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Dietary methionine restriction ameliorates atherosclerosis by remodeling the gut microbiota in apolipoprotein E-knockout mice†

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Dietary methionine restriction (MR) has been shown to reduce the risk of atherosclerosis, but the specific regulatory effects and mechanisms remain unclear. This research intends to investigate the effects of MR on atherosclerosis in apolipoprotein E-knockout (ApoE^{-/-}) mice fed a high-fat, high-cholesterol, high-choline diet and their mechanisms. ApoE^{-/-} mice were fed a normal diet (0.86% methionine + 4.5% fat + 0% cholesterol + 0.2% choline), a high-fat, high-cholesterol, high-choline diet (0.86% methionine + 20% fat + 1% cholesterol + 1% choline), or a high-fat, high-cholesterol, high-choline + MR diet (0.17% methionine + 20% fat + 1% cholesterol + 1% choline) for 8 consecutive weeks. The results show that MR reduced body weight, fat mass, fat deposition in the liver and adipocytes, and plasma lipid levels; improved the morphological structure of the aorta; and reduced the aortic lesion area and lipid levels. In addition, MR downregulated aortic pro-inflammatory cytokine levels, upregulated aortic anti-inflammatory cytokine levels, and improved aortic oxidative stress. Moreover, metagenomic sequencing results suggested that MR improved the gut microbiota composition, particularly through increased relative abundance of short-chain fatty acid (SCFA)-producing bacteria, and changed the relative abundance of inflammation-, lipid metabolism-, and bile acid metabolism-related bacteria at the species level. Furthermore, MR promoted SCFA production and bile acid metabolism, and reduced cell adhesion molecules and foam cell formation in the aorta. Thus, our findings indicated that MR improved the gut microbiota composition, especially increased SCFA production, and ameliorated oxidative stress and inflammation in the aorta, thereby preventing atherosclerosis.

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1 Introduction

Obesity has developed into a widespread worldwide epidemic and public health concern,¹ acting as a significant risk factor for various complex metabolic imbalances, for instance, cardiovascular diseases, diabetes, hyperlipidemia, and certain cancers.^{2,3} Cardiovascular diseases are highly prevalent globally and are the main reason for morbidity and mortality worldwide.^{4,5} Atherosclerosis (AS) serves as the principal pathophysiological basis for the occurrence of most cardiovascular diseases, characterized by lipid metabolism disorders and inflam-

mation, which promote atherosclerotic lesion formation within arterial walls.^{6,7} A commonly acknowledged environmental risk that promotes AS development is a diet abundant in fat, cholesterol, and choline.^{8,9} This dietary pattern contributes to lipid metabolism disorders, inflammation, oxidative stress, over-expression of cell adhesion molecules, and foam cell formation, all of which promote AS development.¹⁰ The gut microbiota plays a vital role in this process, particularly in short-chain fatty acid (SCFA) metabolism and bile acid metabolism.^{11,12} AS is associated with a decreased relative abundance of SCFA-producing bacteria and a reduction in SCFA production.¹³ SCFAs reduce inflammatory responses and promote lipid metabolism, thereby improving AS.¹⁴ Cholesterol is a precursor of bile acids, which are crucial for cholesterol metabolism.¹⁵ Intestinal microorganisms metabolize bile acids, promoting their excretion and reducing cholesterol accumulation in the arterial walls, which can alleviate AS.¹⁶ Therefore, modulating the intestinal microbiome and its metabolites has been verified as a key target for reducing lipid deposition in the arterial walls and improving AS.

Dietary methionine restriction (MR), following dietary energy and protein restriction, has emerged as a promising

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dietary intervention that has been extensively researched.^{17,18} MR lowers lipid and glucose levels, improves cognitive function, inhibits tumors and cancers, delays aging, and prolongs lifespan.^{19,20} MR reduces inflammatory responses by upregulating anti-inflammatory cytokine levels and downregulating pro-inflammatory cytokine levels in middle-aged obese mice.²¹ Since inflammatory response commonly accompanies oxidative stress, and the two are closely related, MR has also been reported to improve oxidative stress in rats through reduced oxidative damage product generation and increased antioxidant enzyme activity.²² We have recently reported that MR decreased lipid accumulation by reducing hepatic and plasma lipid levels in mice fed a high-fat diet or a high-choline diet.^{23,24} As a pro-angiogenic trigger, MR increased capillary density and promoted angiogenesis in mice.²⁵ Taking into account these preliminary investigations, MR may serve as a key factor in ameliorating AS, though further experimental validation is necessary. We used 16S rDNA high-throughput sequencing and found that MR could improve the intestinal microbiota composition at the genus level in mice fed with high-choline or high-fat diets.^{26,27} However, the effect of MR on microbiota at the species level remains unknown. Additionally, whether MR can improve AS and if its underlying mechanisms involve gut microbiota remodeling remain unclear.

Thus, we hypothesized that MR ameliorates AS by remodeling the gut microbiota. Apolipoprotein E-knockout (ApoE^{-/-}) mice serve as a pre-clinical AS model, mimicking the human metabolic characteristics of the disease and exhibiting increased aortic atherosclerotic lesions. Feeding ApoE^{-/-} mice a high-fat, high-cholesterol, high-choline diet is a widely employed method in experiments to rapidly induce AS.^{28–30} To verify our hypothesis, we assessed the effects of MR on the aortic lesion area, lipid deposition, inflammation, cell adhesion molecules, foam cell formation, gut microbiota composition (*via* metagenomic sequencing), and SCFA production in AS mice to explore the underlying mechanism through which MR ameliorates AS in ApoE^{-/-} mice fed a high-fat, high-cholesterol, high-choline diet.

2 Materials and methods

2.1 Animals and experimental design

The animal research was cleared through the Animal Care and Use Committee of Biological Engineering, Henan University of Technology, Henan, China (no. HAUTETHI-20220309), and followed the National Guidelines for the Ethical Review of Experimental Animal Welfare. Twenty-four ApoE^{-/-} mice (male, 6 weeks old) were supplied by GemPharmatech Co., Ltd (Jiangsu, China), production license number: SCXK (Su) 2018-008, and housed at Henan University of Technology within a monitored animal laboratory (humidity regulated within 50 ± 10%, a 12 h light–dark cycle, and a constant temperature of 22 ± 1 °C) with free availability of water and food. Mice were allocated into three groups (*n* = 8): (a) the ND group (fed a normal

diet with 0.86% methionine + 4.5% fat + 0% cholesterol + 0.2% choline); (b) the AS group (fed a high-fat, high-cholesterol, high-choline diet with 0.86% methionine + 20% fat + 1% cholesterol + 1% choline); and (c) the AS + MR group (fed a high-fat, high-cholesterol, high-choline diet + MR diet with 0.17% methionine + 20% fat + 1% cholesterol + 1% choline). The total study duration was 8 weeks. Throughout the study, body weight was documented on a weekly basis, and daily food intake and water intake were documented during weeks 4 and 8. The comprehensive research diet ingredients can be found in Table S1.†

2.2 Whole-body composition analysis

By the conclusion of week 8, as previously described,³¹ an analysis of the mice's whole-body composition was conducted on day 57 using the MesoMR23-060H-I NMR Analyzer (Shanghai Niumai Electronic Technology Co., Ltd, Shanghai, China). The NMR Analyzer parameters were as follows: magnetic field strength 0.5 ± 0.08 T, resonance frequency 21.3 MHz, and probe coil diameter 60 mm.²⁴ Fat and lean mass were measured and converted into percentages using the following equation: percent fat or lean mass (%) = [(fat or lean mass)/body weight] × 100.

2.3 Sample collection

Based on a previous method,³² fecal samples were collected under sterile conditions over a span of three days in a row (days 58–60). After a one-day rest and a 12 hour overnight fast, mice were anesthetized on day 62 through inhaling isoflurane (dose 3%, 3 minutes). Whole blood samples were collected from the eyeball and preserved in tubes containing the anti-coagulant heparin sodium. The samples were allowed to stand for 30 min and were then centrifuged at 3500*g* for 15 min at 4 °C to separate plasma, which was refrigerated at –80 °C for further analysis. Mice were then sacrificed *via* cervical dislocation, and their livers, peritesticular fat, perirenal fat, mesenteric fat, and brown adipose tissue (BAT) were harvested and weighed, and the ileum and colon tissues were also collected. The liver was separated into two portions, with one assigned for histopathological analysis and the other maintained at –80 °C for biochemical index measurement. Colonic contents were used for metagenomic sequencing. Subsequently, the colon and ileum tissues, peritesticular fat tissue, and BAT were fixed in 4% paraformaldehyde and maintained at 4 °C for histopathological analysis. The heart and upper aorta sections were also collected. The heart was placed under a stereo microscope, the terminal portion of the aortic sinus was identified and separated based on the absence of the three aortic valve leaflets, and the aortic sinus was stained with hematoxylin and eosin (H&E) and oil red O (ORO). The upper aorta section was incised longitudinally and stained with ORO. The lower aorta section was used for real-time quantitative polymerase chain reaction (RT-qPCR) analysis. Finally, forelimb, shoulder, hindlimb, and hip muscles were collected for measurement of crude protein levels.

