Diabetes, Metabolic Syndrome and Obesity Dovepress

Open Access Full Text Article ORIGINAL RESEARCH

Hesperidin and Fecal Microbiota Transplantation Modulate the Composition of the Gut Microbiota and Reduce Obesity in High Fat Diet Mice

Ting Liu[1,*], Chao Lei[1,*], Qinhong Huang[2,*], Weiqi Song[3], Chen Li[1], Ning Sun[4,5], Zhihua Liu[6]

[1]Key Laboratory of Biological Targeting Diagnosis, Therapy and Rehabilitation of Guangdong Higher Education Institutes, the Fifth Affiliated Hospital of Guangzhou Medical University, Guangzhou, Guangdong, 510799, People's Republic of China; [2]The First Clinical College, Guangzhou Medical University, Guangzhou, 511400, People's Republic of China; [3]Department of Public Health, Guangzhou Medical University, Guangzhou, People's Republic of China; [4]Guangzhou 11th People's Hospital, Guangzhou Cadre and Talent Health Management Center, Guangzhou, 510530, People's Republic of China; [5]The State Key Laboratory of Chemical Biology and Drug Discovery, Department of Applied Biology and Chemical Technology, the Hong Kong Polytechnic University, 999077, Hong Kong, China; [6]Department of Anorectal Surgery, the Tenth Affiliated Hospital of Southern Medical University (Dongguan People's Hospital), Dongguan, People's Republic of China

*These authors contributed equally to this work

Correspondence: Zhihua Liu; Ning Sun, Email liuzhihua83@hotmail.com; ning.sun@connect.polyu.hk

Introduction: Obesity, which is associated with gut microbiota dysbiosis, low-grade chronic inflammation and intestinal barrier dysfunction, can cause a variety of chronic metabolic diseases. Phytochemical flavonoids have a variety of biological activities, among which there may be safe and effective anti-obesity solutions.

Methods: We tested a plant-derived flavonoid hesperidin and fecal microbiota transplantation (FMT) to alleviate diet-induced obesity. High-fat diet (HFD)-fed mice were treated with hesperidin (100 and 200 mg/kg BW) and FMT.

Results: Results indicated that hesperidin had the effects of reducing obesity as indicated by reduction of body weight, fat accumulation and blood lipids, reducing inflammation as indicated by reduction of pro-inflammation factors including TNFα, IL-6, IL-1βand iNOS, and improving gut integrity as indicated by increasing colon length, reducing plasma gut permeability indicators iFABP and LBP, increased mRNA expression of mucus protein Muc2, tight junction p Claudin 2, Occludin and ZO-1 in the HFD-fed mice. The anti-obesity effects of hesperidin treatment have a dose-dependent manner. In addition, 16S rRNA-based gut microbiota analysis revealed that hesperidin selectively promoted the growth of *Lactobacillus salivarius, Staphylococcus sciuri and Desulfovibrio C21_c20* while inhibiting *Bifidobacterium pseudolongum, Mucispirillum schaedleri, Helicobacter ganmani* and *Helicobacter hepaticus* in the HFD-fed mice. Horizontal feces transfer from the normal diet (ND)-fed mice to the HFD-fed mice conferred anti-obesity effects and transmitted some of the HFD-modulated microbes.

Conclusion: We concluded that hesperidin and FMT both affect the reduction of body weight and improve HFD-related disorders in the HFD-fed mice possibly through modulating the composition of the gut microbiota.

Keywords: hesperidin, high-fat diet, obesity, gut microbiota, fecal microbiota transplantation

Introduction

Obesity is considered to be a disease condition associated with high risk of numerous health problems. The increasing prevalence of obesity has been becoming a major threat to public health and administration of obesity is a main challenge for modern societies.[1] Unfit diet especially high-fat diet (HFD), inadequate exercise, neuronal and hormonal factors, genetic and epigenetic mechanisms all contribute to obesity development, and among them, HFD is the major cause.[2,3] Nature products like the flavonoid have been proving to be novel measures to manage chronic metabolic disease like obesity.[4,5]

HFD resulted in obesity, which is characterized by fat mass accumulation, chronic subclinical inflammation, imbalanced gut microbiota and increased intestinal permeability.[6,7] Gut microbiota was involved in the molecular crosstalk between the HFD, host energy metabolism, host immune system and the gut barrier function in the context of obesity and metabolic disease.[8,9] HFD

Received: 17 June 2024
Accepted: 30 September 2024
Published: 7 October 2024

modulates microbiota and induces alteration in intestinal barrier leading to an increase in absorption and circulating levels of lipopolysaccharide (LPS) and a reduction in short-chain fatty acids (SCFAs) such as acetate, propionate, and butyrate. LPS binds and activates Toll-like receptor 4 (TLR4), and the subsequent pathway finally leads to subclinical inflammation, insulin resistance and an increase in adipose mass.[10,11] SCFAs act via G protein coupled receptors (GPCR)—Gpr41, Gpr43, Gpr109a and Olfr78—to modulate a wide range of cellular pathway related to adiposity, inflammation and intestinal permeability.[12] As the research of gut microbiota progress on, more microbiota-derived signals other than LPS and SCFAs have been found to mediate fat accumulation, inflammation and intestinal permeability.[13,14]

The orange peel, when fermented in a special way, is called Chenpi, which is considered to be a traditional Chinese medicine, and is well known for the functions of reducing lipid and anti-inflammation, but less known for its specific mechanism. Hesperidin is the main bioactive component of Chenpi.[15] Hesperidin is a flavanone glycoside that belongs to flavonoids, which is also rich in citrus fruits and quite a few vegetables.[15] In vivo and in vitro studies have shown beneficial effects of hesperidin including vitamin-like activity, antioxidant and anti-inflammatory properties, glucose-lowering, dyslipidemia-, atherosclerosis-, and obesity-preventing, antihypertensive, antiallergic and anticarcinogenic effects.[16–19] Moreover, hesperidin has been reported to protect intestinal barrier function.[20,21] However, there is still controversy about these effects, especially whether hesperidin could ameliorate adiposity, as several clinical trials reporting no effects of hesperidin and orange juice which is rich in hesperidin on the lipid parameters.[22,23]

Many phytochemicals such as hesperidin are combined with sugar to form glycosides, which are not absorbed by the small intestine but reach the large intestine to be metabolized by the gut microbiota, where sugar is removed to become absorbable small molecules, or converted into other bioactive molecules with stronger activity. Thus, the gut microbiota which is highly variable depending on host genetics and diet provides a window to explain the mechanism of phytochemicals and the differences in effects among individuals. In addition, phytochemicals may selectively inhibit some harmful bacteria or promote the growth of some beneficial bacteria. Changes in these bacteria and their metabolites may indirectly mediate the efficacy of phytochemicals.[19] In the present study, we aim to examine whether hesperidin could decrease obesity in the HFD-fed mice, whether the anti-obesity effect is mediated by gut microbiota and which specific microbes are candidates that mediate the anti-obesity effect.

Materials and Methods

Murine

Animal experiments were approved and performed in accordance with the guidelines of Laboratory Animal centre of Guangzhou Medical University (Document no. 2018–083). Eight-week-old male mice of the C57BL/6 were purchased from Guangdong Medical Laboratory Animal Centre (GDMLAC) and kept under controlled temperature and light conditions (25°C, 12h light–dark cycle), with free access to food and water. Mice were randomly distributed into eight groups containing six animals each. Mice were housed in groups of three animals per cage. Three groups were fed with a normal diet (ND, 13.5% of energy from fat; D12450; GDMLAC, China) and five groups were fed with a high-fat diet (HFD, 40% of energy from fat; D12451; GDMLAC, China). The formula of the diet was shown in Supplementary Table 1.

From the fifth week of feeding, the ND-fed mice were subjected to three treatments: sterile saline, 200 mg/kg Body Weight (BW) hesperidin (Aladdin, CAS#520-26-3) and fecal bacteria of HFD-fed mice. The HFD-fed mice were subjected to four treatments: sterile saline, 100 mg/kg BW hesperidin, 200 mg/kg BW hesperidin, fecal bacteria of ND-fed mice. All the treatments were conducted every other day by intragastric gavage for six weeks. The groups of mice treated with hesperidin have drinking water with saturated hesperidin.

