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Highly Branched RG-I Domain Enrichment are Indispensable for Pectin Mitigating Against High-Fat Diet-Induced Obesity

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Abstract

Obesity is associated with gut microbiome dysbiosis. Our previous research has shown that highly branched RG-I enriched pectin (WRP, 531.5 kDa, 70.44% RG-I, Rha:(Gal+Ara)=20) and its oligosaccharide with less branched (DWRP, 12.1 kDa, 50.29% RG-I, Rha:(Gal+Ara)=6) are potential prebiotics. The present study is conducted to uncover the impact by which the content, molecular size and branch degrees of RG-I on the inhibiting effect of high-fat diet (HFD)-induced obesity. The commercial pectin (CP, 496.2 kDa, 35.77% RG-I, Rha:(Gal+Ara)=6), WRP and DWRP were orally administered to HFD-fed C57BL/6J mice (100mg kg\(^{-1}\) d\(^{-1}\)) to determine their individual effects on obesity. WRP significantly prevented bodyweight gain, insulin resistance, and inflammatory responses in HFD-fed mice. No obvious anti-obesity effect was observed in either CP or DWRP supplementation. Mechanistic study revealed that CP and DWRP could not enhance the diversity of gut microbiota, while WRP treatment positively modulated the gut microbiota of obese mice by increasing the abundance of *Butyrivibrio*, *Roseburia*, *Barnesiella*, *Flavonifractor*, *Acetivibrio*, and *Clostridium* cluster IV. Furthermore, the WRP significantly promoted browning of white adipose tissue in HFD-fed mice, while CP and DWRP did not. WRP can attenuate the HFD-induced obesity by modulation of gut microbiota and lipid metabolism. Highly branched RG-I domain enrichment are essential for pectin mitigating against the HFD-induced obesity.
Introduction

The prevention of obesity is a challenge of global proposition. Evidence has shown that obesity is associated with reduced gut bacterial diversity or altered proportions of bacterial species. Consumption of plant polysaccharides revealed a significant and positive effect on adiposity-induced lipid metabolic disorders and gut microbiota dysbiosis. Among them, pectin and derived-oligosaccharides are good candidate modulators of obesity due to their fermentation potential by various probiotic microorganisms to modulate the obesity due to their complex structure that fermented by various of probiotics.
disaccharide of \([\rightarrow 2]-\alpha-L-Rhap-(1\rightarrow 4)-\alpha-D-GalAp-(1\rightarrow] \) residues with Ara and Gal residues attached to the \( O-4 \) or \( O-3 \) position of \( \alpha-L-Rhap \) backbone units. It is usually removed from commercial pectin preparations by hot acid treatment as it is considered a hinder for pectin gelling. However, accumulating evidence has illustrated that pectin containing RG-I regions from various sources can modulate the composition of obesity-related intestinal microbiota and increase the production of butyrate, which is a dominant protective agent against obesity. Recent findings suggested has been reported that polysaccharide utilization loci (PULs) of gut bacteria activated by different RG-I domains can recruit a myriad of glycoside hydrolases (GHs) and polysaccharides lyases (PLs) for metabolism of RG-I pectin molecules. Thus, RG-I is hypothesized to contribute significantly to bacterial fermentation in the colon leading to favorable changes in gut microbiota composition. Particularly, Khodaei and his colleagues have confirmed that potato RG-I pectin stimulated the growth of \( \textit{Lactobacillus} \) spp. and \( \textit{Bifidobacterium} \) spp. Reduction in these two species are proposed biomarker of gut dysbiosis and found to be decreased under high-fat diet (HFD) conditions. A recent research also indicated the pectin containing RG-I can modulate the composition of obesity-related gut microbiota and upregulate the production of butyrate—a dominant protective agent against obesity. Moreover, apple pectin rich in RG-I strongly promoted \( \textit{Bifidobacterium} \), \( \textit{Bacteroides} \), and \( \textit{Lactobacillus} \) in HFD-fed mice colon, subsequently producing short-chain fatty acids (SCFAs) which limits the secretion of proinflammatory cytokines and alleviated the
obesity caused inflammation. However, up to now the impact of RG-I content in pectin on the HFD-induced obesity is still unclear.

Apart from the RG-I content, another factor affected the bioactivity of the RG-I pectin is molecular size. A recent study on citrus pectic oligosaccharides containing RG-I with a molecular weight of 3~4 kDa have shown hypocholesterolemic effects on HFD-fed mice by modulating specific gut bacterial groups. Besides, the report from Gómez et al. has shown pectic oligosaccharides (5.9~22.8 kDa) containing relative high content RG-I (37.65%) caused better shifts prebiotic properties than high Mw pectin (51.4~82 kDa), confirming the essential of molecular size in functional properties of RG-I pectin. However, most of the research merely focused on the preparation and probiotic effect of pectic oligosaccharides primarily consists of HG, less studies were conducted on RG-I enriched oligosaccharides. Furthermore, a greater proportion of side chains in RG-I pectin was confirmed that can promote the growth of Bacteroides species. The arabino/galacto-oligosaccharides derived from the side chains of RG-I were proved to be more fermented by Bifidobacterium than those from backbone of RG-I. These observations indicate that the neutral sugar branching chains may have a great impact on the gut microbial composition improvement of RG-I enriched oligosaccharides. Since the neutral sugar side chains were degraded significantly during the pectic oligosaccharide preparation process. There is also necessary to take the branching degrees into account for assessment the RG-I oligosaccharides’ beneficial effects in the gut microbiota.
In our previous study, RG-I enriched oligosaccharides (DWRP, 50.29% RG-I content, 12.1 kDa, Rha: (Gal+Ara)=1:6) degraded from citrus canning processing basic water recovered pectin (WRP, 70.44% RG-I, 531.5 kDa, Rha: (Gal+Ara)=1:20) were obtained by metal-free Fenton reaction. DWRP can significantly enriched *Bifidobacterium* and *Lactobacillus* populations, and WRP can improve the *Bacteroides*, *Desulfovibrio* and Ruminococcaceae in mice. The results evidenced both the highly branched RG-I enriched pectin with large Mw and RG-I oligosaccharides with less branching degree can modulate the gut microbiota. However, these effects on gut microbe of obesity mouse is still unclear.