2.4 Histological analysis of the aorta, hepatic, and intestinal tissues

Paraffin sections of the aortic sinus, hepatic tissues, BAT, peritesticular fat tissue, ileum tissues, and colon tissues were stained with H&E, using previously described methods.^{19,24,33} Frozen hepatic tissues, the aortic sinus, and the aorta were sliced and stained using the manufacturer's protocols (Beyotime, Shanghai, China). Segments stained with both H&E and ORO were examined under a CX31 RTSF microscope (Olympus Corporation, Tokyo, Japan) and analyzed using Image-Pro Plus version 7.0 (Media Cybernetics). The lesion area (%), en face lesion area (%), and hepatic fatty infiltration area ratio (%) were calculated utilizing the following formula: (default color range/total area) $\times$ 100%. Cell volumes of the peritesticular fat tissue and BAT were calculated as described previously.³⁴ Briefly, the number of cells was quantified by counting 6 random fields for each membrane and the average volume of cells per field was calculated as described in a previous published study, and the cell volume of the AS and AS + MR groups was normalized to that of the ND group.³⁵

2.5 Biochemical analysis

Total antioxidant capacity (T-AOC), total superoxide dismutase (T-SOD), malondialdehyde (MDA), glutathione peroxidase (GSH-Px), total glutathione (T-GSH), and reduced glutathione/oxidized glutathione (GSH/GSSG) levels in the plasma and hepatic tissues were assayed using assay kits (Nanjing Jiancheng Bioengineering Institute, China). Plasma levels of interleukin 6 (IL-6), interleukin 10 (IL-10), interleukin 1 β (IL-1 β), and tumor necrosis factor α (TNF- α) were assayed using enzyme-linked immunosorbent assay kits obtained from Xiamen Huijia Bioengineering Institute (Xiamen, China).

Plasma and hepatic tissue levels of high-density lipoprotein cholesterol (HDL-C), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and triglyceride (TG) were assayed using assay kits (Nanjing Jiancheng Bioengineering Institute, China). The AS index (AI) was calculated as a percentage using the following formula: AI (%) = (TC – HDL-C)/HDL-C. Crude protein content (hindlimb, forelimb, shoulder, and hip muscles) was measured through Kjeldahl analysis employing a constant of 6.25.

Bile acid levels in the feces and plasma were assayed using assay kits (Nanjing Jiancheng Bioengineering Institute, China). SCFA (acetate, propionate, and butyrate) levels in both feces and plasma were analyzed *via* gas chromatography-mass spectrometry (GC-MS-QP2010, Shimadzu, Japan) using an external standard method, based on a previously described protocol.³⁶

2.6 RT-qPCR data analysis

Based on a previous method,³⁷ total RNA from the aorta was obtained utilizing the Trizol reagent (Vazyme Biotech Co., Ltd, Nanjing, China). After assessing the purity and quantity of the extracted RNA through measurement of the A260/A280 ratio, which ranged from 1.80 to 2.00, and performing agarose gel

electrophoresis, the total RNA was reverse transcribed to cDNA, employing MultiScribe Reverse Transcriptase (Applied Biosystems). The mRNA expression levels of nuclear factor erythroid-2-related factor 2 (*Nrf2*), Kelch-like ECH-associated protein 1 (*Keap1*), heme oxygenase 1 (*HO-1*), NADH-quinone oxidoreductase-1 (*NQO-1*), *IL-6*, *IL-10*, *IL-1 β* , *TNF- α* , monocyte chemotactic protein-1 (*MCP-1*), vascular cell adhesion molecule-1 (*VCAM-1*), intercellular cell adhesion molecule-1 (*ICAM-1*), E-selectin, cluster differentiation 36 (*CD36*), class A1 scavenger receptor (*SR-A1*), *CD68*, *F4/80*, G-protein-coupled receptor-41 (*GPR41*), G-protein-coupled receptor-43 (*GPR43*), and histone deacetylase 3 (*HDAC3*) were assessed using RT-qPCR. The levels of gene expression are expressed as values relative to β -actin levels. Primer sequences can be found in Table S2.†

2.7 Metagenomic analysis

Metagenomic DNA was extracted from the colonic contents according to a previously reported method.³⁸ It was then sent to GENEWIZ (Suzhou, China) for sequencing, as described in previous studies.³⁹ Sequencing libraries were prepared utilizing Illumina's TruSeq ChIP Library Preparation kit (Illumina, San Diego, CA, USA), and barcoded libraries were sequenced on an Illumina HiSeq2000 platform based on the fragment size. Gene functions were annotated and quantified based on orthologous genes from the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. R (Version 3.1.1) and QIIME (Version 1.9.1) were utilized to perform the α -diversity and β -diversity analyses, respectively, and Heml (Version 1.0.3.3) was used to create a heat map. Box plots were constructed to indicate the relative abundance of bacteria with significant discrepancies.

2.8 Statistical analysis

Statistical analyses were employed using SPSS 17.0. All results were presented as the mean $\pm$ standard error of the mean (SEM). Metagenomic sequencing data at the genus level and KEGG data were analyzed using Student's *t*-tests, while species-level data were assessed using STAMP analysis. One-way analysis of variance (ANOVA) was conducted to assess the remaining data discrepancies, subsequently employing Tamhane's T2 test (when equal variance could not be assumed) or Tukey's HSD test (when equal variance could be assumed). The findings were deemed significant at $p < 0.05$ and highly significant at $p < 0.01$.

3 Results

3.1 MR reduced the body weight and fat mass but did not change the lean mass of AS mice

The animal experimental layout and schedule can be found in Fig. 1A. Before the start of the research study, the ND, AS, and AS + MR group mice showed no notable differences in body weight (Fig. 1B) ($p > 0.05$). However, in comparison with the ND group mice, the body weight of the AS group mice markedly increased at weeks 4 and 8 ($p < 0.05$). Conversely, com-

