFA production in different mice groups. It was noted that HAMS brought a remarkable elevation of total SCFA in comparison with HFD-fed mice and the amount was numerically slightly higher in the PS group, which was probably due to de-esterified propionate from introduced propionyl groups.

3.5. PS supplementation modulated gut microbiota diversity and populations

The gut microbial composition may relate to metabolic dysbiosis. Here, to assess the influence of PS on GM alteration of HFD-fed mice, partial 16S rRNA gene (V3–V4) sequencing was applied to investigate the alteration in GM of four experimental groups, and the overlapping ASV data from the Venn diagram before and after intervention are shown in Fig. 4 A and C respectively. The number of common microbes among different mice groups before intervention was 2664, accounting for 10 %. Notably, only 517 ASVs coexisted in all groups after 8 weeks of feeding, suggesting that GM composition responded significantly to PS supplementation, and manifested on unweighted UniFrac PCoA. As presented in Fig. 4 B and D, the closest distance was observed among four experimental groups at the initial week, whereas the ND group exhibited clear segregation with the HFD group after feeding with HFD for eight consecutive weeks, suggesting that HFD exhibited an obvious impact on the structure of GM. As expected, significant separation of GM between groups HFD and HAMS or PS was detected in PCoA and hierarchical clustering (Fig. 4E). PERMANOVA results on unweighted UniFrac distance metrics before and after intervention were summarized in Tables S2 and S3. These results indicate that HAMS and PS supplementation altered the structure of GM in HFD-fed mice.

To explore the microbiota response of PS in the HFD-fed mice, detailed composition alterations of GM were further visualized at different levels (phylum and genus), and the results are shown in Fig. 5. At the phylum level, five dominant phyla, including Bacteroidetes, Firmicutes, Proteobacteria, Actinobacteria, and TM7 were identified (Fig. 5A). The total relative levels of Bacteroidetes and Firmicutes were more than 90 % in the gut bacterial communities, while Actinobacteria and Proteobacteria contributed lower proportions. The Firmicutes/Bacteroidetes (F/B) was generally used as an indicator of GM dysbiosis

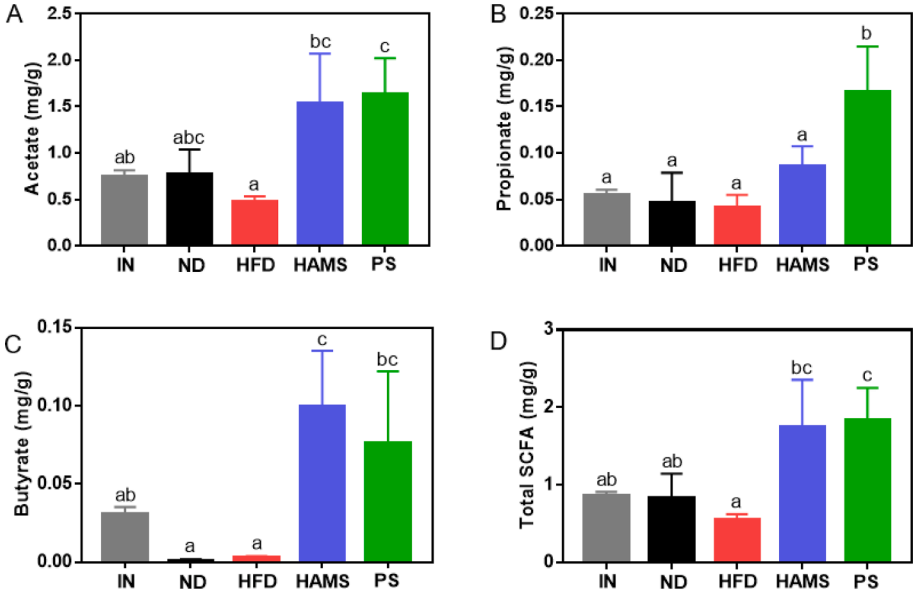

Fig. 3. Effects of PS on acetate (A), propionate (B), butyrate (C) and total SCFA (D) content of HFD-fed mice. Values marked with different letters indicate significant differences ($p < 0.05$) within each section. (IN, before intervention; ND, normal diet; HFD, high-fat diet; HAMS, high-amylose maize starch; PS, propionylated high-amylose maize starch).