At end of the tenth week, animals were fasted for 12 hours before sacrifice. Mice were deeply anaesthetized with 1% pentobarbital sodium (50 mg/kg BW) and whole blood was withdrawn through ventral aorta in tubes containing anticoagulant KEDTA. The mesentery adipose tissues, epididymal adipose tissues and the liver were removed and weighed. The colorectum was removed and its length was measured. The faeces in cecum were squeezed out. The blood was centrifuged at 500 g for 5 min and the supernatant (plasma) was collected. All samples were immersed in liquid nitrogen and stored at −80°C for further analysis.

Fecal Microbiota Transplantation

Fecal microbiota of six HFD-fed mice was transplanted to six ND-fed mice, and fecal microbiota of six ND-fed mice was transplanted to six HFD-fed mice, respectively. Stools from six donor mice of each diet group were collected under a laminar flow hood in sterile conditions and 100 mg stool was suspended in 3mL of sterile saline. The solution was vigorously mixed and centrifuged at 2000g for 3 min. The deposit was re-suspended in 3mL of sterile saline and used as transplant material. Fresh transplant material was prepared on the same day of transplantation within 10 min before oral gavage (300 mg/kg BW) to prevent changes in bacterial composition. Six recipient mice were inoculated every other day for 6 weeks before being killed for subsequent analysis.

Measurement of Plasma Cytokines

Plasma interleukin (IL)-6, tumor necrosis factor-alpha (TNF-α), intestinal fatty acid binding protein (iFABP), lipopolysaccharide-binding protein (LBP) were determined by commercial ELISA kits: mouse IL-6 high sensitivity ELISA kit (Cat# EK206HS-96, Multi Sciences, China), mouse TNF-α high sensitivity ELISA kit (Cat# EK282HS-96, Multi Sciences, China), mouse LBP ELISA kit (Cat# CSB-EL012775MO, CUSABIO biotech CO., LTD, China), mouse iFABP ELISA kit (Cat# CSB-E08025m, CUSABIO biotech CO., LTD, China), according to the manufacturer's instructions.

Measurement of Plasma Lipids

The total cholesterol (TCho), triglyceride (Trig), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) were determined by commercial ELISA kits (Roche Diagnostics, USA): Cholesterol Gen 2 (Cat$^{\#}$ 05168538190), Triglycerides (Cat$^{\#}$ 05171407190), HDL-Cholesterol plus 3rd generation (Cat$^{\#}$ 05168805190), LDL-Cholesterol Gen 3 (Cat$^{\#}$ 07005768190), according to the manufacturer's instructions.

Caecal Microbiota Analysis

At the end of the experiment when mice were sacrificed, cecum feces of all mice were sampled for microbiota analysis. The caecal microbiota DNA was extracted using a Stool DNA Kit (Guangzhou IGE biotechnology, China) and applied to amplification of the V3-V4 regions of the 16S rRNA gene. The caecal microbiota composition was assessed using Illumina 2500 sequencing of 16S rRNA amplicon and QIIME-based microbiota analysis. The high-quality reads for bioinformatics analysis were selected and all of the effective reads from all samples were clustered into OTUs based on 99% sequence similarity according to QiimeUclust. The OTUs were annotated through RDP Classifier (Version 2.2), confidence cutoff 0.8 according to the GreenGene database, then composition and abundance information of each sample at different classification levels were statistically summarized. The datasets generated for this study can be found in the Sequence Read Archive (SRA), PRJNA602132.

Quantitative Real-Time Reverse-Transcription (qRT-PCR)

qRT-PCR was conducted to determine mRNA levels of inflammatory genes iNOS, TNFα and IL-6; gut barrier genes Muc2, ZO-1, Claudin 2 and Occludin 2. β-actin was used as reference genes. The total RNA was isolated using UNIQ-10 column trizol total RNA isolation kit (Sangon Biotech, China). The equal amount of total RNA was used to synthesize cDNA with the PrimeScript^{TM} RT reagent kit with gDNA Eraser (Cat$^{\#}$ RR047A, TAKARA, Japan). The qRT–PCR was performed in triplicate using TB Green^{TM} premix Ex Taq^{TM}II(Cat$^{\#}$ RR820A, TAKARA, Japan), 96-well plates and the 7500 Real-Time PCR System (Applied Biosystems). Each well was loaded with a total of 20 μL containing 2μL of cDNA, 2μL of target primers, 6μL of water and 10μL of TAKARA TB green premix. The hot-start PCR was performed for 40 cycles, with each cycle consisting of denaturation for 15s at 95°C, annealing for 30s at 60°C and elongation for 10s at 72°C. The Applied Biosysterm software (life technologies) was used for data analysis. Expression was normalized against the housekeeping gene β-actin. Mean expression levels of ND-fed mice were set as 100%. The primers used were shown in Supplementary Table 2.

Statistical Analysis

The statistical analyses of data were performed using GraphPad Prism (Version 7.00). The normality of the data was confirmed by Shapiro–Wilk test and comparisons of treatment groups were done by one-way ANOVA. All data were presented as mean

± SD. P-values < 0.05 were considered significant (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, **** $P < 0.0001$). Graphs were generated using the software GraphPad Prism.

Results

Hesperidin Alleviates HFD Induced Obesity in Mice

We verified the role of hesperidin on HFD induced obesity (Figure 1A). Results showed that HFD feeding for 10 weeks led to significant increases in body weight, epididymal and mesenteric fat accumulation, content of the plasma lipids including total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol and slight increases