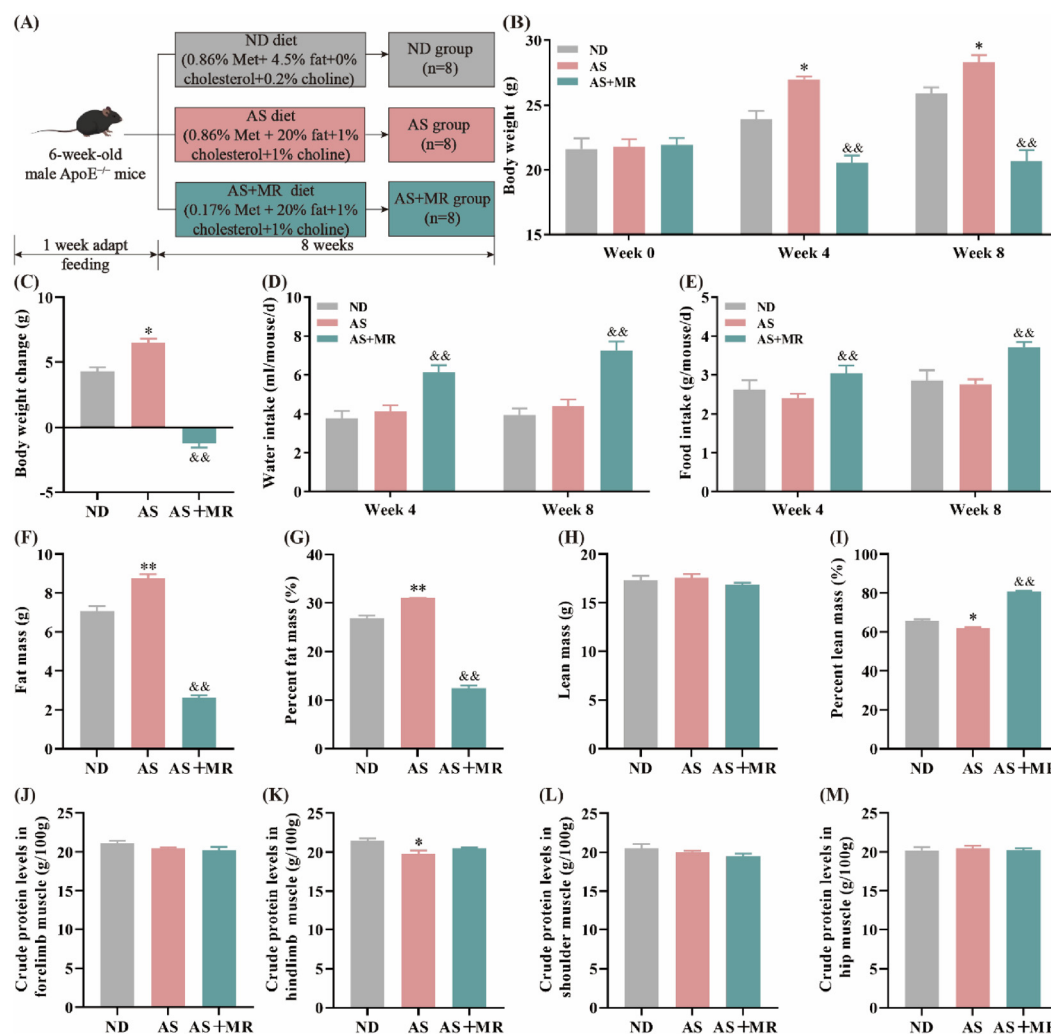


Fig. 1 Animal experiment design diagram and schedule (A); one-week later acclimatization period, ApoE^{-/-} mice (6-week-old, male, $n = 24$) were assigned to three groups, each comprising eight mice. The total duration of the study was eight weeks. The effects of MR on body weight (B) at weeks 0, 4, and 8 in AS mice. The effects of MR on water intake (D) and food intake (E) at weeks 4 and 8 in AS mice. The effects of MR on body weight change (C), fat mass (F), percent fat mass (G), lean mass (H), and percent lean mass (I), and crude protein levels in the forelimb (J), hindlimb (K), shoulder (L), and hip muscle (M) at week 8 in AS mice. Met, methionine. * $p < 0.05$, ** $p < 0.01$ (AS versus ND); $^{\circ}p < 0.05$, $^{\circ\circ}p < 0.01$ (AS + MR versus AS).

pared to the AS group mice, the body weight of the AS + MR group mice markedly reduced at weeks 4 and 8 ($p < 0.01$). In addition, compared with the ND group mice, the body weight change (Fig. 1C) in the AS group mice significantly increased upon finishing the study ($p < 0.05$). In comparison with the AS group mice, the body weight change in the AS + MR group mice markedly reduced upon finishing the study ($p < 0.01$). No notable differences in water intake (Fig. 1D) and food intake (Fig. 1E) were detected between the ND and AS group mice at weeks 4 and 8 ($p > 0.05$). In comparison with the AS group mice, the water intake and food intake in the AS + MR group mice markedly increased at weeks 4 and 8 ($p < 0.01$). Moreover, in comparison with the ND group mice, the AS group mice exhibited markedly increased fat mass (Fig. 1F) and percent fat mass (Fig. 1G), as well as reduced percent lean

mass (Fig. 1I) and crude protein levels in hindlimb muscle (Fig. 1K) ($p < 0.05$). Compared to the AS group mice, the AS + MR group mice exhibited markedly reduced fat mass and percent fat mass, as well as increased percent lean mass ($p < 0.01$). There were no marked variations in lean mass (Fig. 1H) and crude protein levels in the forelimb (Fig. 1J), shoulder (Fig. 1L), and hip (Fig. 1M) among the ND, AS, and AS + MR group mice upon finishing the study ($p > 0.05$).

3.2 MR reduced the aortic lesion area in AS mice

To visually demonstrate the influences of MR on fat deposition in the aorta of AS mice, H&E and ORO staining of the aorta and aortic sinus were utilized. Representative photomicrographs of the aortic sinus stained with H&E and ORO are presented in Fig. 2A and B, respectively. Quantitative analysis of

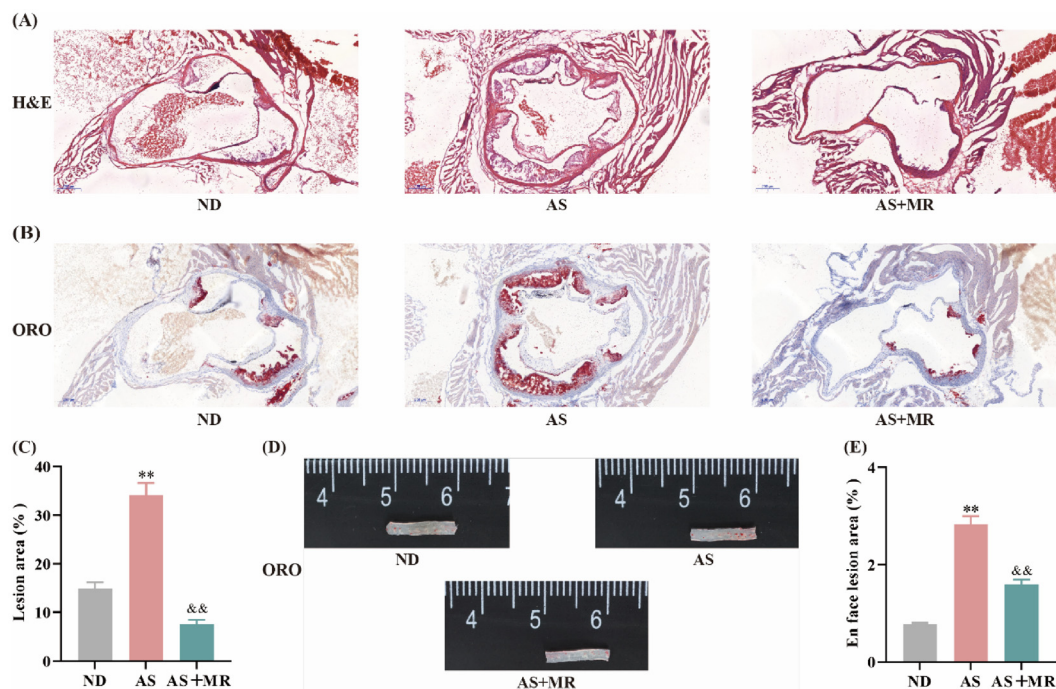


Fig. 2 The effects of MR on representative photomicrographs of the aortic sinus from ND, AS, and AS + MR group mice stained with H&E (A) and ORO (B), magnification $\times 5$. Quantitative analysis of the aorta sinus lesion area from B (C). The effects of MR on representative photomicrographs of en face images of the aorta from the ND, AS, and AS + MR group mice stained with ORO (D). Quantitative analysis of the aorta en face lesion area from D (E). * $p < 0.05$, ** $p < 0.01$ (AS versus ND); $^{\&\&}p < 0.05$, $^{\&\&\&}p < 0.01$ (AS + MR versus AS).

the lesion area in the aortic sinus presented in Fig. 2B was performed and is shown in Fig. 2C. The AS group mice exhibited notably increased lesion area in the aortic sinus in comparison with the ND group mice ($p < 0.01$). Compared to the AS group mice, the AS + MR group mice exhibited markedly reduced lesion area in the aortic sinus ($p < 0.01$). The ORO staining of a representative aorta is presented in Fig. 2D. Quantitative analysis of the en face lesion area of the aorta presented in Fig. 2D was performed and is shown in Fig. 2E. In comparison with the ND group mice, the en face lesion area of the aorta in the AS group mice markedly increased ($p < 0.01$). In comparison with the AS group mice, the en face lesion area of the aorta in the AS + MR group mice notably lowered ($p < 0.01$).

3.3 MR reduced plasma and hepatic lipid levels in AS mice

To clarify the influences of MR on lipid levels in both plasma and hepatic tissues in AS mice, the TG, TC, LDL-C, and HDL-C levels in both the plasma and the liver were determined, and the morphology of hepatic tissues was examined employing H&E and ORO staining. The AS group mice showed notably increased plasma TG (Fig. 3A), TC (Fig. 3B), and LDL-C (Fig. 3C) levels, as well as reduced plasma HDL-C (Fig. 3D) levels ($p < 0.05$) in comparison with the ND group mice, while the AS + MR group mice showed markedly lowered plasma TG, TC, and LDL-C levels, and increased plasma HDL-C levels in comparison with the AS group mice ($p < 0.05$). In addition, based on the aforementioned data, we further calculated the AI (Fig. 3E), and the result exhibited that the AS group mice

exhibited notably increased AI compared to the ND group mice ($p < 0.01$). Compared to the AS group mice, the AS + MR group mice exhibited markedly reduced AI ($p < 0.01$). Moreover, the AS group mice exhibited markedly increased hepatic TG (Fig. 3G), TC (Fig. 3H), and LDL-C (Fig. 3K) levels, and reduced hepatic HDL-C (Fig. 3L) levels in comparison with the ND group mice ($p < 0.05$). Compared to the AS group mice, the AS + MR group mice exhibited markedly reduced hepatic weight (Fig. 3F) and hepatic TG, TC, and LDL-C levels and higher hepatic HDL-C levels ($p < 0.05$). Furthermore, H&E and ORO staining of hepatic tissues is shown in Fig. 3I and J, respectively. The hepatic fatty infiltration area ratio was calculated and is presented in Fig. 3M. In comparison with the ND group mice, the hepatic fatty infiltration area ratio in the AS group mice markedly increased ($p < 0.01$), while in comparison with the AS group mice, the hepatic fatty infiltration area ratio in the AS + MR group mice notably reduced ($p < 0.01$).