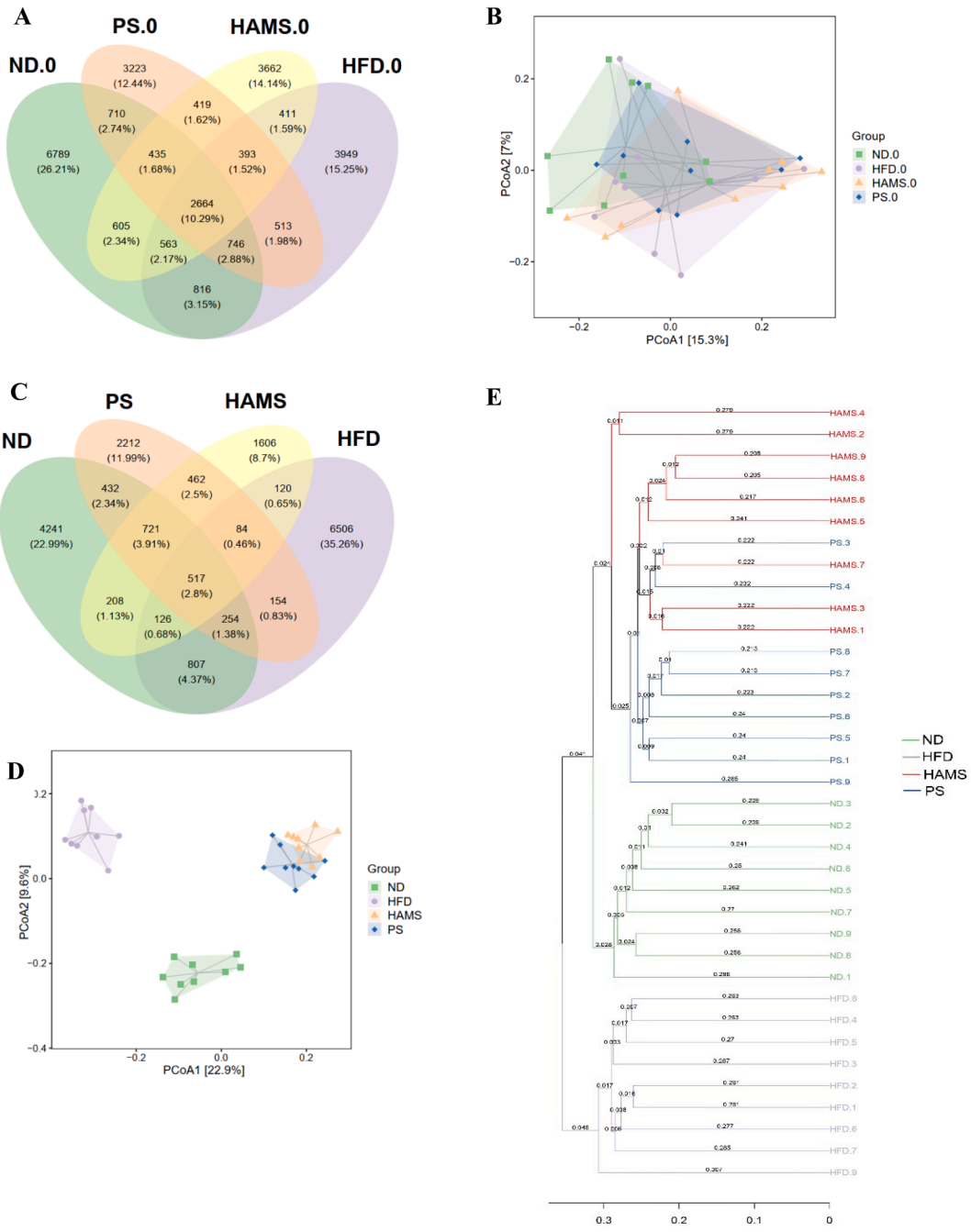

Fig. 4. Venn diagram (A and C), PCoA (B and D) and hierarchical clustering (E) of the gut microbiota structure before and after intervention in different experimental groups. (ND, normal diet; HFD, high-fat diet; HAMS, high-amylose maize starch; PS, propionylated high-amylose maize starch; 0, before intervention).

in HFD-fed mice, and the higher F/B ratio is highly linked to the energy harvest of the host and the potential development of obesity (Turnbaugh et al., 2006). As shown in Fig. 5B, the F/B ratio of mice fed with HFD was higher than that of the ND-fed mice, whereas HAMS and PS supplementation gave a marked decrease in the F/B ratio relative to the HFD group. The result agrees with most previous studies, indicating that a lower F/B ratio might relate to anti-obesity effects both in mouse models as well as in clinical interventions (Chang, Lin, Lu, Martel, Ko, Ojcius, & Young, 2015; Ridaura et al., 2013). Proteobacteria was elevated to 12.30 % with HFD after 8 weeks of feeding, but such an increase driven by the HFD-feeding was regulated by HAMS, which might be related to its modulatory effects on the concentrations of serum LPS (Fig. 2L) (Rizzatti, Lopetuso, Gibiino, Binda, & Gasbarrini, 2017).