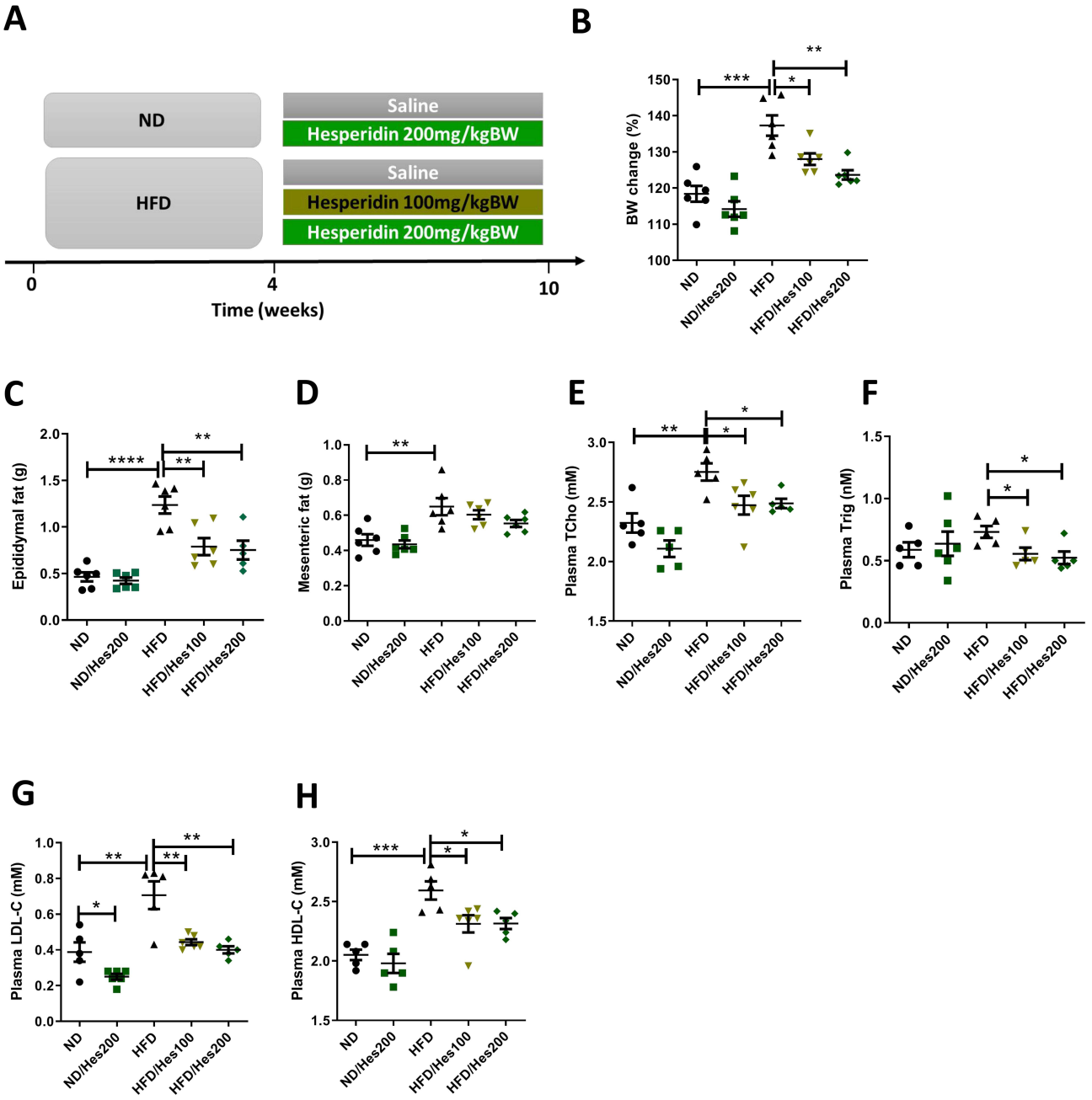

Figure 1 Hesperidin reduced body weight, fat accumulation and plasma lipids in HFD-fed mice. (**A**) ND- and HFD-fed mice were treated every other day with 100 mg/kg BW or 200mg/kg BW by intragastric gavage for 6 weeks (n=6 for each group). (**B**) Body weight gain. (**C**) Epididymal fat. (**D**) Mesenteric fat. (**E**) Plasma total cholesterol. (**F**) Plasma triglyceride. (**G**) Plasma low-density lipoprotein cholesterol (LDL-C). (**H**) Plasma high-density lipoprotein cholesterol (HDL-C). Data are expressed as mean ± SEM. All differences were analyzed using unpaired two-tailed Student's *t*-test (*P<0.05, **P<0.01, ***P<0.001, ****P<0.0001).

in content of plasma triglyceride (Figure 1B–H). Hesperidin did not produce any significant effects in the ND-fed mice except for decreasing plasma low-density lipoprotein cholesterol (Figure 1B–H). The higher concentration of hesperidin (200mg/kgBW) decreased more weight gain, fat accumulation and plasma lipids than the lower concentration (100 mg/kgBW) in HFD-fed mice (Figure 1B–H). These results showed that hesperidin reduced weight gain, fat accumulation and plasma lipids in the HFD-fed mice.

Hesperidin Reduced Inflammation in HFD-Fed Mice

It has been reported that obesity was characterized by low-grade inflammation with higher pro-inflammatory cytokines including tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6) and interleukin-1-beta (IL-1β).[24] Thus, we measured plasma protein levels of TNF-α and IL-6 and colonic messenger RNA (mRNA) levels of TNF-α, IL-6 and IL-1β after 10 weeks of feeding with and without hesperidin supplementation. Protein levels of TNF-α and IL-6 were significantly higher in plasma of HFD-fed mice compared with ND-fed mice (Figure 2A and B). mRNA levels of TNF-α was significantly higher, and IL-6 and IL-1β were non-significantly higher in colons of HFD-fed mice compared with ND-fed mice (Figure 2C–E), while the expression level of most of these cytokines was reduced non-significantly by low concentration and significantly by high concentration of hesperidin (Figure 2A–E). Inducible NO-synthase (iNOS) is a key pro-inflammatory mediator.[25] iNOS mRNA expression increased non-significantly in colons of HFD-fed mice compared to ND-fed mice but decreased following treatment with hesperidin in a dose dependent manner (Figure 2F). These results indicated that hesperidin reduced inflammation in HFD-fed mice.

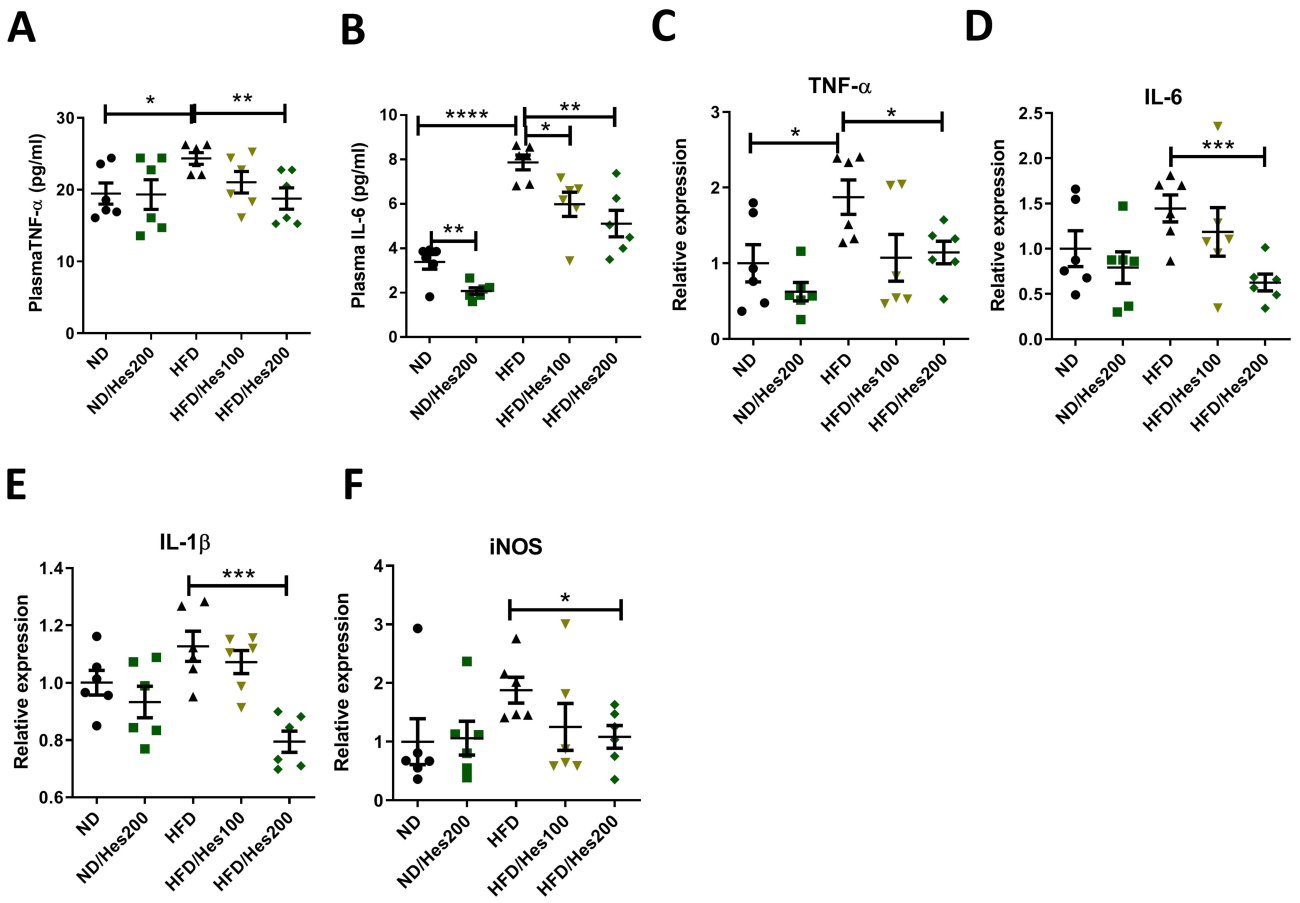

Figure 2 Hesperidin reduced system and colon pro-inflammatory cytokines in HFD-fed mice. ND- and HFD-fed mice were treated every other day with 100 mg/kg BW or 200mg/kg BW by intragastric gavage for 6 weeks (n=6 for each group). (**A**) Levels of TNF-α in plasma. (**B**) Levels of IL-6 in plasma. (**C**) Relative mRNA levels of TNF-α in colon. (**D**) Relative mRNA levels of IL-6 in colon. (**E**) Relative mRNA levels of IL-1β in colon. (**F**) Relative mRNA levels of iNOS in colon. Data are expressed as mean ± SEM. All differences were analyzed using unpaired two-tailed Student's *t*-test (n.s, not significant, *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001).

Hesperidin Maintained Intestinal Integrity in HFD-Fed Mice

Previous studies have shown that gut microbiota dysbiosis caused by HFD increased gut permeability and subsequently resulted in releasing of bacterial endotoxin into the circulation.[26] Thus, we examined the effect of hesperidin on gut integrity by measuring its key markers including the colon length, plasma protein content of lipid binding protein (LBP), plasma intestinal fatty acid binding protein (iFABP), colonic mRNA levels of muc2, tight junction components (claudin 2, occludin and zonula occludens-1). HFD significantly reduced colon length, the expression of the tight junction components, increased plasma protein content of LBP and iFABP, while all these effects were reversed by hesperidin supplementation, in which higher concentration conferred more significant effects than the lower concentration (Figure 3A–G). These results suggested that hesperidin improved intestinal barrier integrity in the HFD-fed mice.