3.4 MR reduced the adipose weight and cell volume in AS mice

In addition, the fat tissue weight and the adipocyte volume in peritesticular fat tissue and BAT were determined to clarify the influences of MR on lipid deposition of AS mice. In comparison with the ND group mice, the peritesticular fat weight (Fig. 4A) in the AS group mice markedly increased ($p < 0.05$). In comparison with the AS group mice, the peritesticular fat and mesenteric fat weight (Fig. 4A) in the AS + MR group mice markedly reduced ($p < 0.05$). Moreover, as presented in Fig. 4D

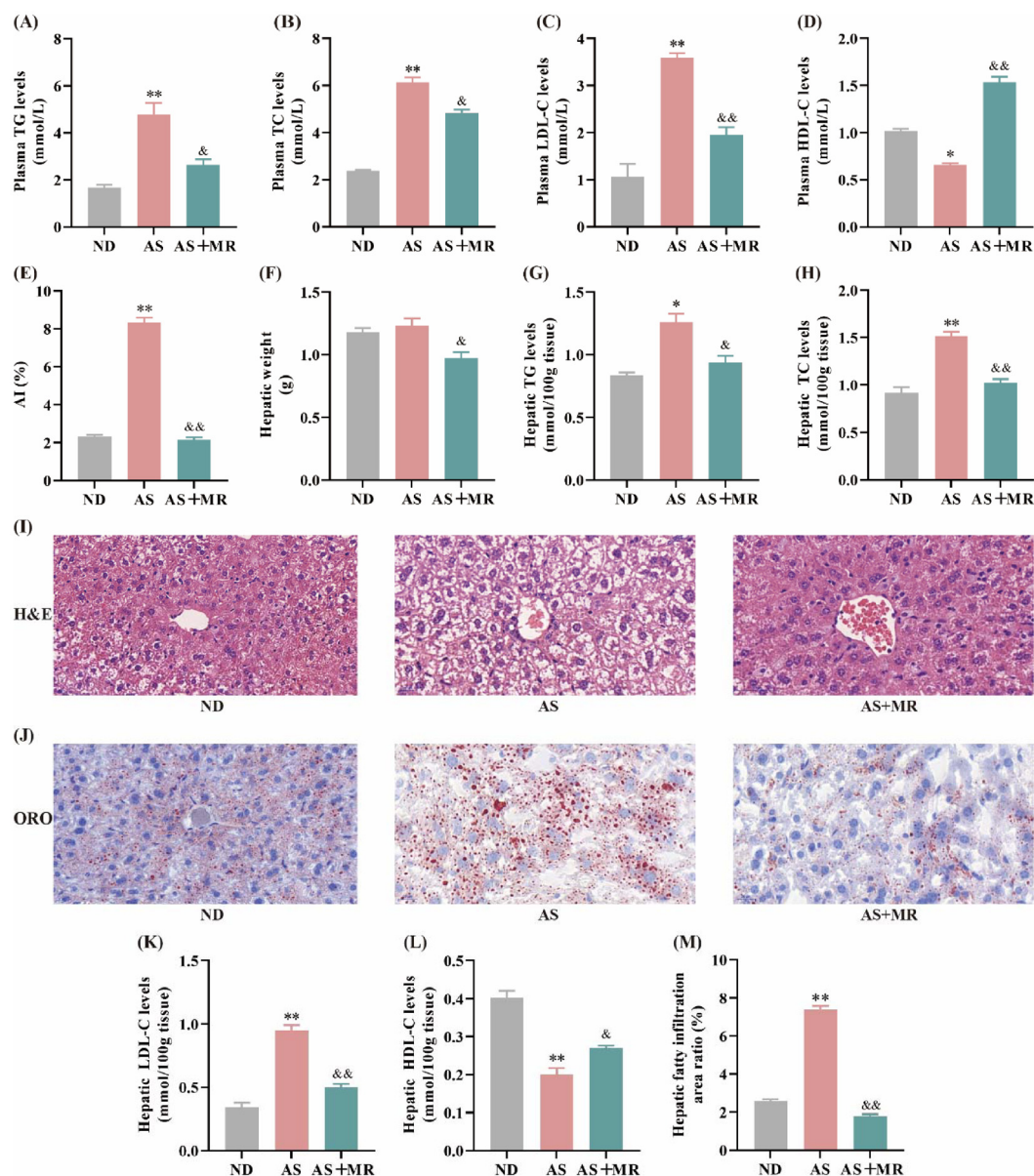


Fig. 3 The effects of MR on plasma TG (A), TC (B), LDL-C (C), and HDL-C (D) levels, AI (E), hepatic weight (F), hepatic TG (G), TC (H), LDL-C (K), and HDL-C (L) levels, and hepatic fatty infiltration area ratio (M) in AS mice. The effects of MR on representative photomicrographs of the liver from the ND, AS, and AS + MR group mice stained with H&E (I) and ORO (J), magnification $\times 40$. Atherogenic index (AI) (%) = (TC – HDL-C)/HDL-C. * $p < 0.05$, ** $p < 0.01$ (AS versus ND); $^{\circ}p < 0.05$, $^{\circ\circ}p < 0.01$ (AS + MR versus AS).

and E, the peritesticular fat tissue and BAT were stained with H&E. Compared to the ND group mice, the adipocyte volume of peritesticular fat tissue (Fig. 4B) and BAT (Fig. 4C) in the AS group mice notably increased ($p < 0.01$). Conversely, compared with the AS group mice, the adipocyte volume of peritesticular fat tissue and BAT in the AS + MR group mice markedly reduced ($p < 0.01$).

3.5 MR ameliorated oxidative stress of the aorta, plasma, and hepatic tissue in AS mice

To clarify the influences of MR on the redox status of the aorta, plasma, and hepatic tissue in AS mice, oxidative stress-

related factors were studied. The AS group mice exhibited notably decreased *Nrf2* (Fig. 5A), *HO-1* (Fig. 5C), and *NQO-1* (Fig. 5D) mRNA expression levels, and increased *Keap1* (Fig. 5B) mRNA expression levels in the aorta in comparison with the ND group mice ($p < 0.01$). Conversely, the AS + MR group mice showed markedly increased *Nrf2*, *HO-1*, and *NQO-1* mRNA expression levels, and reduced *Keap1* mRNA expression levels in the aorta in comparison with the AS group mice ($p < 0.01$). Moreover, compared with the ND group mice, the AS group mice exhibited markedly reduced plasma T-AOC (Fig. 5E), T-SOD (Fig. 5F), T-GSH (Fig. 5I), and GSH/GSSG (Fig. 5J) levels, and increased plasma MDA (Fig. 5G) levels ($p <$

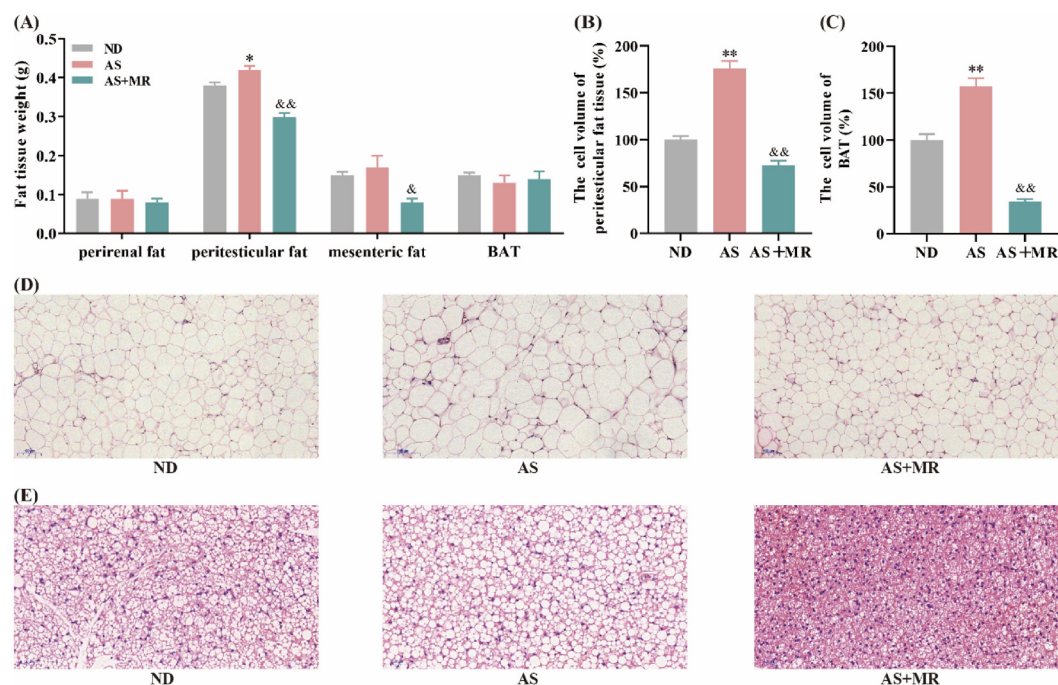


Fig. 4 The effects of MR on fat tissue weight (A), cell volume of peritesticular fat tissue (B), and cell volume of BAT (C) in AS mice. Representative photomicrographs of peritesticular fat tissue (D, scale bars, 100 μ m) and BAT (E, scale bars, 50 μ m) from the ND, AS, and AS + MR group mice stained with H&E. * p < 0.05, ** p < 0.01 (AS versus ND); & p < 0.05, && p < 0.01 (AS + MR versus AS).