Genus level composition in individual mice were shown in Fig. 5C. In the 8th week of the experiment, the genera unclassified S24-7, *Allobaculum*, unclassified Clostridiales, *Oscillospira*, and *Lactobacillus* accounted for a high proportion in the ND groups. It was noteworthy that HFD resulted in a great alteration of GM composition at the genus level relative to the ND group, with the decreased abundance of two main genera (unclassified S24-7 and *Allobaculum*) and the promotion of the genera unclassified clostridiales, *Oscillospira*, *Lactobacillus*, unclassified Desulfovibrionaceae, unclassified Lachnospiraceae, *Helicobacter*, unclassified Bacteroidales, unclassified Ruminococcaceae, unclassified F16, *[Ruminococcus]*, and unclassified Rikenellaceae. Supplementation with HAMS and PS decreased the relative abundance of the genera promoted by HFD and enhanced HFD-induced reduction in the level of unclassified S24-7 and *Bifidobacterium*. *Bifidobacterium* can produce acetate as their major fermentation end-products and is suggested to be

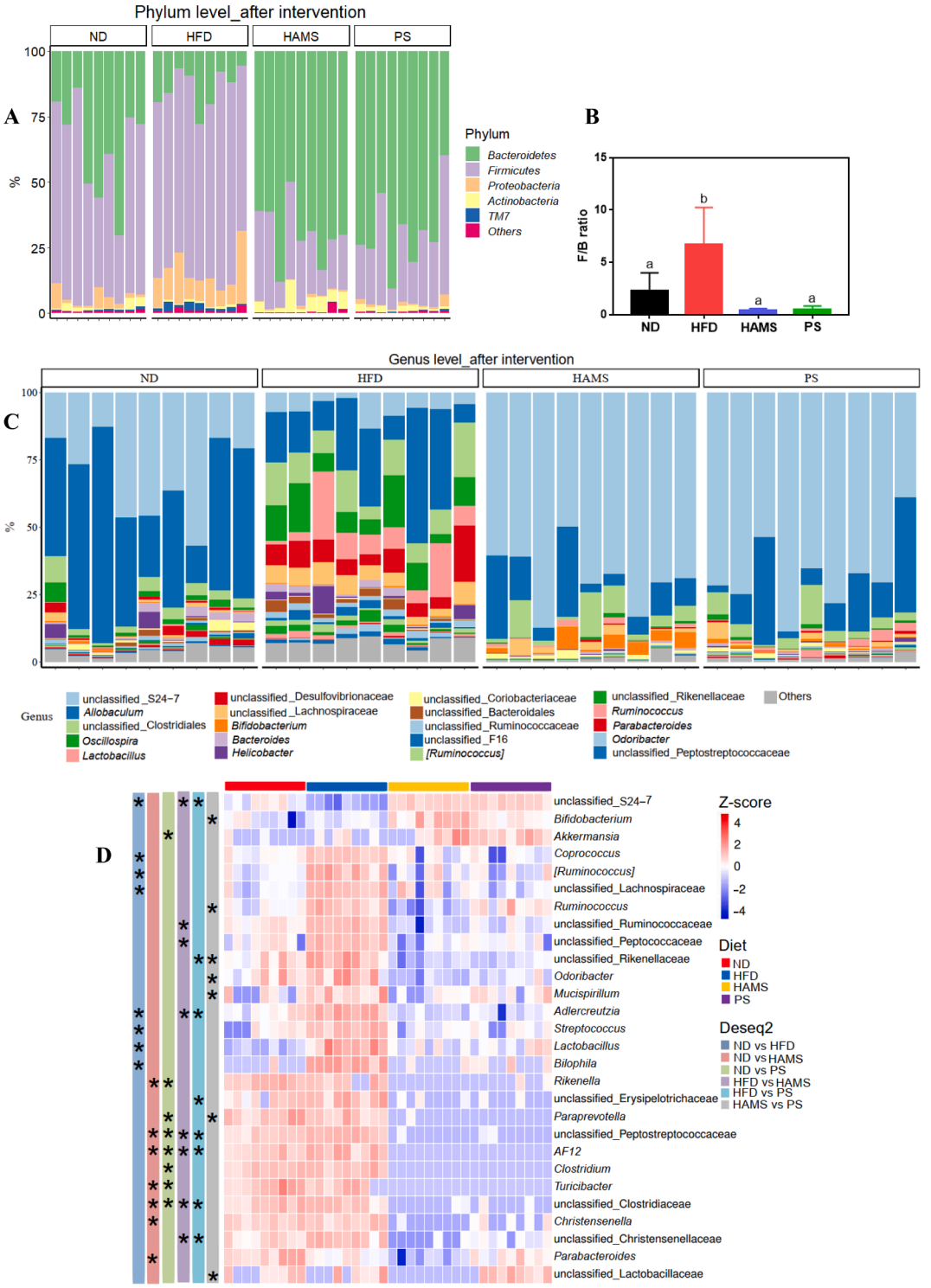

Fig. 5. Phylum level composition (A), F/B ratio change (B), genus level composition (C) and differentially abundant taxa between different experimental groups (D) after 8 weeks intervention. Values marked with different letters in Fig. 5B indicate significant differences ($p < 0.05$). For Fig. 5D, differentially abundant taxa were determined by Deseq2 and labeled with * ($p < 0.05$) in the heatmap between-group comparison, and the color of the heatmap from blue to red represents the abundance of each tax from low to high. (ND, normal diet; HFD, high-fat diet; HAMS, high-amylose maize starch; PS, propionylated high-amylose maize starch). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