Hesperidin Restored Part of HFD-Induced Gut Dysbiosis

The obese mice and human are characterized by a dysbiosis of gut microbiota.[27,28] Thus, we examined the effects of hesperidin on gut microbiota by performing a pyrosequencing-based analysis of bacterial 16S rRNA (V3–V4 region) using caecal feces samples. UniFrac-based principal coordinates analysis (PCoA) showed a distinct clustering of microbiota composition of ND and HFD-fed mice, HFD-fed mice with and without hesperidin treatments, however, there was no distinct clustering of microbiota composition of ND-fed mice with and without hesperidin treatments (Figure 4A). The gut microbiota richness was significantly reduced by HFD compared to ND. HFD-induced decreasing of microbiota richness was improved by hesperidin (Figure 4B). Microbiota diversity was also increased by hesperidin as

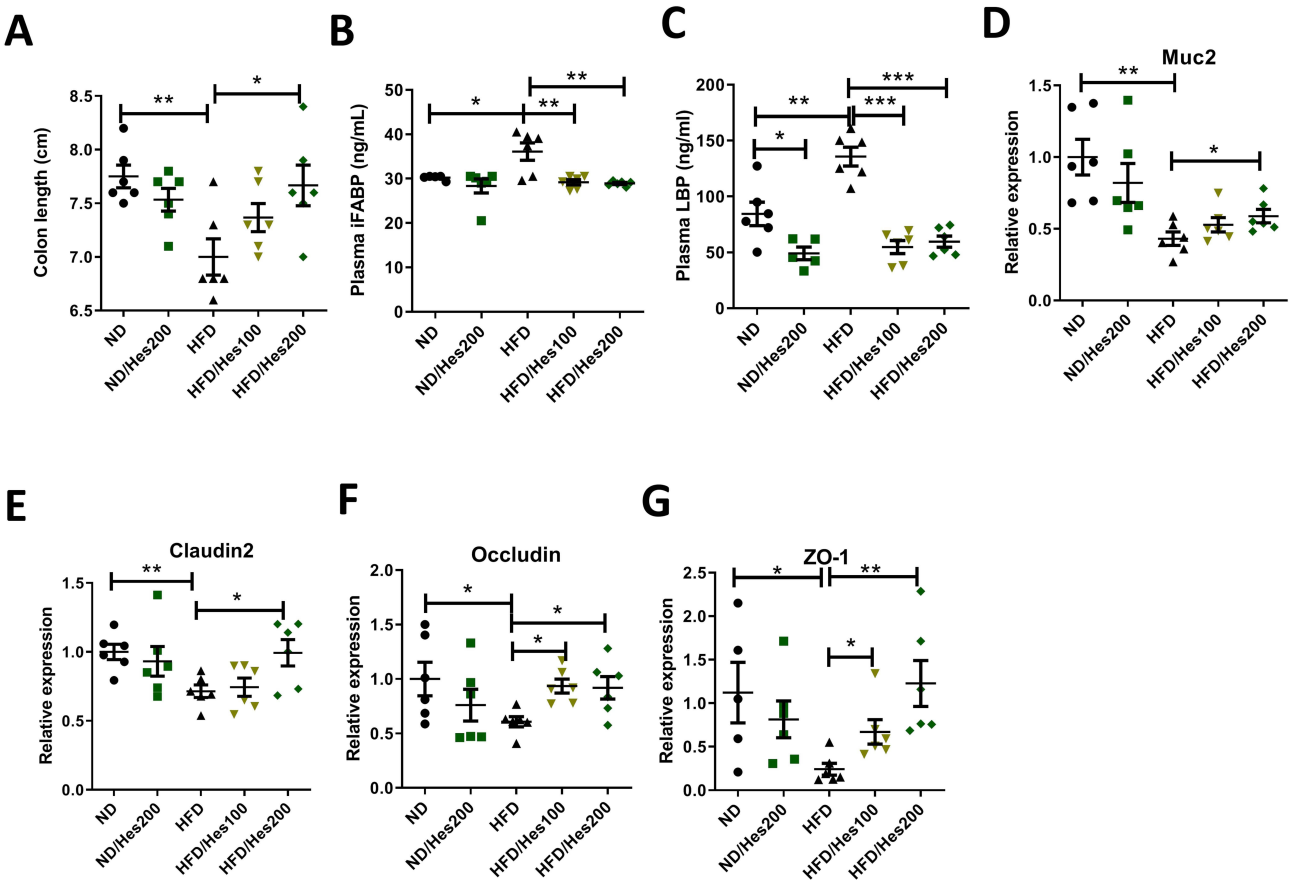

Figure 3 Hesperidin protected intestinal barrier integrity in HFD-fed mice. ND- and HFD-fed mice were treated every other day with 100 mg/kg BW or 200mg/kg BW by intragastric gavage for 6 weeks (n=6 for each group). (**A**) Colon length. (**B**) Levels of lipid binding protein (LBP) in plasma. (**C**) Levels of intestinal fatty acid binding protein (iFABP) in plasma. (**D**) Relative mRNA levels of Muc2 in colon. (**E**) Relative mRNA levels of claudin 2 in colon. (**F**) Relative mRNA levels of occludin in colon. (**G**) Relative mRNA levels of ZO-1 in colon. Data are expressed as mean ± SEM. All differences were analyzed using unpaired two-tailed Student's *t*-test (*P<0.05, **P<0.01, ***P<0.001).