Therefore, the main aim of this study was to uncover the contribution by which RG-I content, molecular size and branching degrees of pectin to the alleviation of HFD-induced obesity and obesity-induce microbiota dysbiosis shaping. The RG-I enriched pectin recovered (WRP), its degradation products (DWRP) and commercial HG dominated pectin (CP, 496.2 kDa, 35.77% RG-I, Rha:(Gal+Ara)=6) were selected in this study. First, investigating the effects of CP, WRP, and DWRP treatment on obesity and obesity-induced metabolic disorders in HFD-fed mice. Then, the contribution of pectin on gut microbiota composition and SCFAs were studied by 16S rRNA and gas chromatography (GC). Moreover, qRT-PCR analysis and immunohistochemistry were also used to analyze the expression of genes and proteins related to brown-like adipocyte formation, respectively.

2. Experimental Section

2.1 Preparation of Pectin
Rhamnogalacturonan-I (RG-I)-enriched pectin (WRP) and its degradation products (DWRP) was recovered from citrus (*Citrus unshiu* Marc.) processing water by sequential acid and alkaline treatments in a previous study. WRP (Mw=531.5 kDa) was recovered from the citrus segments material and was previously reported to have 70.44% of RG-I content with high degree of side chain branching (Rha: (Gal+Ara)=1:20), while its depolymerized fraction DWRP (Mw=12.1 kDa, 56.29% RG-I content) with less side chain branching (Rha: (Gal+Ara)=1:6). Commercial pectin (CP) was bought from Sigma-Aldrich (Shanghai, China) and is mainly composed of HG (52.55%) with an average Mw of 496.2 kDa and low degree of side chain branching (Rha: (Gal+Ara)=1:6) was used in the present study.

### 2.2 Animal Experiments

Fifty C57BL/6J male mice (SPF, 6-8 weeks old, IACUC-20180917-02) were kept under specific-pathogen-free conditions in a 12-hour light/dark cycle with free access to standard chow diet (CD; 12% of energy from fat; Rodent diet, SHOBREE, Jiangsu Synergy Pharmaceutical Biological Engineering Co, Ltd, Nanjing China) and sterile drinking water in a temperature-controlled room (21 °C±2 °C). After an accommodation period of 1 week, the mice were randomly divided into five groups (10 mice/group) and were fed for 8 weeks with CD, high fat diet (HFD, 60% of energy from fat; Research Diets D12492, Opensource Diets, USA), HFD with 100mg/kg CP (HFD-CP), HFD with 100mg/kg WRP (HFD-WRP), HFD with 100mg/kg DWRP (HFD-DWRP). A certain amount of pectin according to the dosages of 100mg/kg was
dissolved in 200 μL distilled water and administrated orally via intragastric gavage once per day. The compositions and energy densities of the diets are listed in Table S1. Body weight and food intake were measured weekly.

The oral glucose tolerance test (OGTT) was performed three days before sacrifice. Overnight-fasted mice were administrated with glucose solution (2 g/kg body weight, 66% solution) by oral gavage, then blood glucose was measured from tail vein blood at 0, 30, 60, 90, 120 min using test strips (ACCU-CHEK Performa) and a portable glucose meter (Roche Diagnostics, Shanghai, China). The blood glucose level before glucose administration represented the fasting glucose concentration. Incremental area under the curve (AUC) was calculated using the trapezoidal method.

Mice were fasting for 12 hours, anaesthetised and sacrificed by cervical dislocation after 9 weeks. Blood and tissues were collected and stored at -80 °C until further use. All procedures were approved by the Institutional Animal Care and Use Committee of Zhejiang University School of Medicine.

2.3 Biochemical analysis and cytokine measurements of serum

Serum was isolated by centrifugation (4 °C, 12,000g, 10 min). Serum total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) were measured using commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer’s instructions. Serum TNF-α, IL-6, LPS, Insulin and adiponectin protein levels were then quantified using commercial ELISA kits (Cloud-clone Crop, USA)
2.4 Liver and epididymal fat histology

The fresh liver, inguinal white adipose tissue (iWAT) and epididymis WAT (eWAT) were isolated and fixed with 4% neutral formalin solution at room temperature for 48 h. After dehydration, eWAT, iWAT and liver were clarified in benzene and embedded in low melting point paraffin wax. Sections (3 nm thick) were cut and stained with haematoxylin and eosin (H&E staining) for light microscopic examination. All of these assays were performed in a blinded manner.

2.5 Immunohistochemistry staining

The paraffin sections of iWAT were subjected to deparaffination, antigen retrieval, endogenous peroxidase activity blocking. Thereafter, slides were incubated with UCP1 primary antibody (Santacruz Biotechnology Inc., USA) and horseradish peroxidase (HRP)-conjugated secondary antibody. After 3, 3-diaminobenzidine (DAB) immunostaining, harris hematoxylin counterstaining, dehydration and coverslipping, the sections were observed in DS-Ri1-U3 Nikon digital imaging system and the positive integral optical density (IOD) of UCP1 in the immunohistochemical pictures was analyzed with the Image J software (National Institute of Health, MD, USA).

2.6 RNA extraction and quantitative real-time PCR analysis

Total RNA was extracted from eWAT, iWAT, and BAT using TRIzol reagent (Invitrogen, CA, USA), which was then used to synthesize cDNA with PrimeScript RT reagent Kit with gDNA Eraser (Takara, Beijing, China). Quantitative real-time PCR was performed using SYBR Green Master Mix (Applied biosystems, CA, USA), 96-
well plates and an Applied Biosystems QuantStudio 3 Real-Time PCR instrument (Life
Technologies, Singapore). qPCR was performed for 40 cycles with following programs:
50 °C for 2 min, 95 °C for 1s, 60 °C for 40s. Relative quantification was done based on
the 2$^{-\Delta\Delta\text{CT}}$ method. Expression was normalized to the housekeeping gene.

2.7 16S rRNA gene analysis

Cecal samples were collected and used for the bacterial 16S rRNA sequencing. Five samples of each group were selected randomly for 16S rRNA analysis. DNA was
extracted from the cecal solid contents of mice by using the E.Z.N.A. ® Stool DNA Kit
(D4015, Omega, Inc., USA) according to manufacturer’s instructions. The total DNA
was eluted in 50 µL of Elution buffer and stored at -80 °C until measurement in the
PCR by LC-Bio Technology Co., Ltd. The V3-V4 region of the prokaryotic (bacterial
and archaeal) small-subunit (16S) rRNA gene was amplified with slightly modified
versions of primers 338F (5'-ACTCCTACGGGAGCAGCAG-3') and 806R (5'-
GGACTACHVGGGTWTCTAAT-3')$^{32}$. The 5' ends of the primers were tagged with
specific barcodes per sample and sequencing universal primers.