0.05). Conversely, the AS + MR group mice showed markedly increased plasma T-AOC, T-SOD, T-GSH, and GSH/GSSG levels, and reduced plasma MDA levels compared to the AS group mice (p < 0.05). Furthermore, the AS group mice showed markedly reduced hepatic T-AOC (Fig. 5K), T-SOD (Fig. 5L), GSH-Px (Fig. 5N), T-GSH (Fig. 5O), and GSH/GSSG (Fig. 5P) levels, and increased hepatic MDA (Fig. 5M) levels compared to the ND group mice (p < 0.05). Conversely, the AS + MR group mice exhibited markedly increased hepatic T-AOC, T-SOD, GSH-Px, T-GSH, and GSH/GSSG levels, and reduced hepatic MDA levels compared with the AS group mice (p < 0.05).

3.6 MR improved the aortic inflammation-, cell adhesion molecule-, and foam cell formation-related gene expression in AS mice

To assess the influences of MR on the aortic plaque formation in AS mice, inflammation-associated and plaque formation-related factors were studied. Compared with the ND group mice, the AS group mice exhibited markedly increased *IL-6* (Fig. 6A), *IL-1 β* (Fig. 6B), *TNF- α* (Fig. 6C), and *MCP-1* (Fig. 6E) mRNA expression levels, and reduced *IL-10* (Fig. 6D) mRNA expression levels in the aorta (p < 0.01). Conversely, the AS + MR group mice showed markedly reduced *IL-6*, *IL-1 β* , *TNF- α* , and *MCP-1* mRNA expression levels, and increased *IL-10* mRNA expression levels in the aorta compared to the AS group mice (p < 0.01). The AS group mice showed markedly increased mRNA expression levels of cell adhesion molecules, including *VCAM-1* (Fig. 6F), *ICAM-1* (Fig. 6G), and *E-selectin* (Fig. 6H) in the aorta in comparison with the ND group mice (p < 0.01).

Conversely, compared to the AS group mice, the AS + MR group mice exhibited markedly reduced *VCAM-1*, *ICAM-1*, and *E-selectin* in the aorta (p < 0.01). Compared with the ND group mice, the AS group mice exhibited markedly increased foam cell formation mRNA expression levels, including *SR-A1* (Fig. 6I), *CD36* (Fig. 6J), *CD68* (Fig. 6K), and *F4/80* (Fig. 6L) in the aorta (p < 0.01). Conversely, in comparison with the AS group mice, the AS + MR group mice exhibited markedly reduced *SR-A1*, *CD36*, *CD68*, and *F4/80* in the aorta (p < 0.01). Compared with the ND group mice, the AS group mice exhibited markedly increased plasma *IL-6* (Fig. 6M), *IL-1 β* (Fig. 6N), and *TNF- α* (Fig. 6O) levels, as well as lowered plasma *IL-10* (Fig. 6P) levels (p < 0.01). Conversely, the AS + MR group mice exhibited markedly reduced plasma *IL-6*, *IL-1 β* , and *TNF- α* levels, as well as increased plasma *IL-10* levels in comparison with the AS group mice (p < 0.01).

3.7 Metagenomic sequencing revealed that MR changed the gut microbiota composition at the phylum level in AS mice

To comprehensively investigate the effects of MR on the gut microbiota composition in AS mice, metagenomic sequencing technology for intestinal microorganisms was performed. The α diversity (ACE, Chao1, Shannon, and Simpson indexes, Fig. 7A) of the microbiome in the colonic contents of the AS group and the AS + MR group mice was measured to assess the structural differences and abundance between the groups. Compared with the AS group mice, the AS + MR group mice exhibited an increasing trend in the ACE, Chao1, Shannon, and Simpson indexes, but no notable vari-

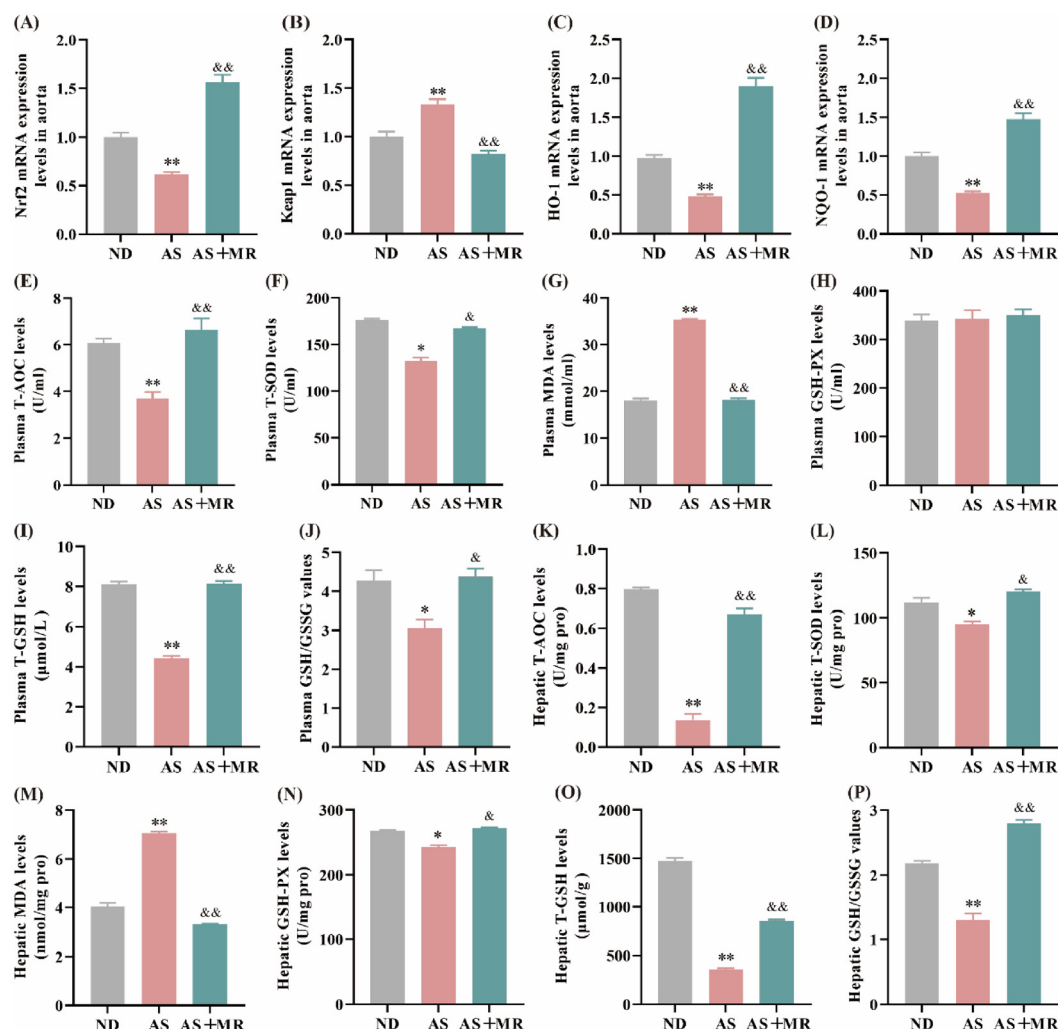


Fig. 5 The effects of MR on oxidative stress-related gene mRNA expression levels in the aorta (A–D), plasma T-AOC (E), T-SOD (F), MDA (G), GSH-Px (H), T-GSH (I), and GSH/GSSG (J) levels, and hepatic T-AOC (K), T-SOD (L), MDA (M), GSH-Px (N), T-GSH (O), and GSH/GSSG (P) levels in AS mice. * $p < 0.05$, ** $p < 0.01$ (AS versus ND); &p < 0.05, &p < 0.01 (AS + MR versus AS).