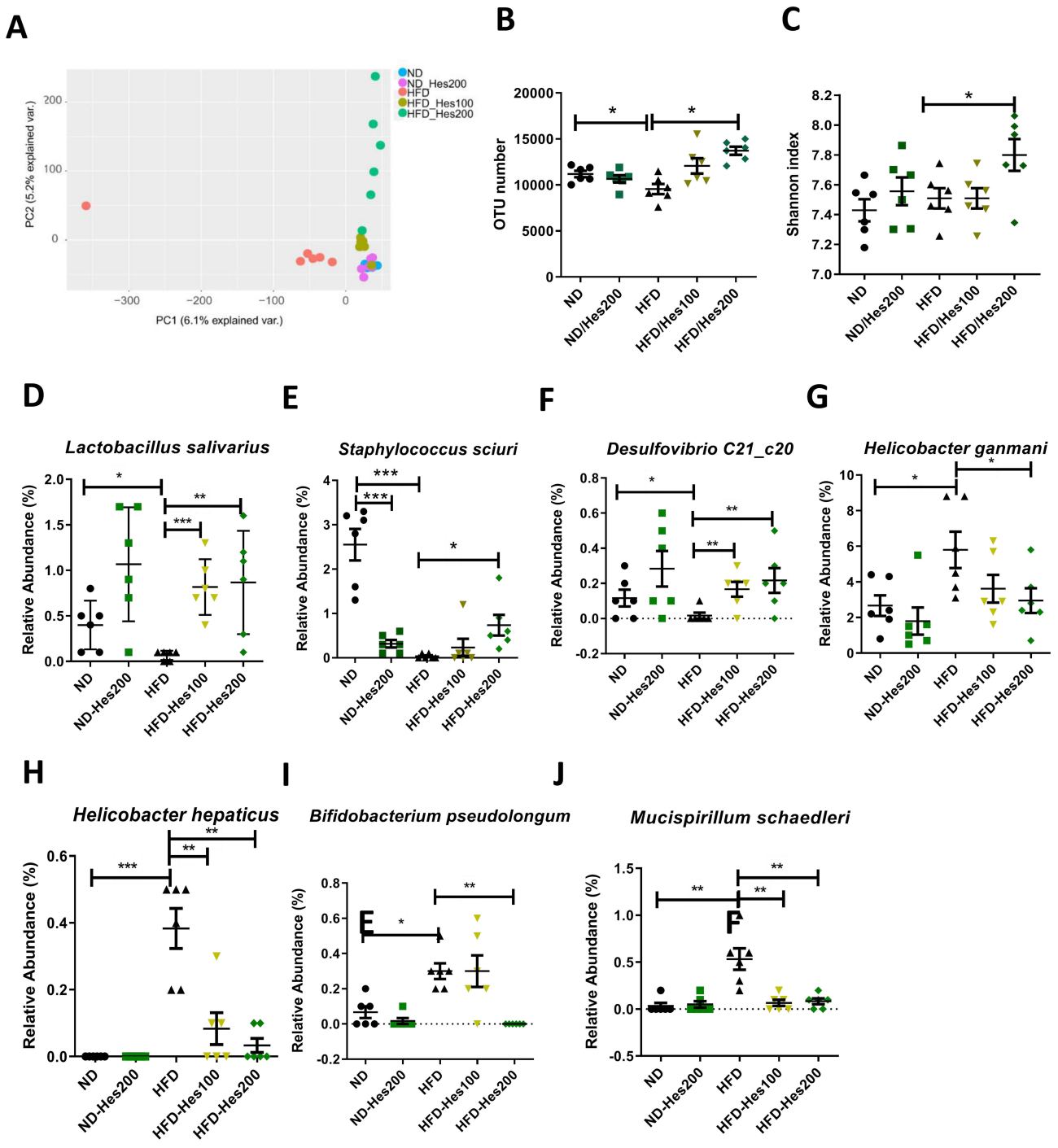

Figure 4 HFD and hesperidin changes gut microbiota in mice. ND- and HFD-fed mice were treated every other day with 100 mg/kg BW or 200mg/kg BW by intragastric gavage for 6 weeks. Fecal samples were taken at the end of experiment for 16S rRNA sequencing (n=6 for each group) **(A)** Principal coordinates analysis (PCoA) based on an unweighted UniFrac analysis of the intestinal microbial composition where samples of mice from different diet and hesperidin supplementation are highlighted with different colors. The position and distance of data points indicates the degree of similarity in terms of both presence and relative abundance of bacterial taxonomies. (**B**) Richness of gut microbiota indicated by number of observed operational taxonomic units (OTUs). (**C**) Diversity of gut microbiota indicated by Shannon index. (**D**) Relative abundance of *Lactobacillus salivarius*. (**E**) Relative abundance of *Staphylococcus sciuri*. (**F**) Relative abundance of*Desulfovibrio _C21_c20*. (**G**) Relative abundance of *Helicobacter ganmani*. (**H**) Relative abundance of *Helicobacter hepaticus*. (**I**) Relative abundance of *Bifidobacterium pseudolongum*. (**J**) Relative abundance of *Mucispirillumschaedleri*. Data are expressed as mean ± SEM.All differences were analyzed using unpaired two-tailed Student's *t*-test (*P<0.05, **P<0.01, ***P<0.001).

https://doi.org/10.2147/DMSO.S474034 DovePress

indicated by Shannon index (Figure 4C). Both microbiota richness and diversity were increased significantly only by high concentration of hesperidin (Figure 4B and C).

A closer look at the microbial community revealed specific influence of hesperidin from the phylum to species levels. The detected OTUs can be annotated to 8 phyla, 13 classes, 15 orders, 22 families, 29 genera and 19 species (Supplementary Figure 1). HFD changes relative abundance of most intestinal microbial taxa. We detected ten species that were significantly different between ND-fed and HFD-fed mice. Six of them including *Lactobacillus salivarius, Lactobacillus vaginalis, Staphylococcus sciuri, Desulfovibrio C21_c20, Corynebacterium stationis* and *Akkermansia muciniphila* were decreased in HFD-fed mice. The other four including *Helicobacter ganmani, Helicobacter hepaticus; Bifidobacterium pseudolongum* and *Mucispirillum schaedleri* were increased in HFD-fed mice (Liu et al, 2020).

Hesperidin enriched *Lactobacillus salivarius, Staphylococcus sciuri* and *Desulfovibrio C21_c20* but depleted *Helicobacter ganmani, Helicobacter hepaticus, Bifidobacterium pseudolongum* and *Mucispirillum schaedleri* in the HFD-fed mice (Figure S1 and Figure 4D–J). However, hesperidin did not change relative abundance of *Lactobacillus vaginalis, Corynebacterium stationis* and *Akkermansia muciniphila* (Figure S1). All these results may indicated that hesperidin could modify the composition of the gut microbiota and reversed a part of HFD-induced gut dysbiosis in a dose-dependent manner in which the higher concentration reversed more than the lower concentration.

Fecal Microbiota Transplantation (FMT) Transferred Obesity, Inflammation and Intestinal Integrity Traits

FMT were reported to be able to transmit donor's traits to recipients.[29] Thus, we tested whether the obesity, inflammation and intestinal integrity traits might also be transferred by FMT. Fecal microbiota from HFD-fed donors treated with saline was transplanted into ND-fed recipients, and fecal microbiota from ND-fed donors treated with saline was transplanted into HFD-fed recipients (Figure 5A). FMT from HFD-fed donors increased obesity traits, inflammation and gut integrity in ND-fed recipients, while FMT from ND-fed donors reduced obesity traits, inflammation and gut integrity in HFD recipients compared with the controls, though most of the indicators were not significant (Figure 5B–M).

FMT Partly Changed Recipients' Gut Microbiota

FMT were reported to be the most efficient way to change the recipients' gut microbiota and to prove causal link between traits and microbiota (Diao et al, 2016). Thus, we tested whether the gut microbiota of ND-fed and HFD-fed mice might also be transferred by FMT. PCoA showed a distinct clustering of microbiota composition of ND-fed mice and ND-fed mice received fecal microbiota of HFD-fed mice, HFD-fed mice and HFD-fed mice received fecal microbiota of ND-fed mice (Figure 6A). The gut microbiota richness of ND-fed mice was not significantly changed by fecal microbiota of HFD-fed donors while that of HFD-fed mice was significantly increased by fecal microbiota of ND-fed donors (Figure 6B). Microbiota diversity was not significantly changed as indicated by Shannon index (Figure 6C).

Among the ten microbes that specifically regulated by HFD, the proportions of *Lactobacillus salivarius* did not change by FMT (Figures 6D), the proportions of *Staphylococcus sciuri, Helicobacter hepaticus* and *Bifidobacterium pseudolongum* of ND-fed mice became closer to the HFD-fed donor following FMT treatment (Figure 6E, H and I), the proportions of *Staphylococcus sciuri, Desulfovibrio C21_c20, Helicobacter hepaticus* and *Mucispirillum schaedleri* of HFD-fed mice became closer to the ND-fed donor following FMT treatment (Figure 6E, F, H and J). These results indicated that over all profiles of gut microbiota was partly changed and a part of the HFD and hesperidin modified microbes were reversed by FMT treatment.

Discussion

Our experiments on HFD-fed mice indicated a reduction in adipose tissue, improved lipid profile and inflammatory status caused by hesperidin, which is consistent with previous in vivo and in vitro studies.[30,31] Moreover, our experiments