The PCR products were purified by AMPure XT beads (Beckman Coulter
Genomics, Danvers, MA, USA) and quantified by Qubit (Invitrogen, USA). The
amplicon pools were prepared for sequencing and the size and quantity of the amplicon
library were assessed on Agilent 2100 Bioanalyzer (Agilent, USA) and with the Library
Quantification Kit for Illumina (Kapa Biosciences, Woburn, MA, USA), respectively.
PhiX Control library (v3) (Illumina) was combined with the amplicon library (expected
at 30%). The libraries were sequenced either on 300PE MiSeq runs and one library was sequenced with both protocols using the standard Illumina sequencing primers, eliminating the need for a third (or fourth) index read.

Samples were sequenced on an Illumina MiSeq platform according to the manufacturer's recommendations, provided by LC-Bio. Paired-end reads was assigned to samples based on their unique barcode and truncated by cutting off the barcode and primer sequence. Paired-end reads were merged using FLASH. Quality filtering on the raw tags were performed under specific filtering conditions to obtain the high-quality clean tags according to the FastQC (V 0.10.1). Chimeric sequences were filtered using Verseach software (v2.3.4). Sequences with $\geq 97\%$ similarity were assigned to the same operational taxonomic units (OTUs) by Verseach (v2.3.4). Representative sequences were chosen for each OTU, and taxonomic data were then assigned to each representative sequence using the RDP (Ribosomal Database Project) classifier. The differences of the dominant species in different groups, multiple sequence alignment was conducted using the PyNAST software to study phylogenetic relationship of different OTUs. OTUs abundance information were normalized using a standard of sequence number corresponding to the sample with the least sequences. Alpha diversity is applied in analyzing complexity of species diversity for a sample through 4 indices, including Chao1, Shannon, Simpson and Observed species. All indices of samples were calculated with QIIME (Version 1.8.0). Beta diversity analysis was used to evaluate differences of samples in species complexity. Beta diversity were calculated by
principle co-ordinates analysis (PCoA) and cluster analysis by QIIME software (Version 1.8.0). The spearman’s rho nonparametric correlations between the gut microbiota and heal-related indexes were determined using R packages (V2.15.3).

Alpha diversity indexes, relative abundance of phyla, principal component analysis (PCA) and linear discriminant analysis (LDA) effect size (LEFse) analysis were assessed.

### 2.8 Ceacal and colonic short-chain fatty acids

Production of SCFA in the ceca and feces of mice was analysed using a 7890A GC (Agilent Technologies, Stockport, UK) using a slightly modified method. Detailed description of these methods is described in a previous study.

### 2.9 Statistical Analysis

Data were expressed as means ± SD. Statistical analysis was performed using GraphPad Prism V.7.04 (GraphPad Software, USA). One-way analysis of variance (ANOVA) for multiple comparisons was conducted, followed by the non-parametric Kruskal–Wallis test with Dunnett’s multiple comparisons test. Significance was set at $p < 0.05$.

### 3. Results

#### 3.1 WRP Prevented Body Weight Gain in HFD-induced Obese Mice

To test the effects of pectin supplementation on body weight, we fed mice with HFD with or without pectin supplementation for 8 weeks. Compared with the CD, mice fed an HFD showed a significant and sustained increase body weight (260%) (Figure 1). Notably, WRP supplementation dramatically prevent the body weight gain caused
by HFD ($p < 0.001$, Figure 1B&C). However, no significant improvement in weight gain was observed in the HFD-CP and HFD-DWRP groups. As shown in Figure 1C–E, HFD significantly induced the weight gain of liver, kidney, inguinal white adipose tissue and epididymal white adipose tissue of mice. In parallel with weight gain caused by HFD (Figure 1E), the weight gain of white adipose tissue and visceral fat of HFD fed mice was prevented decreased observably when intervened by WRP (iWAT, $p < 0.001$; eWAT, $p < 0.01$). Besides, WRP apparently reduced macrosteatosis, hepatocyte ballooning in the livers of obese mice. The liver and fat tissue morphology in HFD-CP and HFD-DWRP groups was the same as in the HFD. The fat tissue morphology failed to maintained in HFD-CP and HFD-DWRP group (Figure 2).

Some reports suggested that fucoidan was reported to affected appetite regulation and subsequent control of body weight. There was no significant difference in food intake between groups (see on Figure S1), indicating that the mitigating effects of WRP on HFD-induced obesity in mice was not through reduced food consumption.
Figure 1. Whole body and tissue weight fed on conventional chow (CD) and high-fat diet (HFD) for 8 weeks. A: Growth curve of mice in different groups; B: The weight gain of mice in each group after 8 weeks of feeding; C–E: shows the weight of liver, kidney, inguinal white adipose tissue, epididymal white adipose tissue and brown adipose tissue of mice, respectively. (Data are presented as means ± SD (n=8 mice per group). *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001; ns, not significant.

3.2 WRP Alleviated HFD-induced Hyperlipidemia, Hyperglycemia, and Inflammatory Responses

As shown in Table 1, the serum level of total triacylglycerol (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-C) and free fatty acids (FFA)
in mice were negatively controlled and the high-density lipoprotein cholesterol (HDL-C), adiponectin and HDL-C/LDL-C content positively controlled by WRP treatment. Pro-inflammatory cytokines have been shown systemic inflammation but also insulin resistance. And bacterial lipopolysaccharide (LPS) is an early factor in the triggering of metabolic diseases induced by obesity. The adipose tissues of obese animals and humans secreted considerable level of pro-inflammatory cytokines and LPS compared with lean individuals. In the present study, supplementation of WRP and DWRP significantly controlled the level of serum LPS and TNF-α in HFD-fed mice. Further, to determine the effect of different pectins on glucose homeostasis and insulin sensitivity, OGTT and fasting insulin test were performed. As shown in Figure 3, HFD treatment impeded the glucose utilization ability as the levels of fasting blood glucose ($p < 0.0001$) and insulin ($p < 0.01$) were significantly increased compared to CD group. Nevertheless, WPR intervened HFD-fed mice exhibited lower glucose levels at all time points up to 120 min after oral glucose challenge and reduced AUC glycemic response. Moreover, WPR and DWPR supplementation lowered the plasma levels of glucose and insulin compared with the HFD group (Figure 3D). Together, WRP effectively alleviated the dyslipidemia of induced by HFD through negatively control of blood lipid and proinflammatory factors content; on the other hand, WRP improved the glucose intolerance and insulin sensitivity.
3.3 WRP Promotes Browning of White Adipocytes in HFD-induced Mice