one of the most important probiotic groups related to the improvement of metabolic disorders (Arboleya, Watkins, Stanton, & Ross, 2016). The promotion of *Bifidobacterium* in the HAMS group might be linked with higher acetate generation (Fig. 3A). Those results indicated that HAMS and PS supplementation could regulate the GM alteration induced by HFD.

Deseq2 was used to identify the differentially abundant genera of between-group comparison. As shown in Fig. 5D, the relative level of the genera *Coprococcus*, unclassified Lachnospiraceae, *[Ruminococcus]*, *Aldercreutzia*, *Streptococcus*, *Lactobacillus*, and *Biophilia* increased significantly in HFD-fed mice, and the genus unclassified S24-7 showed a remarkable reduction in comparison to the ND group. In comparison

with the ND group, mice in HAMS and PS groups presented a lower abundance of the genera *Rikenella*, *Paraprevotella*, unclassified Peptostreptococcaceae, *AF12*, *Clostridium*, *Turicibacter*, unclassified Clostridiales, *Christensenella*, and *Parabacteroides*. PS remarkably promoted *Akkermansia* compared to the ND group, which is consistent with the previous finding showing that dietary intervention can promote the establishment of *Akkermansia* (Song et al., 2021). Differentially abundant taxa between the model group and starch groups (HFD vs HAMS and HFD vs PS) were also shown in Fig. 5D, and the results of the two comparisons were consistent. HAMS and PS supplementation promoted the abundance of the genus unclassified S24-7 but decreased the genera unclassified Ruminococcaceae, unclassified Peptococcaceae, unclassified Rikenellaceae, *Aldercreutzia*, unclassified Erysipelotrichaceae, unclassified Peptostreptococcaceae, *AF12*, unclassified Clostridiaceae, and unclassified Christensenellaceae in comparison with the mice fed with HFD, which is consistent with the findings observed in Fig. 5C. Notably, PS significantly increased the abundance of *Ruminococcus*, *Mucispirillum*, and unclassified Lactobacillaceae compared with HAMS. *Ruminococcus* has been demonstrated to join the degradation of RS (Hooda et al., 2012), which might be related to the promotion of the propionate concentration (Fig. 3B).

3.6. Correlation between microbial changes and different indexes

Pearson's correlation was adopted to show the relationship between microbial changes at the genus level and obesity-related indicators in mice. As shown in Fig. 6, three genera (*[Ruminococcus]*, unclassified Clostridiales, and unclassified_Lachnospiraceae) were positively correlated with LDL-C level, and the genera unclassified_Rikenellaceae and unclassified_Bacteroidales were positively linked to NEFA concentration. Six genera (*Oscillospira*, unclassified_Desulfovibrionaceae, *[Ruminococcus]*, *Lactobacillus*, unclassified Clostridiales, and unclassified_Lachnospiraceae) were positively correlated with inflammatory index (TNF-α, IL-6, and LPS). It was well shown that *Desulfovibrio* could produce a high level of LPS in the intestinal lumen and result in systemic and targeted inflammation, which is consistent with the inflammatory cytokines observed in Fig. 2L (Sawin et al., 2015). It has also been documented that the increase in the abundances of *Lactobacillus,* Lachnospiraceae, and Rikenellaceae is positively associated with obesity, exerting metabolic disorders, including glycemic and inflammatory effects (Cui, Hu, Li, & Yuan, 2018). The acetate and total SCFA production were positively related to the genus unclassified_S24-7 but negatively linked to *Helicobacter* in our results. Previous research revealed that the genus *Helicobacter* is generally linked with patients with stomach diseases, and a lower proportion of *Helicobacter* is preferred (Shen, Shen, Zhao, Zhu, Yang, Lu, & Wang, 2017). However, GM ferment dietary fibers producing SCFAs that exhibit physiological effects might be linked into a complex network, and the changes in SCFA profile might come from the cooperation of several bacterial species in the community.