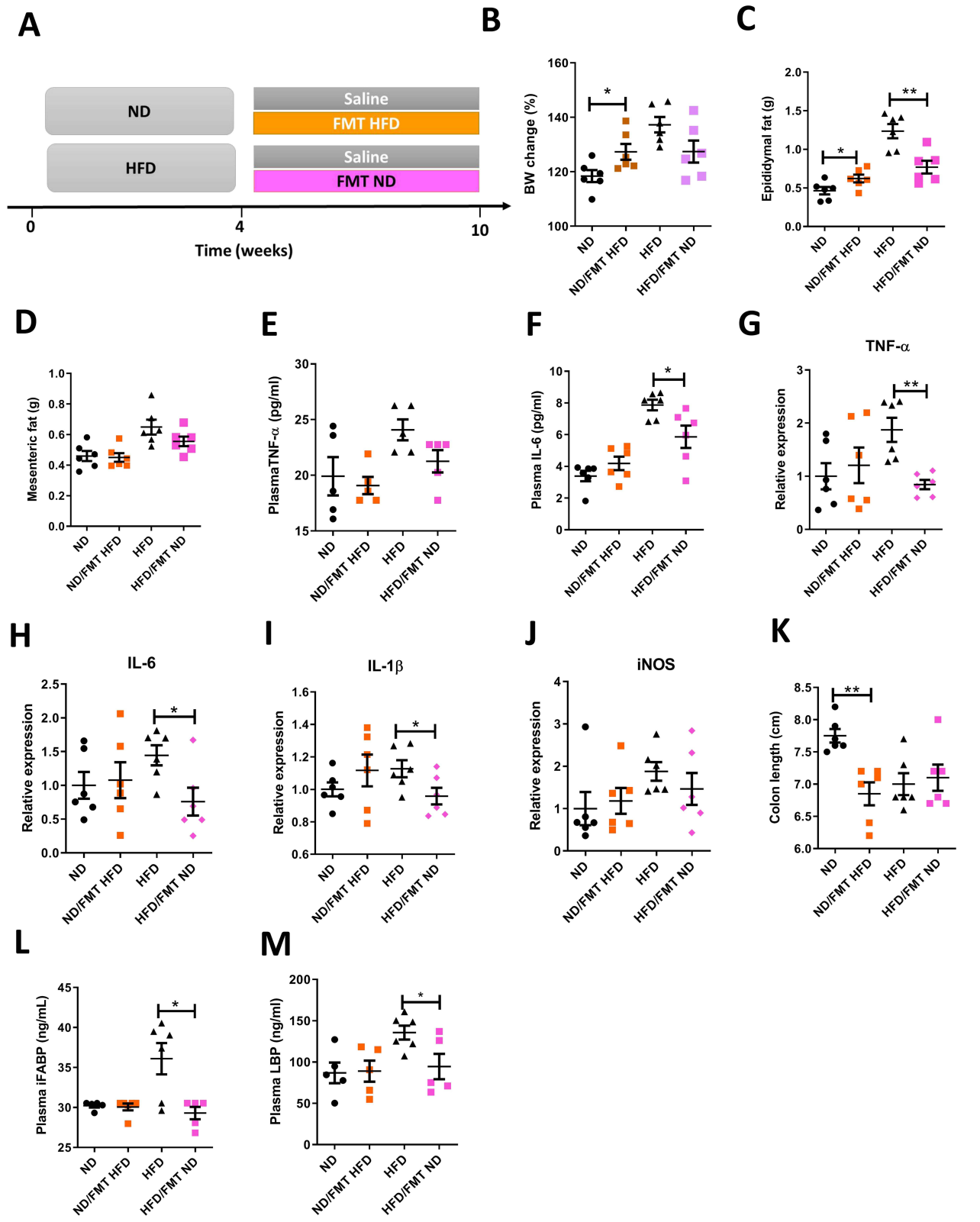

Figure 5 Obesity, inflammation and Intestinal barrier dysfunction were partly reversed by FMT from donor to recipient mice. (**A**) ND- and HFD-fed mice were treated every other day with either saline or fecal bacteria (300 mg/kgBW) from donor mice by intragastric gavage for 6 weeks (n=6 for each group). (**B**) body weight gain. (**C**) Epididymal fat. (**D**) Mesenteric fat. (**E**) Plasma levels of TNF-α. (**F**) Plasma levels of IL-6. (**G**) Relative mRNA levels of TNF-α in colon. (**H**) Relative mRNA levels of IL-6 in colon. (**I**) Relative mRNA levels of IL-1β in colon. (**J**) Relative mRNA levels of iNOS in colon. (**K**) Colon length. (**L**) Plasma levels of lipid binding protein(LBP) (**M**) Plasma levels of intestinal fatty acid binding protein (iFABP). Data are expressed as mean ± SEM. All differences were analyzed using unpaired two-tailed Student's *t*-test (*P<0.05, **P<0.01).

https://doi.org/10.2147/DMSO.S474034 DovePress

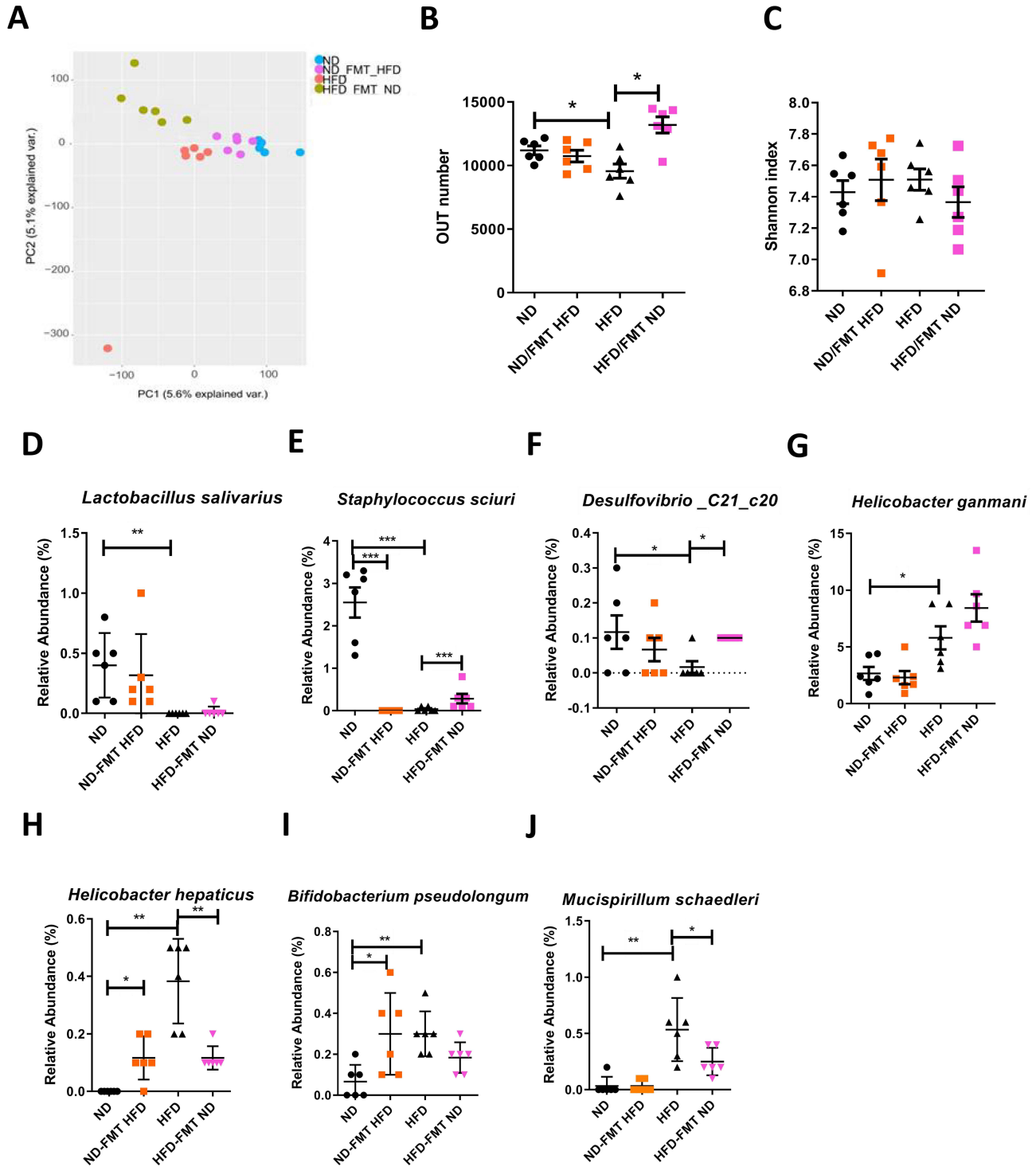

Figure 6 FMT transferred gut microbes from donor to recipient mice. ND- and HFD-fed mice were treated every other day with either saline or fecal bacteria (300 mg/kgBW) from donor mice by intragastric gavage for 6 weeks Fecal samples were taken at the end of experiment for 16S rRNA sequencing (n=6 for each group). (**A**) Principal coordinates analysis (PCoA) based on an unweighted UniFrac analysis of the intestinal microbial composition. (**B**) Richness of gut microbiota indicated by number of observed operational taxonomic units (OTUs). (**C**) Diversity of gut microbiota indicated by Shannon index. (**D**) Relative abundance of *Lactobacillus salivarius*. (**E**) Relative abundance of *Staphylococcus sciuri*. (**F**) Relative abundance of *Desulfovibrio _C21_c20*. (**G**) Relative abundance of *Helicobacter ganmani*. (**H**) Relative abundance of *Helicobacter hepaticus*. (**I**) Relative abundance of *Bifidobacterium pseudolongum*. (**J**) Relative abundance of *Mucispirillumschaedleri*. Data are expressed as mean ± SEM.All differences were analyzed using unpaired two-tailed Student's *t*-test (*P<0.05, **P<0.01, ***P<0.001).

showed that hesperidin improved intestinal barrier function in HFD-fed mice, and the role of hesperidin in decreasing intestinal inflammation and restoring intestinal barrier function was also reported in DSS-induced colitis mice.[20]

However, it's frustrating that a few clinical trials reported no effects of treatments with hesperidin or with orange juice on lipid profile and adiposity in humans.[19,22,23] Mas-Capdevila et al suggested that some of hesperidin's contradictory effects in human trials are partly due to the gut microbiota composition, which impacts the bioavailability of hesperidin.[19] The fact that there were more contradictory effects in human trials than in animal models is probably because of a larger variation in individual human microbiota. Therefore, gut microbiota is a non-negligible aspect when evaluating the function of hesperidin.