The average cell size of iWAT in HFD group was significantly larger than CD group (Figure 4A). WRP supplementation reduced the size of iWAT significantly lower than HFD group, but no obvious difference in size of iWAT was observed in both HFD-CP and HFD-DWRP groups. Under some stimulation (cold condition or β-3 adrenergic agonist), the content of mitochondria in WATs increased dramatically and enhances thermogenic properties. This process was called “browning”, and brown-like adipocytes expressed large amounts of uncoupling protein 1 (UCP1) to enhance energy expenditure in WATs [39]. As expected, the immunohistochemistry staining results revealed that the expression level of UCP1 protein in iWAT was remarkably upregulated in HFD-WRP group compared to HFD group (Figure 4A). Consistent with these changes, qPCR analysis confirmed that WRP increased the mRNA level of UCP1 in iWAT (5.65-fold v.s. HFD group, $p < 0.001$) (Figure 4B). In addition, supplementation of WRP also remarkably increased the expression of some...
thermogenic genes and beige adipocyte-selective markers in iWAT, such as PRDM16 ($p < 0.0001$), PGC-1α ($p < 0.01$), ERRα ($p < 0.05$), mtTFA ($p < 0.05$), Tmem26 ($p < 0.05$), CD137 ($p < 0.01$), and Cidea ($p < 0.01$) (**Figure 4B&C**). These findings demonstrated that supplementation of WRP stimulates browning of iWAT and increased adaptive thermogenesis in HFD-fed mice.

**Figure 3.** Effects of CP, WRP and DWRP on the development of insulin resistance in HFD-fed mice. (A) Fasting blood glucose; (B) fasting insulin; (C) blood glucose; and (D) AUC of OGTT are shown. (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$; ns, not significant.)
**Figure 4.** RG-I enriched pectin promoted thermogenesis and browning in iWAT of HFD-fed C57BL/6J mice. (A) Immunohistochemistry for UCP1 protein in iWAT of HFD-fed C57BL/6J mice (magnification 200 times). (B) Thermogenic genes and (C) beige adipocyte-selective markers in iWAT. Relative expression of UCP1, PRDM16, PGC-1α, Nuclear respiratory factor-1 (NRF-1), estrogen-related receptor α (ERRα), Cytochrome c (Cytoc), mitochondrial transcription factor A (mtTFA), Cidea, CD137 and TMEM26 was assessed by qRT-PCR and was Compared to the HFD group. Results are expressed as mean± SD (n ≥ 5). ns, not significant. (*)(*)(**)(***)$p < 0.05$, 0.01, 0.001, Compared to the HFD group based on one-way analysis of variance (ANOVA) with Duncan’s range tests.

### 3.4 WRP Prevents HFD-induced Gut Dysbiosis in Mice

Sequencing analysis of caecal samples from WRP-intervened mice produced an average of 1179±156 observed species compared to the HFD group (960±51) (see on Table S3). Next, α-diversity analysis was performed to determine the community richness and diversity. Significant differences in the richness (Chao estimator) and
diversity index (Shannon and Simpson index) were detected with the HFD and HFD-WRP groups. In addition, both CP and DWRP shown no remarkable effect on the gut microbiota richness. WRP mitigated the phenomenon of extremely reduced gut microbiota species richness and diversity caused by HFD (see on Table S3). The β-diversity analysis based on principal coordinate analysis (PCoA) plots of weighted UniFrac distance and UPGMA showed significant separation was observed between HFD-WRP and HFD groups, and there was also a clear dividing line among the CD, HFD and HFD-WRP groups (Figure 5A&B). Collectively, these results indicated that WRP alleviate intensively the gut microbiota dysbiosis in HFD-fed mice compared to CP and DWRP.

At the phylum level (Figure 5C), HFD markedly increased the Firmicutes/Bacteroidetes (F/B) ratio to 8.78 compared to 1.44 in CD group. WRP supplementation reduced F/B ratio to 1.72 in obese mice which comparable to that of CD group, while HFD-CP and HFD-DWRP groups with a ratio of 8.52 and 6.30 respectively (Figure 5C, Table S4). The relative abundance of Proteobacteria in HFD-fed mice was significantly higher. Nevertheless, Proteobacteria level showed an inconspicuous decline under the intervention of CP, WRP and DWRP. At class level (Figure 5D), the Bacteroidia (Bacteroidetes phylum) comprised 36.27%±14.43%, 15.72%±12.05%, 8.83%±4.73%, 23.49%±5.41% and 12.16%±7.12% of gut microbiota in CD, HFD, HFD-CP, HFD-WRP and HFD-DWRP groups. WRP significantly enhanced the abundance of Bacteroidia in the caecum of HFD-fed mice (p < 0.05, see
on Table S4). In contrast, WRP reversed the increase tendency on the abundance of Clostridia (Firmicutes phylum) caused by HFD. Additionally, predominant bacteria in the caecum of mice were Lachnospiraceae (Firmicutes phylum) and Porphyromonadaceae (Bacteroidetes phylum) at family level (see on Figure S2). On Table S4, the relative abundance of Lachnospiraceae in CD, HFD, and HFD-WRP group were 40.38%±7.23%, 61.98%±8.26%, and 46.42%±7.85%, respectively. On the other side, the relative abundance of Porphyromonadaceae in CD, HFD, and HFD-WRP group were 23.80%±4.64%, 4.14%±1.43%, and 15.92%±6.78%. The results based on the class and family level also explained the decrease of F/B ratio with WRP supplementation. At the genus level, Un-Lachnospiraceae, and Acetatifactor were predominant bacteria (average relative abundance above 10%), the rest consisted of Lachnoclostridium, Acetatifactor, Olsenella, Lactobacillus, Butyrivibrio, Alistipes, Desulfovibrio, and Bacteroides with an average relative abundance below 5% (Figure 5E). HFD feeding significantly increased the obesity-related bacteria in the cecum of mice, such as Acetatifactor and Olsenella, while WRP significantly declined the relative abundance of these bacteria (Figure 4E, Table S4). In addition, the obesity negative-related bacteria (Barnesiella and Butyrivibrio) were enriched under the intervention of WRP.