ation was detected ($p > 0.05$). In addition, β -diversity metrics were examined to certify the variations in the colonic microbial community structure between the AS and AS + MR group mice utilizing an unweighted UniFrac cluster tree (Fig. 7B) and 3D principal component analysis (Fig. 7C). These analyses exhibited a clear separation and distinctly clustered the mice into two groups. In all, 43 bacterial phyla were detected in the colonic contents at the phylum level, among which 10 bacterial phyla had a relative abundance $>1\%$ (Fig. 7D). Further investigation revealed that 4 of the 10 bacterial phyla exhibited notable discrepancies. As shown in Fig. 7F, in comparison with the AS group mice, the AS + MR group mice exhibited markedly increased relative abundance of *Firmicutes* and *Actinobacteria*, and reduced relative abundance of *Verrucomicrobia* and *Proteobacteria* ($p < 0.05$). Moreover, the AS + MR group mice showed a markedly increased *Firmicutes/Bacteroidetes* (F/B) (Fig. 7E) ratio compared to the AS group mice ($p < 0.01$).

3.8 Metagenomic sequencing revealed that MR improved the gut microbiota composition at the genus level in AS mice

To further analyze the influences of MR on the intestinal microbial composition in the colonic contents of AS mice, the gut microbiome at the genus level was analyzed. In all, 517 bacterial genera were identified in the colonic contents at the genus level, among which 64 bacterial genera had a relative abundance $>0.1\%$ (Fig. 8A). Further analyses revealed that 20 of the 64 bacterial genera exhibited notable discrepancies. As shown in Fig. 8B, the AS + MR group mice exhibited markedly decreased relative abundance of *Faecalibaculum*, *Akkermansia*, *Bacteroides*, *Desulfovibrio*, *Mailhella*, and *Bilophila*, and increased relative abundance of *Oscillibacter*, *Roseburia*, *Pseudoflavonifractor*, *Bifidobacterium*, *Olsenella*, *Flavonifractor*, *Anaerotruncus*, *Faecalitalea*, *Collinsella*, *Intestinimonas*, *Libanicoccus*, *Enterococcus*, *Bacillus*, and *Clostridioides* in comparison with the AS group mice ($p < 0.05$).

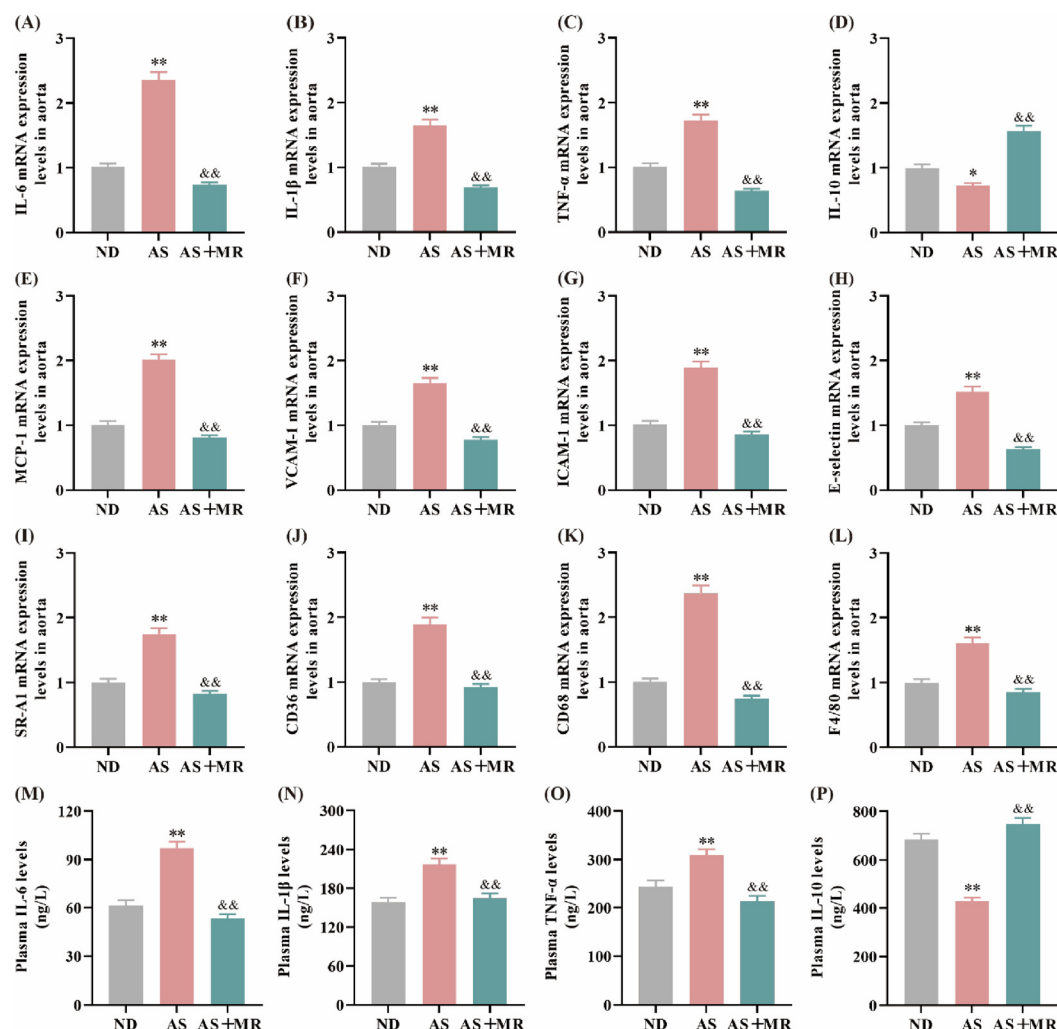


Fig. 6 The effects of MR on mRNA expression levels of inflammatory factors *IL-6* (A), *IL-1 β* (B), *TNF- α* (C), *IL-10* (D), and *MCP-1* (E) in the aorta of AS mice. The effects of MR on mRNA expression levels of cell adhesion molecules *VCAM-1* (F), *ICAM-1* (G), and *E-selectin* (H) in the aorta of AS mice. The effects of MR on mRNA expression levels of foam cell formation *SR-A1* (I), *CD36* (J), *CD68* (K), and *F4/80* (L) in the aorta of AS mice. The effects of MR on plasma *IL-6* (M), *IL-1 β* (N), *TNF- α* (O), and *IL-10* (P) levels in AS mice. * $p < 0.05$, ** $p < 0.01$ (AS versus ND); – $p < 0.05$, –– $p < 0.01$ (AS + MR versus AS).

3.9 Metagenomic sequencing revealed that MR improved the gut microbiota composition at the species level in AS mice

Moreover, to further explore the effects of MR on the intestinal microbial composition in the colonic contents of AS mice, the gut microbiome at the species level was studied. In all, 2349 bacterial species were identified in the colonic contents at the species level, 45 bacterial species showed significant discrepancy, and these are presented in Fig. 9B–E. These 45 bacteria were categorized into four groups: SCFA-producing bacteria (Fig. 9B), inflammation-related bacteria (Fig. 9C), lipid metabolism-related bacteria (Fig. 9D), and bile acid metabolism-related bacteria (Fig. 9E). In comparison with the AS group mice, the AS + MR group mice exhibited markedly increased relative abundance of SCFA-producing bacteria, including *Bifidobacterium pseudolongum*, *Roseburia* sp. 1XD42-69, *Roseburia* sp. 40_7, *Clostridiales bacterium*, *Butyrivococcus* sp.