4. Conclusion

In summary, this study aimed to evaluate the anti-obesity effects of PS in HFD-fed mice and reveal the potential mechanisms. Our findings confirmed that propionylation of HAMS is a practical way to produce more propionate in HFD-fed mice, and PS supplementation could alleviate obesity and regulate related parameters by altering GM composition. The body weight of HFD-fed mice remarkably decreased after supplementing PS, and improved glucose stability, as well as decreased insulin resistance, were observed. Besides, PS exhibited great serum lipid regulation effects in HFD-fed mice, as the serum lipid indicators including TC, TG, HDL-C, and NEFA were reduced. The inflammatory response generated by HFD was suppressed and the serum levels of TNF-α, LPS, and IL-6 decreased in PS group. It's worth noting that the content of propionate in mice feces fed with PS showed an increasing trend in comparison with HAMS, proving that introduced propionyl groups could be released through propionylation after being metabolized. The V3–V4 16S rRNA high-throughput sequencing results suggested that PS had a great influence on the microbiota composition in mice fed with HFD for consecutive 8 weeks. The relative abundances of the family S24-7 and genus *Ruminococcus* were relatively promoted, and several harmful genera were suppressed, which might improve health by affecting inflammatory response and weight-reducing effect. Our results would provide a valuable reference for the alleviation of obesity and related metabolic dysbiosis by PS to a greater extent than with HAMS alone.

Ethical statements

The experimental design was approved by Institutional Animal Care and Use Committee (Approval NO. SHRM-IACUC-024), and all the experimental procedures followed the Animal Ethical and Welfare Approval.

CRediT authorship contribution statement

Zhuqing Xie: Investigation, Methodology, Formal analysis, Writing – original draft. **Minghua Yao:** Investigation, Methodology, Writing – review & editing, Resources, Funding acquisition. **Josué L. Castro-**

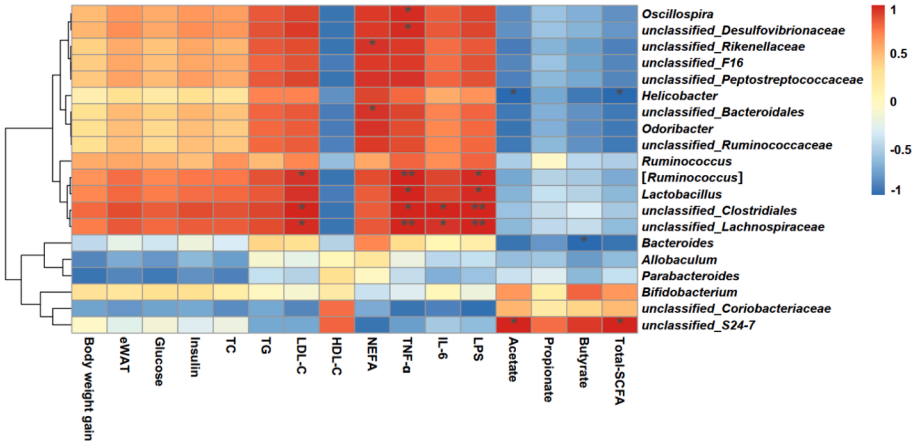

Fig. 6. Pearson's correlation analysis between GM taxa and different indexes. The colors range from blue (negative correlation) to red (positive correlation), and significant correlations are marked by $*p < 0.05$, $**p < 0.01$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Mejía: Writing – review & editing. **Ming Ma:** Investigation, Writing – review & editing. **Yuyan Zhu:** Writing – review & editing. **Xiong Fu:** Writing – review & editing. **Qiang Huang:** Writing – review & editing. **Bin Zhang:** Conceptualization, Supervision, Methodology, Writing – review & editing.

Declaration of Competing Interest

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2023.105447.

References

Anhe, F. F., Roy, D., Pilon, G., Dudonne, S., Matamoros, S., Varin, T. V., … Marette, A. (2015). A polyphenol-rich cranberry extract protects from diet-induced obesity, insulin resistance and intestinal inflammation in association with increased Akkermansia spp. population in the gut microbiota of mice. *Gut, 64*(6), 872–883.

Arboleya, S., Watkins, C., Stanton, C., & Ross, R. P. (2016). Gut bifidobacteria populations in human health and aging. *Frontiers in Microbiology, 7*, 1204.

Backhed, F., Ding, H., Wang, T., Hooper, L. V., Koh, G. Y., Nagy, A., … Gordon, J. I. (2004). The gut microbiota as an environmental factor that regulates fat storage. *Proceedings of the National Academy of Sciences, 101*(44), 15718–15723.

Barter, P. J., Gotto, A. M., Larosa, J. C., Maroni, J., Szarek, M., Grundy, S. M., … Fruchart, J. C. (2007). HDL cholesterol, very low levels of LDL cholesterol, and cardiovascular events. *The New England Journal of Medicine, 357*(13), 1301–1310.

Belobrajdic, D. P., King, R. A., Christophersen, C. T., & Bird, A. R. (2012). Dietary resistant starch dose-dependently reduces adiposity in obesity-prone and obesity-resistant male rats. *Nutrition & Metabolism,* 9(1), 93.

Brouns, F., Kettlitz, B., & Arrigoni, E. (2002). Resistant starch and "the butyrate revolution". *Trends in Food ence & Technology, 13*(8), 251–261.