Figure 6 shown pathogenic taxa Firmicutes, Clostridia, Clostridiales, Lachnospiraceae, Acetatifactor, and Desulfovibrio were higher in HFD group compared to CD group, while CD group enriched the beneficial phylotypes Bacteroidates,
Bifidobacterium, Butyrivibrio, Porphyromonadaceae, Alloprevotella, Anaerostipes, and Anaerotaenia were enriched in CD group. After treating with CP, there were only 2 significant different OUT units. Notably, 19 remarked different OUT units were observed in HFD-WRP group (LDA score threshold > 3 were listed). Figure 6C presented the dominate bacteria taxon in the caecum of HFD-fed mice intervened by WRP. Specifically, WRP supplementation significantly increase the abundance of Bacteroidia, Bacteroidales, Barnesiella, Butyrivibrio, Roseburia, Prophyromonadaceae, Flavonifractor, Acetivibrio, and Clostridium cluster IV, while HFD-DWRP group enriched the obesity-related Butyrivibrio and Mucispirillum genus. The gut dysbiosis induced by HFD was effectively modulated after treatment of WRP, which was due to the more complex RG-I domain stimulating the growth of intestinal microorganisms.
Figure 5. Structural composition of gut microbiota. (A) PCoA plot of cecal microbiota in HFD-fed mice based on weighted UniFrac metric. (B) UPGMA analysis of cecal microbiota of HFD-fed mice. Cecal microbiota in CD, HFD, HFD-CP, HFD-WRP and HFD-DWRP groups at phylum (C), class (D) and genus (E) level
Figure 6. LEfSe comparison of gut microbiota. (A) The LDA score between CD and HFD groups, with LDA score > ±3.6; (B) The LDA score between CD, HFD-WRP and HFD-CP groups, with LDA score > 3.3; (C) The LDA score between CD, HFD-WRP and HFD-DWRP groups, with LDA score > 3.3

3.5 WRP Treatment Promotes Generation of SCFAs in HFD-induce Mice

The gut microbiota was modulated by WRP supplementations in HFD-fed mice, thus, we investigated the effect of WRP on SCFAs-microbial metabolites. HFD significantly inhibited total SCFAs, acetate and propionate in obese mice caecum but had no effect on butyrate generation (Figure 7). CP, WRP and DWRP treatment remarkably prevented the suppression of ceecal total SCFAs, acetate and valerate. The fermentation of galacturonic acid and xylose were reported to promote the production
of acetate and butyrate, while the production of propionate being promoted by arabinose and glucose fermentation. The increase production of propionate in HFD-WRP and HFD-DWRP may be due to the higher content of arabinose and rhamnose in RG-I pectin compared to CP. Compared with CD group, HFD significantly decreased the acetate, butyrate and valerate levels in the colon feces except propionate. Notably, only HFD-WRP group contained significantly higher concentrations of all kinds of SCFAs in obese mice colon compared to HFD group. However, no significant improvement of these SCFAs was observed in both HFD-CP and HFD-DWRP groups.

**Figure 7.** The concentration (μmol/g) of acetic, propionate, butyrate, I-butyrate, valerate, and I-valerate in the cecal contents (A) and colon feces (B) of pectin treated group and chow diet group.

### 3.6 Correlation of gut microbiota with obesity-related indexes
The Spearman’s correlation analysis was performed to clarify the correlation among the microbiota and obesity-related indexes (Figure 8). *Pseudoflavonifrator, Desulfovibrionaceae, Acetatifactor, Lachnoclostridium*, and *Robinsoniella* were strongly positively correlated with body weight, epididymal fat weight, TG, TC, and insulin, whereas they were strongly negatively correlated with the gut tissue index ($p < 0.01$ or $p < 0.05$), suggesting that they may be the most significant genera for the development of obesity. In addition, the Simpson index, *Bifidobacterium, Prorphyromonadaceae, Acetanaerobacterium, Alloprevotella, Prevotella, Acetivibrio, Acetatifactor, Prevotella, Gemella, Eubacterium, Ruminococcus, Butyrivibrio*, and *Clostridium_sensu_stricto* were highly positively correlated with the gut tissue index, while they were highly negatively correlated with body weight, epididymal fat weight, TG, total cholesterol and LPS ($p < 0.01$ or $p < 0.05$), indicating that they may play the most important role in the obesity.
Figure 8. Heatmap of Sperman’s correlation between cecal microbiota and health-related indexes.

The indexes of $\alpha$ diversity including observed species; the Shannon, Simpson, and Chao1 indexes;
The colors range from blue (negative correlation) to red (positive correlation). Significant correlations are marked by *$p < 0.05$ and **$p < 0.01$

4. Discussion

4.1 RG-I Content, Molecular Weight and Branching Chains are Key Factors of Pectin to Prevent Obesity

In our previous research, we found WRP and DWRP had a positive modulation of gut prebiotic microbiota, which stimulated our interests in the potential anti-obesogenic effects of RG-I enriched pectin and its oligosaccharides $^{29, 31}$. However, the gut...
microbiota of mice changed dramatically under the condition of HFD feeding, and in turn might affect the ferment of pectin in the microbiota. In the present study, we first found that WRP with a high molecular size (531.5 kDa) and RG-I content (70.44%) mitigated against the HFD-induced body weight gain, adipocyte hypertrophy, fatty liver, and hyperlipidemia without suppressing the food intake. On the contrary, no significant decrease in body weight gain was discovered after oral administration of CP (rich in HG domain) and DWRP (depolymerized from WRP). This indicates that the content and molecular structure of RG-I enriched pectin are important for its biological function. Bacterial species characteristic of lean hosts, such as Butyrivibrio, Roseburia, Prophyromonadaceae, Barnesiella, Flavonifractor, Acetivibrio, and Clostridium cluster IV were largely enriched in HFD-WRP group. The anti-obesogenic effects would be explained by the production of SCFAs which could regulate energy homeostasis. Meanwhile, CP and DWRP supplementation barely motivated the enrichment of beneficial bacteria or the diversity of gut microbiota in obese mice.