1XD8-22, *Faecalitalea cylindroides*, *Lachnoclostridium* sp. An138, *Oscillibacter* sp. CAG:155, *Oscillibacter ruminantium*, *Intestinimonas massiliensis*, *Intestinimonas butyriciproducens*, *Anaerotruncus* sp. G3(2012), and *Olsenella umbonate* ($p < 0.05$); markedly decreased relative abundance of pro-inflammatory-related bacteria, including *Akkermansia muciniphila*, *Desulfovibrionaceae bacterium*, and *Akkermansia muciniphila* CAG:154; increased relative abundance of anti-inflammatory-related bacteria, including *Acetatifactor muris*, *Flavonifractor plautii*, *Parolsenella catena*, *Clostridioides difficile*, and *Dubosiella newyorkensis* ($p < 0.05$); markedly reduced relative abundance of lipid metabolism negatively related bacteria, including *Bacteroides* sp. CAG:927 and *Lachnospiraceae bacterium 3-1*; increased relative abundance of lipid metabolism positively related bacteria, including *Firmicutes bacterium M10-2*, *Lachnospiraceae bacterium 10-1*, *Libanicoccus massiliensis*, *Firmicutes bacterium* CAG:129, *Oscillibacter* sp. PC13,

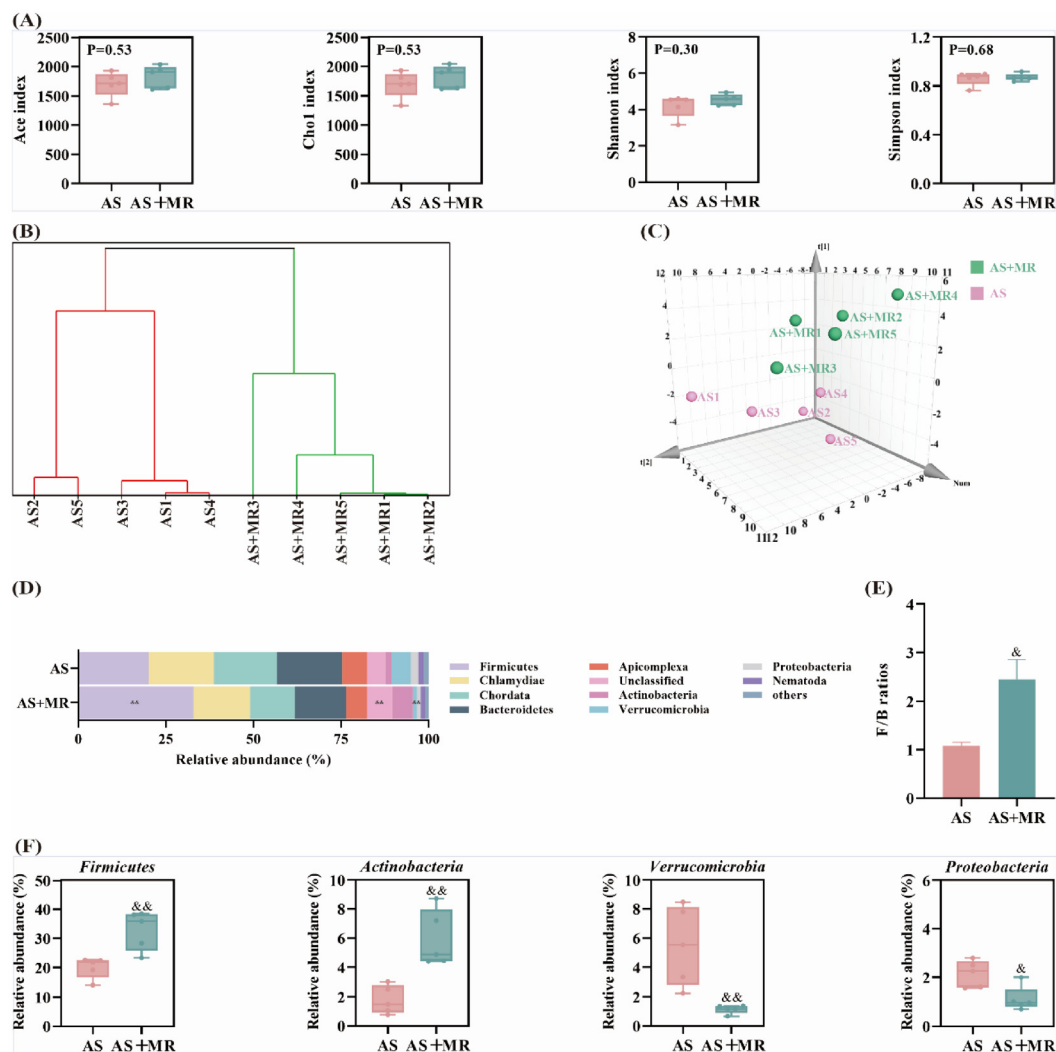


Fig. 7 The effects of MR on the results of α -diversity (A) including the Ace index, Chao1 index, Shannon index, and Simpson index and β -diversity analysis comprising the unweighted UniFrac cluster tree (B) and 3D principal component analysis score (C) of microbiota in AS mice colonic contents. Profiling of the phylum-level colonic contents microbiota composition (D). For the phylum-level results, only major phyla comprising over 1% of the relative abundance in the colonic contents microbiota dataset are indicated. Significantly different phylum-level genomic bins identified between the AS + MR and AS group mice are shown in (F). The effects of MR on the F/B ratio (E) in colonic contents of AS mice. F, *Firmicutes*; B, *Bacteroidetes*. $^{\circ}p < 0.05$, $^{\circ\circ}p < 0.01$ (AS + MR versus AS).

Pseudoflavonifractor sp. An85, *Pseudoflavonifractor* sp. An44, *Pseudoflavonifractor* sp. An187, *Pseudoflavonifractor* sp. An176, and *Pseudoflavonifractor* sp. An184 ($p < 0.05$); markedly reduced relative abundance of bile acid metabolism negatively related bacteria, including *Faecalibaculum rodentium* and *Bilophila wadsworthia*; and increased relative abundance of bile acid metabolism positively related bacteria, including *[Clostridium] innocuum* and *Coriobacteriaceae bacterium* ($p < 0.01$). According to the composition analysis of the KEGG categories at the species level, the top 10 KEGG categories and their average relative contents are presented in Fig. 9A, including global and overview maps, carbohydrate metabolism, energy metabolism, nucleotide metabolism, amino acid metabolism, metabolism of cofactors and vitamins, translation, replication and repair, membrane transport, and signal

transduction. In comparison with the AS group mice, the carbohydrate metabolism, energy metabolism, nucleotide metabolism, amino acid metabolism, and membrane transport in the AS + MR group mice markedly increased ($p < 0.05$).

3.10 MR increased SCFA production and promoted bile acid metabolism in AS mice

To assess the influences of MR on SCFA production and bile acid metabolism linked to the gut microbiota in AS mice, SCFA and bile acid levels in feces and plasma were separately determined. Compared to the ND group mice, the AS group mice exhibited markedly reduced acetate (Fig. 10A and E), propionate (Fig. 10B and F), butyrate (Fig. 10C and G), and total SCFA (Fig. 10D and H) levels in the feces and plasma, reduced bile acid levels in the feces (Fig. 10L), and increased bile acid levels in the plasma