Callahan, B. J., Mcmurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A., & Holmes, S. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods, 13*(7), 581–583.

Cani, P. D., Amar, J., Iglesias, M. A., Poggi, M., Knauf, C., Bastelica, D., … Chabo, C. (2007). Metabolic Endotoxemia Initiates Obesity and Insulin Resistance. *Diabetes, 56*(7), 1761.

Chambers, E. S., Byrne, C. S., Morrison, D. J., Murphy, K. G., Preston, T., Tedford, C., … Frost, G. S. (2019). Dietary supplementation with inulin-propionate ester or inulin improves insulin sensitivity in adults with overweight and obesity with distinct effects on the gut microbiota, plasma metabolome and systemic inflammatory responses: A randomised cross-over trial. *Gut, 68(8), 1430-1438.*

Chambers, E. S., Viardot, A., Psichas, A., Morrison, D. J., Murphy, K. G., Zac-Varghese, S. E., … Frost, G. (2015). Effects of targeted delivery of propionate to the human colon on appetite regulation, body weight maintenance and adiposity in overweight adults. *Gut, 64*(11), 1744–1754.

Chang, C., Lin, C., Lu, C., Martel, J., Ko, Y., Ojcius, D. M., … Young, J. D. (2015). Ganoderma lucidum reduces obesity in mice by modulating the composition of the gut microbiota. *Nature Communications,* 6(1), 7489–7489.

Cheng, H. H., & Lai, M. H. (2000). Fermentation of resistant rice starch produces propionate reducing serum and hepatic cholesterol in rats. *Journal of Nutrition, 130*(8), 1991–1995.

Clarke, J. M., Topping, D. L., Christophersen, C. T., Bird, A. R., Lange, K., Saunders, I., & Cobiac, L. (2011). Butyrate esterified to starch is released in the human gastrointestinal tract. *The American Journal of Clinical Nutrition, 94*(5), 1276–1283.

Cui, H., Hu, Y., Li, J., & Yuan, K. (2018). Hypoglycemic mechanism of the berberine organic acid salt under the synergistic effect of intestinal flora and oxidative stress. *Oxidative Medicine and Cellular Longevity, 2018*, 8930374.

Den Besten, G., Bleeker, A., Gerding, A., van Eunen, K., Havinga, R., van Dijk, T. H., … Reijngoud, D.-J. (2015). Short-chain fatty acids protect against high-fat diet–induced obesity via a PPARγ-dependent switch from lipogenesis to fat oxidation. *Diabetes, 64*(7), 2398–2408.

Flint, H. J., Scott, K. P., Louis, P., & Duncan, S. H. (2012). The role of the gut microbiota in nutrition and health. *Nature Reviews Gastroenterology & Hepatology, 9*(10), 577–589.

Fuenteszaragoza, E., Sanchezzapata, E., Sendra, E., Sayas, E., Navarro, C., Fernandezlopez, J., & Perezalvarez, J. A. (2011). Resistant starch as prebiotic: A review. *Starch-Stärke, 63*(7), 406–415.

Gibson, G. R. (1999). Dietary modulation of the human gut microflora using the prebiotics oligofructose and inulin. *Journal of Nutrition, 129*(7), 1438–1441.

Harte, A. L., Silva, N. F. D., Creely, S. J., Mcgee, K. C., Billyard, T., Youssefelabd, E. M., … Sharada, H. M. (2010). Elevated endotoxin levels in non-alcoholic fatty liver disease. *Journal of Inflammation,* 7(1), 15.

He, Y., Shi, L., Qi, Y., Wang, Q., Zhao, J., Zhang, H., … Chen, W. J. C. P. (2022). Butylated starch alleviates polycystic ovary syndrome by stimulating the secretion of peptide tyrosine-tyrosine and regulating faecal microbiota. *Carbohydrate Polymers, 287*, 119304.

Hooda, S., Boler, B. M. V., Serao, M. C. R., Brulc, J., Staeger, M. A., Boileau, T. W., … Swanson, K. S. (2012). 454 Pyrosequencing reveals a shift in fecal microbiota of healthy adult men consuming polydextrose or soluble corn fiber. *Journal of Nutrition, 142*(7), 1259–1265.

Hooper, L. V., Littman, D. R., & Macpherson, A. J. (2012). Interactions between the microbiota and the immune system. *Science, 336*(6086), 1268–1273.

Hotamisligil, G. S. (2006). Inflammation and metabolic disorders. *Nature, 444*(7121), 860–867.

Hu, J., Zheng, P., Qiu, J., Chen, Q., Zeng, S., Zhang, Y., … Zheng, B. J. I. J.o. M. S. (2022). High-amylose corn starch regulated gut microbiota and serum bile acids in high-fat diet-induced obese mice. *International Journal of Molecular Sciences, 23*(11), 5905.