Notably, data published by our group exactly had shown convincingly that CP intervention has not potentially beneficial effect on the gut microbiota of the chow diet feeding mice. We tentatively linked the reason to easy accessibility of pectic backbone (due to less RG-I branches) to microbial degrading enzymes and single monosaccharide composition, which activated much less microbiota species into the fermentation of CP. Multiple species from Bacteroidetes phylum harbored very broad potential to utilize the RG-I domain from pectin. As described by Li el at., RG-I
pectin purified from *Fructus Mori* promoted the growth of *Bacteroides thetaiotaomicron*, a dominant gut bacteria strain shown to be beneficial to the intestinal mucosa of human. The intricate RG-I pectin comprised complex side-chain components (arabinan, galactan, and arabinogalactan) contributed significantly to the gut microbiota fermentation and favourable changes in microbiota composition. Therefore, the high RG-I domain content contributed significantly to the capacity of pectin in HFD-induced obesity modulation.

As highlighted by others, pectic oligosaccharides better promoted growth of beneficial bacteria and had the potential to reduce metabolic conditions such as obesity. In here, WRP supplementation mitigated the gut microbiota of HFD-fed mice dramatically. Paradoxically, no modulation effect of gut microbiota was observed in HFD-DWRP group (Figure 6). Specifically, many reports have confirmed that a greater proportion of Ara and Gal in RG-I pectin promoted the growth of *Bacteroides* species. Compared to WRP (Rha: (Gal+Ara) ratio of around 1:20), DWRP (Rha: (Gal+Ara)=1:6) had much less degree of Ara and Glu side chains, so large Mw WRP might better regulate the gut microbiota of obese mice. It’s also worth noting that the fermentation effect of DWRP in obese mice gut may be different from pectic oligosaccharides which mainly composed of GalA studied in most articles. Concretely, *Barnesiella, Clostridium* cluster IV which negatively correlated to obesity were restored in HFD-WRP group, while HFD-DWRP group enriched the *Mucispirillum* genus (Figure 6). Since the increased levels of *Mucispirillum* was closely correlated...
with obesity, which suggested that no anti-obesity effect was observed in HFD-DWRP group may due to the *Mucispirillum* induced gut dysbiosis. This could also give a clue as to why DWRP could not inhibit the HFD-induced body weight gain: the low branching degree structural RG-I domain and low Mw of DWRP led a halfway rectification of gut dysbiosis in obese mice.

Our results showed that highly branched RG-I enriched pectin with large molecular size is a strong candidate in prevent of HFD-induced obesity.

### 4.2 WRP alleviates the HFD-induced Obesity via regulation gut dysbiosis and adipocytes thermogenesis

In this study, HFD caused an increase in the Firmicutes/Bacteroidetes ratio (F/B), which was reported to be related to obesity. Whereas WRP supplementation reduced the F/B ratio remarkably (Table 1, Figure 5), since the capacity to utilize RG-I was widespread among colonic Bacteroidetes but relatively uncommon among Firmicutes. Furthermore, we observed changes in the level of Lachospiraceae, *Acetatifactor*, *Desulfovibrio*, *Olsenella*, *Alistipes*, *Mucispirillum* were higher in HFD group. Bacteroidales, *Butyrivibrio*, *Roseburia*, Prophyromonadaceae, *Barnesiella*, *Flavonifractor*, *Acetivibrio*, and *Clostridium* cluster IV, which were restored with WRP supplementation. With regard to the Firmicutes phylum, HFD produced an increase in Lachnospiraceae, which is a potent Firmicutes family related to the regulation of immune and obesity. Here, we observed changes in the level of Lachnospiraceae was significant higher in HFD group, but HFD-WRP group presented lower level of Lachnospiraceae. At a genus level, WRP supplementation presented a decrease in
Acetatificactor which was significantly increased by HFD. As described by several authors, Acetatificactor was found abundant in the intestine of obese mouse and has been related to arthritis progression in mice \(^1\). Specially, key members of luminal Clostridium cluster IV were known to be butyrate producers, which are closely associated with both obesity and weight loss clearly \(^{50-51}\). The presence Clostridia species from clusters IV of has consistently been shown leads to the increase of regulatory T cells which secrete the anti-inflammatory cytokine IL-10 \(^{52}\). In line with this, WRP supplementation led to enhanced Clostridium cluster IV enrichment, which was positively related to butyrate in caecum and negatively related to TNF-\(\alpha\) \((p < 0.05)\) and LPS \((p < 0.05)\) (Figure 8).

The most remarkable differences caused by WRP was in the Bacteroidetes phylum. Consistently, we also found that WRP led to enhanced Bacteroidales abundance, which was positively related to butyrate in caecum and gut index \((p < 0.05)\), and negatively correlated with the body weight gain, TC \((p < 0.01)\), insulin \((p < 0.05)\), and LPS levels \((p < 0.01)\). Furthermore, HFD caused by a decrease in the abundance of the Porphyromonadaceae family and, in particular, the Barnesiella genus which has been proved to positively impact on reducing the pro-inflammatory milieu in the gut \(^{53}\). Chiu et al. has reported higher levels of Barnesiella in lean individuals compared to obese patients \(^{54}\). These variables were consistently confirmed by us, WRP elevated the levels of Barnesiella and Porphyromonadaceae which were negatively correlated with body weight and TC \((p < 0.05)\), positively correlated with gut index \((p < 0.05)\). In contrast,
an increase in *Alistipes* genus of HFD-fed mice was observed in this study. Gram-negative *Alistipes* was the foremost noteworthy taxon related to obesity and was found to be expanded beneath HFD conditions.

In addition, we have also detected an enrichment of *Roseburia*. The *Roseburia* (Actinobacteria phylum) was a typical SCFAs-producing bacterium within the intestine that can anaerobically degrade polysaccharides such as arabinoxylan into SCFAs, which protected against HFD-induced obesity by activating the release of gut hormone and enhancing intestinal barrier function. Several reports pointed out that the content of acetic acid and propionic acid were associated with an increased abundance of *Olsenella* in pectin or fructo-oligosaccharides-fed mice. However, as highlighted by some researchers, *Olsenella* could be a target bacterial flora for obesity since it was enriched considerably in HFD-fed mice. Based on our findings, the abundance of *Olsenella* was enriched by HFD while it reduced to the level close to the CD group after WRP supplementation. Our results exhibit high level of *Acetivibrio* in HFD-WRP group and negatively correlated to body weight ($p < 0.01$), TC ($p < 0.05$).