Previous studies screened out functional microbiota at the taxon level of genus or higher than the genus. However, not all microbes in the same genera play the same role. Different bacterial species possess different characteristics, which may be related to beneficial or harmful traits. This could be another factor generating inconsistencies across studies in addition to biological and technological factors. For example, *Lactobacillus* such as *Lactobacillus plantarum* and *Lactobacillus paracasei* are associated with thinness, while species such as *Lactobacillus reuteri* are associated with obesity.[32] Therefore, it is necessary to identify the specific microbial taxa at species or strain level when exploring causal links to a specific trait.

Previous studies have proved that colon *Bifidobacterium pseudocatenulatum* converts hesperidin to its active form by the α-rhamnosidase activity of the microbiota, releasing the rutinose moiety and hesperetin for further absorption by the colonocytes. There are only a few literature reported that hesperidin promoted the growth of some beneficial bacteria species, mainly with a key role in the SCFA production (*Bifidobacterium spp., Lactobacillus spp*., or *Akkermansia muciniphila*), and inhibited the proliferation of detrimental bacteria, such as *Escherichia coli, Pseudomonas aeruginosa, Prevotella spp., Porphyromonas gingivalis*, and *Fusobacterium nucleatum*.[31]

This study screen-outed 7 different species that were significantly influenced by HFD and hesperidin. Among them, *Desulfovibrio C21-c20* was reported to be enriched in cisplatin-induced mucositis of Male Wistar rats and depleted by alkaloids of *Rhizoma Coptidis*, and the later conferred antihyperlipidemic effects to high-fat- and high-cholesterol-induced hyperlipidemic B6 mice.[33,34] *Staphylococcus sciuri* was reported as a human opportunistic pathogen in nosocomial diseases and related infections.[35] *Helicobacter hepaticus* was a pathogen that causes typhlitis, colitis, and hepatitis.[36] *Helicobacter ganmani* may also be a pathogen since infection by this microbe was associated with a significant increase in the expression of the proinflammatory cytokine IL12/23p40 in the IL10-deficient mice.[36] *Lactobacillus salivarius* was a promising probiotic since it is known to have certain abilities such as enhancement of the immune system, attenuation of gut inflammation and antimicrobial activity against some pathogenic bacteria.[37,38] *Bifidobacterium pseudolongum* was reported to protect gut barrier.[39,40] *Mucispirillum schaedleri*was reported to be a protection against *Salmonella* colitis in mice by competing for anaerobic respiration substrates in the gut.[41]

From our study, we found that when compared to the ND-fed controls, *Lactobacillus salivarius, Staphylococcus sciuri* and *Desulfovibrio C21_c20* decreased, while *Bifidobacterium pseudolongum, Mucispirillumschaedleri, Helicobacter ganmani* and *Helicobacter hepaticus* increased in the HFD-fed mice. Hesperidin that reduced obesity could reverse these changes caused by HFD. However, the anti-obesogenic role of these microbes has not yet been reported. There are thousands of species in the gut microecosystem. It is important to know whether there are key players that contribute to the development of diseases and the beneficial effects of intervention concerns still needs further exploration.[42] More specifically, it is important to investigate whether one or several of detected microbes together with the undetected microbes actually contribute to the development of obesity and the beneficial effects of hesperidin intervention.

FMT is a common way to study causal links between gut microbiota, diseases and effects of drugs, traditional herbal medicines, bioactive compounds and functional foods.[43,44] Our research indicated that FMT transmitted donors' traits to the receptors since six out of seven HFD and hesperidin modulated microbes including *Lactobacillus salivarius, Staphylococcus sciuri, Desulfovibrio C21_c20, Mucispirillum schaedleri, Helicobacter hepaticus* and *Bifidobacterium pseudolongum* can be transmitted from the donor to recipient mice. These results provided one more piece of evidence for gut microbiota mediated effects of bioactive chemicals and FMT as an effective therapy for diseases.

Conclusions

The present study revealed that hesperidin reduced obesity, inflammation, improved gut integrity and modified a few gut microbiota species in the HFD-fed mice. The anti-obese effects and most hesperidin modified gut microbiota species were transmissible through horizontal fecal transplantation. The results obtained indicated that hesperidin may take a role to reduce body weight and reverse HFD-related disorders in the HFD-fed mice by modulating the microbes. However, the anti-obesogenic role of these microbes needs further verification by live strain supplementation.

Data Sharing Statement

The datasets generated for this study can be found in NCBI, Sequence Read Archive (SRA) through the accession number PRJNA602132 or the link: http://www.ncbi.nlm.nih.gov/bioproject/602132.

Acknowledgments

This manuscript has been released as a pre-print at Research Square (Liu et al, 2020). The authors would like to thank Dr Yu-bin Zhou (from Guangzhou IGE Biotechnology Ltd.) for the kind assistance with microbiota sequencing and analysis. The authors also appreciate Prof. Yong-qin Li (from South China Botanical Garden, Chinese Academy of Sciences) for proofreading this manuscript. This work was financially supported by National Natural Science Foundation of China (No. 81670480), Guangzhou Science and Technology Plan Joint Foundation of City and University (202102010085), University-Industry Cooperation Innovation Foundation of Science and Technology Development Center of the Ministry of Education of China (2021JH016), Key Laboratory of Guangdong Higher Education Institutes (2021KSYS009) and Guangzhou Workstation Open Project of State Key Laboratory of Dampness Syndrome of Chinese Medicine (No. SZGZZ20240012). This paper has been uploaded to Research Square as a preprint: https://www.researchsquare.com/article/rs-11687/v2.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Chooi YC, Ding C, Magkos F. The epidemiology of obesity. *Metabolism*. 2019;92:6–10. doi:10.1016/j.metabol.2018.09.005
2. Rohde K, Keller M, la Cour Poulsen L, Blüher M, Kovacs P, Böttcher Y. Genetics and epigenetics in obesity. *Metabolism*. 2019;92:37–50. doi:10.1016/j.metabol.2018.10.007
3. Oussaada SM, van Galen KA, Cooiman MI, et al. The pathogenesis of obesity. *Metabolism*. 2019;92:26–36. doi:10.1016/j.metabol.2018.12.012
4. Nakajima VM, Macedo GA, Macedo JA. Citrus bioactive phenolics: role in the obesity treatment. *LWT Food Sci Technol*. 2014;59(2):1205–1212. doi:10.1016/j.lwt.2014.02.060
5. Corrêa TAF, Rogero MM, Hassimotto NMA, Lajolo FM. The Two-Way Polyphenols-Microbiota Interactions and Their Effects on Obesity and Related Metabolic Diseases. *Frontiers in Nutrition*. 2019;6:188. doi:10.3389/fnut.2019.00188
6. Pindjakova J, Sartini C, Lo Re O, et al. Gut Dysbiosis and Adaptive Immune Response in Diet-induced Obesity vs. *Syst Inflamm Front Microbiol*. 2017;8:1157. doi:10.3389/fmicb.2017.01157
7. Muñoz-Garach A, Cornejo-Pareja IM, Tinahones FJ. *Encyclopedia of Endocrine Diseases*. *Second Edition*. Huhtaniemi I, Martini L. editors. Oxford: Academic Press; 2019. doi:10.1016/B978-0-12-801238-3.65351-5 353–362
8. Boulangé CL, Neves AL, Chilloux J, Nicholson JK, Dumas ME. Impact of the gut microbiota on inflammation, obesity, and metabolic disease. *Genome Med*. 2016;8(1):42. doi:10.1186/s13073-016-0303-2
9. Jiao N, Baker SS, Nugent CA, et al. Gut microbiome may contribute to insulin resistance and systemic inflammation in obese rodents: a meta-analysis. *Physiol Genom*. 2018;50(4):244–254. doi:10.1152/physiolgenomics.00114.2017
10. Saad MJ, Santos A, Prada PO. Linking Gut Microbiota and Inflammation to Obesity and Insulin Resistance. *Physiology*. 2016;31(4):283–293. doi:10.1152/physiol.00041.2015