Jiao, A., Yu, B., He, J., Yu, J., Zheng, P., Luo, Y., … Wang, H. J. N. (2021). Sodium acetate, propionate, and butyrate reduce fat accumulation in mice via modulating appetite and relevant genes. *Nutrition, 87*, 111198.

Katoh, K., Misawa, K., Kuma, K., & Miyata, T. (2002). MAFFT: A novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research, 30*(14), 3059–3066.

Kimura-Todani, T., Hata, T., Miyata, N., Takakura, S., Yoshihara, K., Zhang, X.-T., … Behavior. (2020). Dietary delivery of acetate to the colon using acylated starches as a carrier exerts anxiolytic effects in mice. Physiology & Behavior, 223, 113004.

Koh, A., De Vadder, F., Kovatchevadatchary, P., & Backhed, F. (2016). From dietary fiber to host physiology: Short-chain fatty acids as key bacterial metabolites. *Cell, 165*(6), 1332–1345.

Koutsari, C. (2006). Free fatty acid metabolism in human obesity. *Journal of Lipid Research, 47*.

Le Chatelier, E., Nielsen, T., Qin, J., Prifti, E., Hildebrand, F., Falony, G., … Pedersen, O. (2013). Richness of human gut microbiome correlates with metabolic markers. *Nature, 500*(7464), 541–546.

Li, L., Cheng, L., Li, Z., Li, C., Hong, Y., & Gu, Z. J. F. (2021). Butyrylated starch protects mice from DSS-induced colitis: Combined effects of butyrate release and prebiotic supply. *Food & Function, 12*(22), 11290–11302.

Li, M., Wang, F., Wang, J., Wang, A., Yao, X., Strappe, P., … Guo, T. J. F. C. (2022). Starch acylation of different short-chain fatty acids and its corresponding influence on gut microbiome and diabetic indexes. *Food Chemistry, 389*, 133089.

Liu, F., Li, P., Chen, M., Luo, Y., Prabhakar, M., Zheng, H., … Zhang, Y. (2017). Fructooligosaccharide (FOS) and galactooligosaccharide (GOS) increase bifidobacterium but reduce butyrate producing bacteria with adverse glycemic metabolism in healthy young population. *Scientific Report, 7*(1), 11789.

Liu, H., Wang, J., He, T., Becker, S., Zhang, G., Li, D., & Ma, X. (2018). Butyrate: A double-edged sword for health? *Advances in Nutrition, 9*(1), 21–29.

Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology, 15*(12), 1–21.

Macfarlane, S., & Macfarlane, G. T. (2003). Regulation of short-chain fatty acid production. *Proceedings of the Nutrition Society, 62*(01), 67–72.

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.journal, 17*(1), 10–12.

Nguyen, T. D., Prykhodko, O., Hallenius, F. F., & Nyman, M. (2017). Effects of monobutyrin and tributyrin on liver lipid profile, caecal microbiota composition and SCFA in high-fat diet-fed rats. *Journal of Nutritional Science, 6*.

Price, M. N., Dehal, P. S., & Arkin, A. P. (2009). FastTree: Computing large minimum evolution trees with profiles instead of a distance matrix. *Molecular Biology and Evolution, 26*(7), 1641–1650.

Qin, J., Li, Y., Cai, Z., Li, S., Zhu, J., Zhang, F., … Shen, D. (2012). A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature, 490*(7418), 55–60.

Qin, H., Xu, H., Yu, L., Yang, L., Lin, C., & Chen, J. (2019). Sesamol intervention ameliorates obesity-associated metabolic disorders by regulating hepatic lipid metabolism in high-fat diet-induced obese mice. *Food & Nutrition Research, 63*.

Ren, D., Hu, Y., Luo, Y., & Yang, X. (2015). Selenium-containing polysaccharides from Ziyang green tea ameliorate high-fructose diet induced insulin resistance and hepatic oxidative stress in mice. *Food & Function, 6*(10), 3342–3350.

Ridaura, V. K., Fa Ith, J. J., Rey, F. E., Cheng, J., Duncan, A. E., Kau, A. L., … Bain, J. R. (2013). Gut microbiota from twins discordant for obesity modulate metabolism in mice. *Science, 341*.

Rizzatti, G., Lopetuso, L. R., Gibiino, G., Binda, C., & Gasbarrini, A. (2017). Proteobacteria: A common factor in human diseases. *BioMed Research International,* 2017, 9351507.

Sawin, E. A., De Wolfe, T. J., Aktas, B., Stroup, B. M., Murali, S. G., Steele, J. L., & Ney, D. M. (2015). Glycomacropeptide is a prebiotic that reduces Desulfovibrio bacteria, increases cecal short-chain fatty acids, and is anti-inflammatory in mice. *American Journal of Physiology-Gastrointestinal and Liver Physiology, 309*(7).