Data published by Yang et al. shown maize-derived feruloylated polysaccharides significantly increased *Acetivibrio* and controlled the weight gain induced by HFD. One hypothesis is that multiple glycoside hydrolase secreted from *Acetivibrio* hydrolyzed arabinose side chain of WRP and stimulated SCFAs production, as cecal butyrate level positively correlated with *Acetivibrio*. Previous research has illustrated that HFD triggered inflammatory responses,
defecting gut barrier integrity, and increasing the cytokines. In our model, WRP treatment significantly decreased the level of serum LPS and TNF-α in HFD-fed mice. Specifically, the LPS-producing microbiota such as Desulfovibrio and Mucispirillum became abundant after HFD feeding. Notably, Mucispirillum has been described as a mucus-associated bacterium that bursts during inflammation in HFD-induced obese mouse model. Desulfovibrio is one kind of genus belonging to Desulfovibrionaceae and Proteobacteria, positively related to obesity-induced inflammation. Moreover, the correlation analysis further verified that the significant correlation between Desulfovibrio and these obesity-related indexes (Figure 8).

On the other hand, butyrate and other short chain fatty acids are known to inhibit fat accumulation and adipocyte dysfunction. Here, we found that WRP intervention significantly enriched the fecal and ceecal butyrate. Moreover, the correlation analysis verified the positive association of microbiota diversity with ceecal butyrate. Several studies reported that Barnesiella, Roseburia, Clostridium cluster IV, and Butyrivibrio which are highly associated with the production of SCFAs, especially the synthesis of butyrate. Moreover, suppression HFD-induced body weight gain of WRP could related to SCFAs secreted by relevant microbes (Figure S2). In line with this, it has been revealed that WRP treatment enriched these microbes and prevented the HFD-induced gut dysbiosis in mice.

Interestingly, a crucial analysis revealed that SCFAs can stimulate the white adipose tissue browning. Consistently, we also found WRP supplementation reduced
the white adipose tissue’s weight, upregulated UCP1 expression, a specific protein uncouples respiration from ATP synthesis and generates heat (Figure 4). Moreover, WRP showed potential in fat browning by activating PGC-1α, a master regulator of mitochondrial biogenesis. Compared with the HFD group, gene expressions of the beige adipocyte-selective markers including UCP1, TMEM26, CD137, and Cidea were markedly increased in HFD-WRP group (Figure 4B&C). The above results indicated that the supplementation of WRP triggered the browning of iWAT in HFD-fed mice.

Overall, we found that WRP can attenuate HFD-induced obesity, inflammation and gut dysbiosis through combined effects of the white adipose browning and gut microbiota modulation, while the CP (HG dominated pectin) and DWRP (less branched RG-I oligosaccharide) have only limited effect on resistance to HFD-induced obesity. Our findings convinced that highly branched RG-I domain enrichment are essential for pectin mitigating against the HFD-induced obesity.

**Abbreviations:** BAT: Brown adipose tissue; CD: Normal chow diet; DWRP: depolymerized fraction of RG-I enriched pectin from citrus segment membrane; HDL: High density lipoprotein; HFD: High fat diet; OTU: Operational taxonomic units; OGTT: Oral glucose tolerance test; PGC-1α: Peroxisome proliferators-activated receptor-γ coactivator 1-alpha; RG-I: Rhamnogalacturonan-I; LDL: Low density lipoprotein; LEfSe: Linear discriminant analysis effect size; TG: Total triacylglycerol; TC: Total cholesterol; TNF-α: Tumor necrosis factor-α; UCP1: Uncoupling protein 1;
WAT: White adipose tissue; WRP: RG-I enriched pectin from citrus segment membrane.

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Ethics statement
The animal study was reviewed and approved by Zhejiang Chinese Medicine University IACUC-20180917-02.

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Author contributions
S.G.C. conceived the study; K. Z. wrote the manuscript; G.Z.M. designed experiments, performed the animal studies and statistical analysis; D.M.W. contributed to sample preparation; C.X.Y. helped to perform the animal studies; H. X. helped to revise the manuscript; X.Q.Y., R. J. L. and C.O. interpreted the data; S.G.C. critically revised the manuscript. All authors read and approved the final manuscript.

Supporting information
The following are available online. Table S1: The compositions and energy densities of the diets; Table S2: The specific formula of the high-fat diet; Table S3: Diversity and richness of cecal microbiota in CP, WRP and DWRP supplements alter the diversity; Table S4: The abundance of key phylotypes of gut microbiota in HFD-
fed C57BL/6J mice; Table S5: PCR primers used in this study; Figure S1: Weekly food intake (A) and average food intake (B) of mice in response to dietary CP, WRP and DWRP; Figure S2: LEfSe comparison of cecal microbiota. LDA score of cecal microbiota between the CD, HFD, HFD-CP, HFD-WRP and HFD-DWRP groups.

References


9. Palou, M.; Sanchez, J.; Garcia-Carrizo, F.; Palou, A.; Pico, C., Pectin...


46. Rosshart, S. P.; Vassallo, B. G.; Angeletti, D.; Hutchinson, D. S.; Morgan, A.
786  P.; Takeda, K.; Hickman, H. D.; McCulloch, J. A.; Badger, J. H.; Ajami, N. J.;
787 Trinchieri, G.; Pardo-Manuel de Villena, F.; Yewdell, J. W.; Rehermann, B., Wild
788 Mouse Gut Microbiota Promotes Host Fitness and Improves Disease Resistance. *Cell*
790
47. Zhao, Q.; Elson, C. O., Adaptive immune education by gut microbiota antigens.
792
793 S., A fucoidan from sea cucumber Pearsonothuria graeffei with well-repeated structure
794 alleviates gut microbiota dysbiosis and metabolic syndromes in HFD-fed mice. *Food
795 Funct* 2018, 9 (10), 5371-5380.
796
49. Pfeiffer, N.; Desmarchelier, C.; Blaut, M.; Daniel, H.; Haller, D.; Clavel, T.,
797 Acetatifactor muris gen. nov., sp. nov., a novel bacterium isolated from the intestine of
798 an obese mouse. *Archives of Microbiology* 2012, 194 (11), 901-907.
799
800 Monti, D.; Satokari, R.; Franceschi, C.; Brigidi, P.; De Vos, W., Through Ageing, and
801 Beyond: Gut Microbiota and Inflammatory Status in Seniors and Centenarians. *PLOS
802 ONE* 2010, 5 (5), e10667.
803
805 Olde Damink, S. W.; Boeckxhoten, M. V.; Smidt, H.; Zoetendal, E. G.; Dejong, C. H.;
806 Blaak, E. E., Effects of Gut Microbiota Manipulation by Antibiotics on Host
807 Metabolism in Obese Humans: A Randomized Double-Blind Placebo-Controlled Trial.
809
811
812 Garcia, E.; Tapia, G.; Comas, I.; Vilaplana, C.; Cardona, P. J., Influence of Gut
813 Microbiota on Progression to Tuberculosis Generated by High Fat Diet-Induced
815
54. Chiu, C.-M.; Huang, W.-C.; Weng, S.-L.; Tseng, H.-C.; Liang, C.; Wang, W.-
817 analysis of the association between gut flora and obesity through high-throughput
819 906168.
820
55. Dalby, M. J.; Ross, A. W.; Walker, A. W.; Morgan, P. J., Dietary Uncoupling
821 of Gut Microbiota and Energy Harvesting from Obesity and Glucose Tolerance in Mice.
822 *Cell Rep* 2017, 21 (6), 1521-1533.
823
824 Fan, G.; Teng, C., Xylan-oligosaccharides ameliorate high fat diet induced obesity and
825 glucose intolerance and modulate plasma lipid profile and gut microbiota in mice.
826 *Journal of Functional Foods* 2020, 64.
827
57. Li, W.; Zhang, K.; Yang, H., Pectin Alleviates High Fat (Lard) Diet-Induced