11. Wisniewski PJ, Dowden RA, Campbell SC. Role of Dietary Lipids in Modulating Inflammation through the Gut Microbiota. *Nutrients*. 2019;11(1):117. doi:10.3390/nu11010117
12. Ratajczak W, Rył A, Mizerski A, Walczakiewicz K, Sipak O, Laszczynska M. Immunomodulatory potential of gut microbiome-derived short-chain fatty acids (SCFAs). *Acta Biochim Pol*. 2019;66(1):1–12. doi:10.18388/abp.2018_2648
13. Federici M. Gut microbiome and microbial metabolites: a new system affecting metabolic disorders. *J Endocrinol Invest*. 2019;42(9):1011–1018. doi:10.1007/s40618-019-01022-9
14. Vallianou N, Stratigou T, Christodoulatos GS, Dalamaga M. Understanding the Role of the Gut Microbiome and Microbial Metabolites in Obesity and Obesity-Associated Metabolic Disorders: current Evidence and Perspectives. *Curr Oobes Rep*. 2019;8(3):317–332. doi:10.1007/s13679-019-00352-2
15. Erlund I. Review of the flavonoids quercetin, hesperetin, and naringenin. Dietary sources, bioactivities, bioavailability, and epidemiology. *Nutr Res*. 2004;24(10):851–874. doi:10.1016/j.nutres.2004.07.005
16. Roohbakhsh A, Parhiz H, Soltani F, Rezaee R, Iranshahi M. Molecular mechanisms behind the biological effects of hesperidin and hesperetin for the prevention of cancer and cardiovascular diseases. *Life Sci*. 2015;124:64–74. doi:10.1016/j.lfs.2014.12.030
17. Li C, Schluesener H. Health-promoting effects of the citrus flavanone hesperidin. *Crit Rev Food Sci Nutr*. 2017;57(3):613–631. doi:10.1080/10408398.2014.906382
18. Hajialyani M, Farzaei MH, Echeverría J, Nabavi SM, Uriarte E, Sobarzo-Sánchez E. Hesperidin as a Neuroprotective Agent: a Review of Animal and Clinical Evidence. *Molecules*. 2019;24(3):648. doi:10.3390/molecules24030648
19. Mas-Capdevila A, Teichenne J, Domenech-Coca C, et al. Effect of Hesperidin on Cardiovascular Disease Risk Factors: the Role of Intestinal Microbiota on Hesperidin Bioavailability. *Nutrients*. 2020;12(5):1488. doi:10.3390/nu12051488
20. Guo K, Ren J, Gu G, et al. Hesperidin Protects Against Intestinal Inflammation by Restoring Intestinal Barrier Function and Up-Regulating Treg Cells. *Mol Nutr Food Res*. 2019;63(11):e1800975. doi:10.1002/mnfr.201800975
21. Zhang J, Lei H, Hu X, Dong W. Hesperetin ameliorates DSS-induced colitis by maintaining the epithelial barrier via blocking RIPK3/MLKL necroptosis signaling. *Eur J Pharmacol*. 2020;873:172992. doi:10.1016/j.ejphar.2020.172992
22. Demonty I, Lin Y, Zebregs E, et al. The citrus flavonoids hesperidin and naringin do not affect serum cholesterol in moderately hypercholesterolemic men and women. *J Nutr*. 2010;140(9):1615–1620. doi:10.3945/jn.110.124735
23. Mohammadi M, Ramezani-Jolfaie N, Lorzadeh E, Khoshbakht Y, Salehi-Abargouei A. Hesperidin, a major flavonoid in Orange juice, might not affect lipid profile and blood pressure: a systematic review and meta-analysis of randomized controlled clinical trials. *PTR*. 2019;33(3):534–545. doi:10.1002/ptr.6264
24. Chawla A, Nguyen KD, Goh YP. Macrophage-mediated inflammation in metabolic disease, *Nature reviews*. *Immunology*. 2011;11(11):738–749. doi:10.1038/nri3071
25. Lind M, Hayes A, Caprnda M, et al. Inducible nitric oxide synthase: good or bad? *Biomed Pharmacothe*. 2017;93:370–375. doi:10.1016/j.biopha.2017.06.036
26. Cani PD, Bibiloni R, Knauf C, et al. Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. *Diabetes*. 2008;57(6):1470–1481. doi:10.2337/db07-1403
27. Khan MJ, Gerasimidis K, Edwards CA, Shaikh MG. Role of Gut Microbiota in the Aetiology of Obesity: proposed Mechanisms and Review of the Literature. *J Obes*. 2016;2016:7353642. doi:10.1155/2016/7353642
28. Tseng CH, Wu CY. The gut microbiome in obesity. *J Formos Med Assoc*. 2019;118 Suppl 1:S3–s9. doi:10.1016/j.jfma.2018.07.009
29. Diao H, Yan HL, Xiao Y, et al. Intestinal microbiota could transfer host Gut characteristics from pigs to mice. *BMC Microbiol*. 2016;16(1):238. doi:10.1186/s12866-016-0851-z
30. Xiong H, Wang J, Ran Q, et al. Hesperidin: a Therapeutic Agent For Obesity. *Drug Des Devel Ther*. 2019;13:3855–3866. doi:10.2147/DDDT.S227499
31. Yari Z, Movahedian M, Imani H, Alavian SM, Hedayati M, Hekmatdoost A. The effect of hesperidin supplementation on metabolic profiles in patients with metabolic syndrome: a randomized, double-blind, placebo-controlled clinical trial. *Eur J Nutr*. 2020;59(6):2569–2577. doi:10.1007/s00394-019-02105-2
32. Delzenne NM, Neyrinck AM, Cani PD. Modulation of the gut microbiota by nutrients with prebiotic properties: consequences for host health in the context of obesity and metabolic syndrome. *Microb Cell Fact*. 2011;10(Suppl 1):S10. doi:10.1186/1475-2859-10-S1-S10
33. He K, Hu Y, Ma H, et al. Rhizoma Coptidis alkaloids alleviate hyperlipidemia in B6 mice by modulating gut microbiota and bile acid pathways. *BBA*. 2016;1862(9):1696–1709. doi:10.1016/j.bbadis.2016.06.006
34. Wu CH, Ko JL, Liao JM, et al. D-methionine alleviates cisplatin-induced mucositis by restoring the gut microbiota structure and improving intestinal inflammation. *Therapeut Adv Med Oncol*. 2019;11:1758835918821021. doi:10.1177/1758835918821021
35. Christo-Foroux E, Vallaeys T, Loux V, et al. Manual and expert annotation of the nearly complete genome sequence of Staphylococcus sciuri strain ATCC 29059: a reference for the oxidase-positive staphylococci that supports the atypical phenotypic features of the species group. *Syst Appl Microbiol*. 2017;40(7):401–410. doi:10.1016/j.syapm.2017.07.002
36. Alvarado CG, Kocsis AG, Hart ML, Crim MJ, Myles MH, Franklin CL. Pathogenicity of Helicobacter ganmani in mice susceptible and resistant to infection with H. hepaticus. *Comparat Med*. 2015;65(1):15–22.
37. Kang MS, Lim HS, Oh JS, et al. Antimicrobial activity of Lactobacillus salivarius and Lactobacillus fermentum against Staphylococcus aureus. *Pathog Dis*. 2017;75(2). doi:10.1093/femspd/ftx009
38. Yeo S, Shin HS, Lee HW, et al. Determination of Optimized Growth Medium and Cryoprotective Additives to Enhance the Growth and Survival of Lactobacillus salivarius. *J Microbiol Biotechnol*. 2018;28(5):718–731. doi:10.4014/jmb.1801.01059
39. Nishiyama K, Kawanabe A, Miyauchi H, et al. Evaluation of bifidobacterial adhesion to acidic sugar chains of porcine colonic mucins. *Biosci Biotechnol Biochem*. 2014;78(8):1444–1451. doi:10.1080/09168451.2014.918491
40. Mangin I, Dossou-Yovo F, Lévêque C, et al. Oral administration of viable Bifidobacterium pseudolongum strain Patronus modified colonic microbiota and increased mucus layer thickness in rat. *FEMS Microbiol Ecol*. 2018;94(11). doi:10.1093/femsec/fiy177
41. Herp S, Brugiroux S, Garzetti D, et al. Mucispirillum schaedleri Antagonizes Salmonella Virulence to Protect Mice against Colitis. *Cell Host Microbe*. 2019;25(5):681–694.e688. doi:10.1016/j.chom.2019.03.004

42. Lam YY, Zhang C, Zhao L. Causality in dietary interventions-building a case for gut microbiota. *Genome Med*. 2018;10(1):62. doi:10.1186/s13073-018-0573-y
43. Ridaura VK, Faith JJ, Rey FE, et al. Gut microbiota from twins discordant for obesity modulate metabolism in mice. *Science*. 2013;341(6150):1241214. doi:10.1126/science.1241214
44. Chang CJ, Lin CS, Lu CC, et al. Ganoderma lucidum reduces obesity in mice by modulating the composition of the gut microbiota. *Nat Commun*. 2015;6(1):7489. doi:10.1038/ncomms8489