Shang, T., Liu, L., Zhou, J., Zhang, M., Hu, Q., Fang, M., … Gong, Z. (2017). Protective effects of various ratios of DHA/EPA supplementation on high-fat diet-induced liver damage in mice. *Lipids in Health and Disease, 16*(1), 65.

Shang, Q., Song, G., Zhang, M., Shi, J., Xu, C., Hao, J., … Yu, G. (2017). Dietary fucoidan improves metabolic syndrome in association with increased Akkermansia population in the gut microbiota of high-fat diet-fed mice. *Journal of Functional Foods, 28*, 138–146.

Shen, W., Shen, M., Zhao, X., Zhu, H., Yang, Y., Lu, S., … Wang, J. (2017). Anti-obesity effect of capsaicin in mice fed with high-fat diet is associated with an increase in population of the gut bacterium *Akkermansia muciniphila*. *Frontiers in Microbiology,* 8, 272.

Shimotoyodome, A., Suzuki, J., Fukuoka, D., Tokimitsu, I., & Hase, T. (2010). RS4-type resistant starch prevents high-fat diet-induced obesity via increased hepatic fatty acid oxidation and decreased postprandial GIP in C57BL/6J mice. *American Journal of Physiology-Endocrinology and Metabolism, 298*(3).

Si, X., Zhou, Z., Strappe, P., & Blanchard, C. (2017). A comparison of RS4-type resistant starch to RS2-type resistant starch in suppressing oxidative stress in high-fat-diet-induced obese rats. *Food & Function, 8*(1), 232–240.

Song, H., Shen, X., Wang, F., Li, Y., & Zheng, X. (2021). Black current anthocyanins improve lipid metabolism and modulate gut microbiota in high-fat diet-induced obese mice. *Molecular Nutrition & Food Research, 65*(6), e2001090.

Singla, P., Bardoloi, A., & Parkash, A. A. J. W. J. O. D. (2010). Metabolic effects of obesity: A review. *World journal of diabetes, 1*(3), 76.

Song, B., Zhong, Y. Z., Zheng, C. B., Li, F. N., Duan, Y. H., & Deng, J. P. (2019). Propionate alleviates high-fat diet-induced lipid dysmetabolism by modulating gut microbiota in mice. *Journal of Applied Microbiology, 127*(5), 1546–1555.

Sonnenburg, J. L., & Backhed, F. (2016). Diet–microbiota interactions as moderators of human metabolism. *Nature, 535*(7610), 56–64.

Tremaroli, V., & Bäckhed, F. (2012). Functional interactions between the gut microbiota and host metabolism. *Nature, 489*(7415), 242–249.

Turnbaugh, P. J., Ley, R. E., Mahowald, M. A., Magrini, V., Mardis, E. R., & Gordon, J. I. (2006). An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature, 444*(7122), 1027–1031.

Vogeser, M., Konig, D., Frey, I., Predel, H. G., Parhofer, K. G., & Berg, A. (2007). Fasting serum insulin and the homeostasis model of insulin resistance (HOMA-IR) in the monitoring of lifestyle interventions in obese persons. *Clinical Biochemistry, 40*(13), 964–968.

Wang, J., Tang, H., Zhang, C., Zhao, Y., Derrien, M., Rocher, E., … Shen, J. (2015). Modulation of gut microbiota during probiotic-mediated attenuation of metabolic syndrome in high fat diet-fed mice. *ISME Journal, 9*(1), 1–15.

Wong, J. M., De Souza, R., Kendall, C. W., Emam, A., & Jenkins, D. J. (2006). Colonic health: Fermentation and short chain fatty acids. *Journal of Clinical Gastroenterology, 40*(3), 235–243.

Xie, Z., Ding, L., Huang, Q., Fu, X., Liu, F., Dhital, S., & Zhang, B. (2021). In vitro colonic fermentation profiles and microbial responses of propionylated high-amylose maize starch by individual Bacteroides-dominated enterotype inocula. *Food Research International, 144*, 110317.

Xie, Z., Wang, S., Wang, Z., Fu, X., Huang, Q., Yuan, Y., … Zhang, B. (2019). In vitro fecal fermentation of propionylated high-amylose maize starch and its impact on gut microbiota. *Carbohydrate Polymers, 223*, Article 115069.

Zaura, E., Keijser, B. J. F., Huse, S. M., & Crielaard, W. (2009). Defining the healthy "core microbiome" of oral microbial communities. *BMC Microbiology,* 9(1), 259.

Ze, X., Duncan, S. H., Louis, P., & Flint, H. J. (2012). *Ruminococcus bromii* is a keystone species for the degradation of resistant starch in the human colon. *The ISME Journal, 6*(8), 1535–1543.

Zhang, M., Bi, L. F., Fang, J. H., Su, X. L., Da, G. L., Kuwamori, T., & Kagamimori, S. (2004). Beneficial effects of taurine on serum lipids in overweight or obese non-diabetic subjects. *Amino Acids, 26*(3), 267–271.