### Table 1 Effects of RG-I pectin on serum biochemical parameters in C57BL/6 Mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>CD</th>
<th>HFD</th>
<th>HFD-CP</th>
<th>HFD-WRP</th>
<th>HFD-DWRP</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC (mmol/L)</td>
<td>4.99±0.65</td>
<td>9.66±0.75</td>
<td>8.58±0.76</td>
<td>5.28±0.42</td>
<td>6.43±0.56</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>1.15±0.27</td>
<td>1.49±0.11</td>
<td>1.41±0.21</td>
<td>1.26±0.30</td>
<td>1.27±0.15</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.52±0.27</td>
<td>2.10±0.37</td>
<td>2.12±1.02</td>
<td>2.65±0.62</td>
<td>2.81±1.47</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>1.99±0.45</td>
<td>4.00±1.78</td>
<td>2.94±0.39</td>
<td>2.01±0.54</td>
<td>2.29±0.77</td>
</tr>
<tr>
<td>FFA (mmol/L)</td>
<td>1.27±0.43</td>
<td>1.72±0.12</td>
<td>1.57±0.47</td>
<td>1.33±0.33</td>
<td>1.20±0.44</td>
</tr>
<tr>
<td>Insulin (ng/mL)</td>
<td>0.38±0.12</td>
<td>0.76±0.27</td>
<td>0.61±0.19</td>
<td>0.50±0.20</td>
<td>0.56±0.15</td>
</tr>
<tr>
<td>LPS (EU/mL)</td>
<td>0.44±0.02</td>
<td>0.54±0.05</td>
<td>0.54±0.14</td>
<td>0.41±0.07</td>
<td>0.39±0.07</td>
</tr>
<tr>
<td>TNF-α (ng/mL)</td>
<td>1.29±0.55</td>
<td>1.58±0.35</td>
<td>0.91±0.39</td>
<td>0.68±0.19</td>
<td>0.52±0.12</td>
</tr>
<tr>
<td>Adiponectin (ug/mL)</td>
<td>51.03±12.95</td>
<td>27.14±3.62</td>
<td>31.52±3.54</td>
<td>47.77±9.45</td>
<td>38.07±4.36</td>
</tr>
<tr>
<td>TG/HDL-C</td>
<td>0.74±0.24</td>
<td>0.75±0.14</td>
<td>1.33±1.25</td>
<td>0.48±0.05</td>
<td>0.55±0.19</td>
</tr>
<tr>
<td>HDL-C/LDL-C</td>
<td>0.78±0.11</td>
<td>0.57±0.22</td>
<td>0.63±0.33</td>
<td>1.38±0.43</td>
<td>1.54±1.19</td>
</tr>
</tbody>
</table>

The means with different superscript letters (a, b, and c) represent statistically significant results (p < 0.05) based on one-way analysis of variance (ANOVA) with Duncan’s range tests, whereas means labeled with the same superscript correspond to results that show no statistically significant differences.
Figure 2. Histological assessment of livers (A) and epididymal white adipose tissue (B) in HFD-induced obesity mice (H&E stain, 200× magnification)
Figure 4. RG-I enriched pectin promoted thermogenesis and browning in iWAT of HFD-fed C57BL/6J mice. (A) Immunohistochemistry for UCP1 protein in iWAT of HFD-fed C57BL/6J mice (magnification 200 times). (B) Thermogenic genes and (C) beige adipocyte-selective markers in iWAT. Relative expression of UCP1, PRDM16, PGC-1α, Nuclear respiratory factor-1 (NRF-1), estrogen-related receptor α (ERRα), Cytochrome c (Cytc), mitochondrial transcription factor A (mtTFA), Cidea, CD137 and TMEM26 was assessed by qRT-PCR and was Compared to the HFD group. Results are expressed as mean± SD (n≥5). ns, not significant; (**)(***)p<0.05, 0.01, 0.001, Compared to the HFD group based on one-way analysis of variance (ANOVA) with Duncan’s range tests.
Figure 5. Structural composition of gut microbiota. (A) PCoA plot of cecal microbiota in HFD-fed mice based on weighted UniFrac metric. (B) UPGMA analysis of cecal microbiota of HFD-fed mice. Cecal microbiota in CD, HFD, HFD-CP, HFD-WRP and HFD-DWRP groups at phylum (C), class (D) and genus (E) level.
Figure 6. LefSe comparison of gut microbiota. (A) The LDA score between CD and HFD groups, with LDA score > ±3.6; (B) The LDA score between CD, HFD-WRP and HFD-CP groups, with LDA score > 3.3; (C) The LDA score between CD, HFD-WRP and HFD-DWRP groups, with LDA score > 3.3.
Figure 7. Heatmap of Sperman’s correlation between cecal microbiota and health-related indexes. The indexes of α diversity including observed species; the Shannon, Simpson, and Chao1 indexes; The colors range from blue (negative correlation) to red (positive correlation). Significant correlations are marked by *p < 0.05 and **p < 0.01