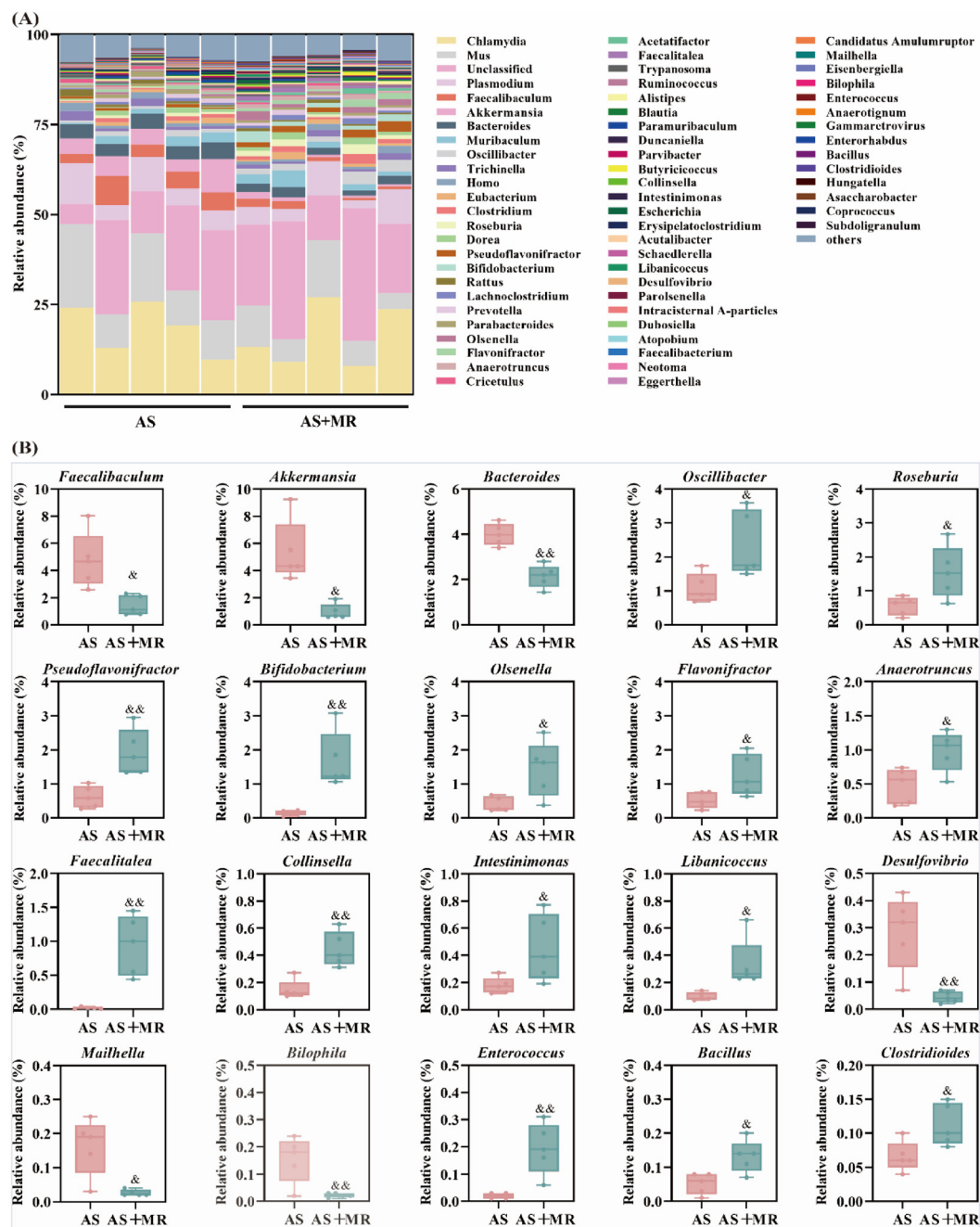


Fig. 8 The relative abundance of microbial species at the genus level in colonic contents (A). For the genus-level data, only major genera comprising over 0.1% of relative abundance in the colonic contents microbiota dataset are indicated. Significantly different genus-level genomic bins identified between the AS + MR and AS groups are shown in (B). $^{\circ}p < 0.05$, $^{\circ\circ}p < 0.01$ (AS + MR versus AS).

(Fig. 10N) ($p < 0.05$), whereas in comparison with the AS group mice, the AS + MR group mice exhibited markedly increased acetate, propionate, butyrate, and total SCFA levels in the feces and plasma, increased bile acid levels in the feces, and reduced bile acid levels in the plasma ($p < 0.05$). Compared with the ND group mice, the AS group mice exhibited markedly reduced *GPR41* (Fig. 10I) and *GPR43* (Fig. 10J) mRNA expression levels, and increased *HDAC3* (Fig. 10K) mRNA expression levels in the aorta ($p < 0.01$). Conversely, the AS + MR group mice showed

markedly increased *GPR41* and *GPR43* mRNA expression levels and reduced *HDAC3* mRNA expression levels in the aorta compared to the AS group mice ($p < 0.01$). H&E staining was used to demonstrate the influences of MR on the morphological structure of the ileum/colon tissues (Fig. 10M) in AS mice. Compared to the ND group mice, the AS group mice exhibited an inflammatory state in the ileum mucosa, with damaged structures and irregular shapes and lengths of the ileal villi, and the colon tissue exhibited loss of goblet cells, distortion and hyperplasia in the crypts,

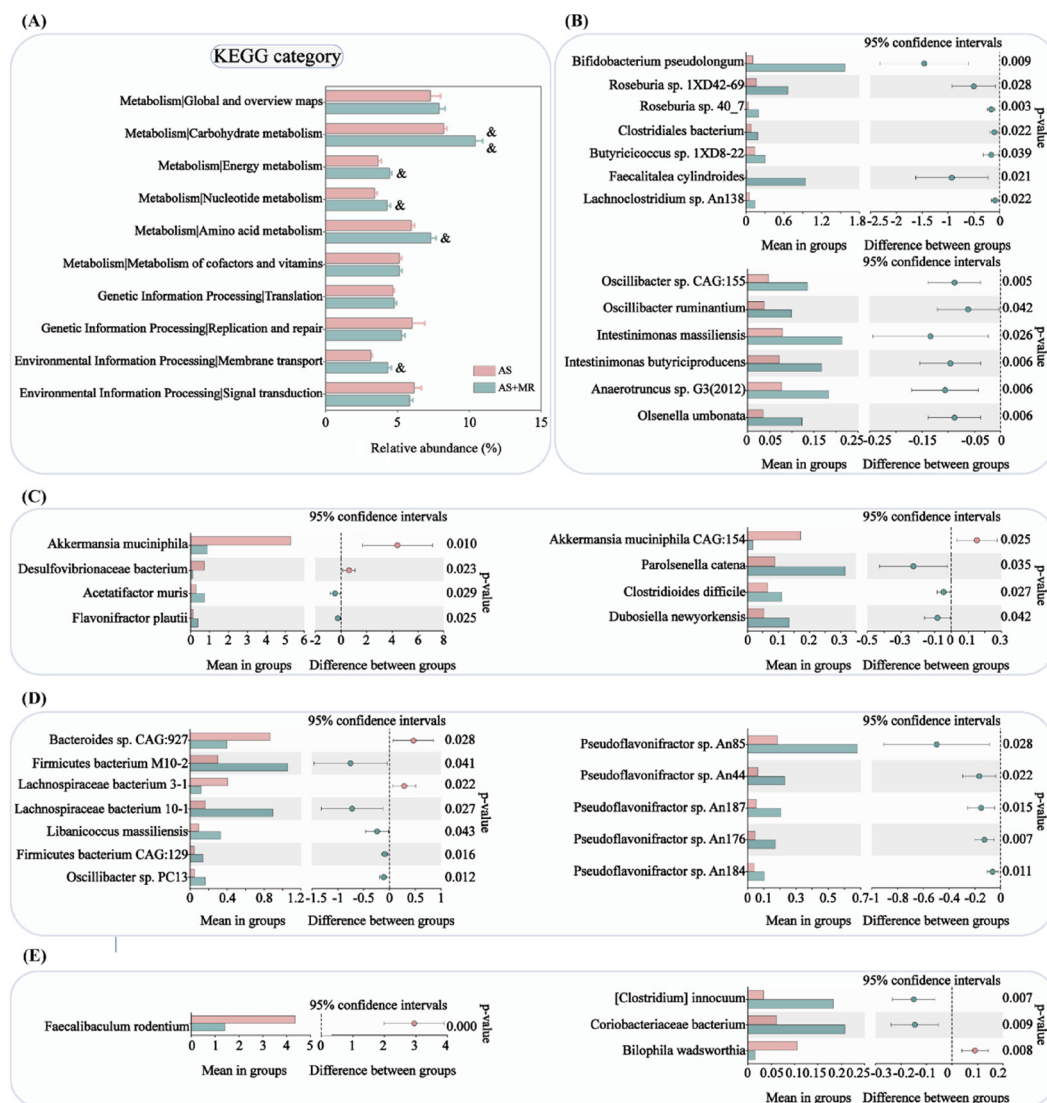


Fig. 9 Top 10 composition of KEGG categories (A). Selected from STAMP analysis, the alterations observed in the gut microbiota at the species level are depicted in (B–E) (Red, AS; Green, AS + MR). $^*p < 0.05$, $^{**}p < 0.01$ (AS + MR versus AS).

and villus atrophy. Conversely, compared with the AS group mice, the AS + MR group mice manifest improved morphology in the intestinal tissue. To further investigate the relationship between AS and SCFA levels, a correlation analysis was conducted between the levels of SCFA in the feces and plasma and AS-related parameters. As shown in Fig. 10O, SCFA levels in the feces and plasma were negatively associated with *IL-6*, *IL-1 β* , *TNF- α* , *MCP-1*, *VCAM-1*, *ICAM-1*, *E-selectin*, *SR-A1*, *CD36*, *CD68*, *F4/80*, AS plaque area, and AI, whereas SCFA levels in the feces and plasma were positively associated with *IL-10*.

4. Discussion

In the current research, we noticed that MR lowered the body weight, fat deposition, aortic lesion area, aortic oxidative stress, aortic inflammation, aortic cell adhesion molecules,

and aortic foam cell formation and improved the gut microbiota composition in AS mice. Specifically, the relative abundance of SCFA production-, inflammation-, lipid metabolism-, and bile acid metabolism-related bacteria was improved. These aforementioned effects were closely associated with the promotion of SCFA production in the intestine and increased plasma SCFA levels. These findings support the hypothesis that MR ameliorates AS by remodeling the gut microbiota.

MR reduced aortic lipid deposition and lesion area. AS is characterized by the formation of atherosclerotic plaques on the inner walls of the aorta, with the primary lesion being lipid accumulation within the aorta.^{40–42} As the disease progresses, the lesion area of the aorta gradually increases.⁴³ In our study, a quantitative analysis of the aorta using ORO-stained photomicrographs showed that MR markedly reduced the en face lesion area in the aorta, indicating that MR decreased the aortic lesion area and improved aortic lipid

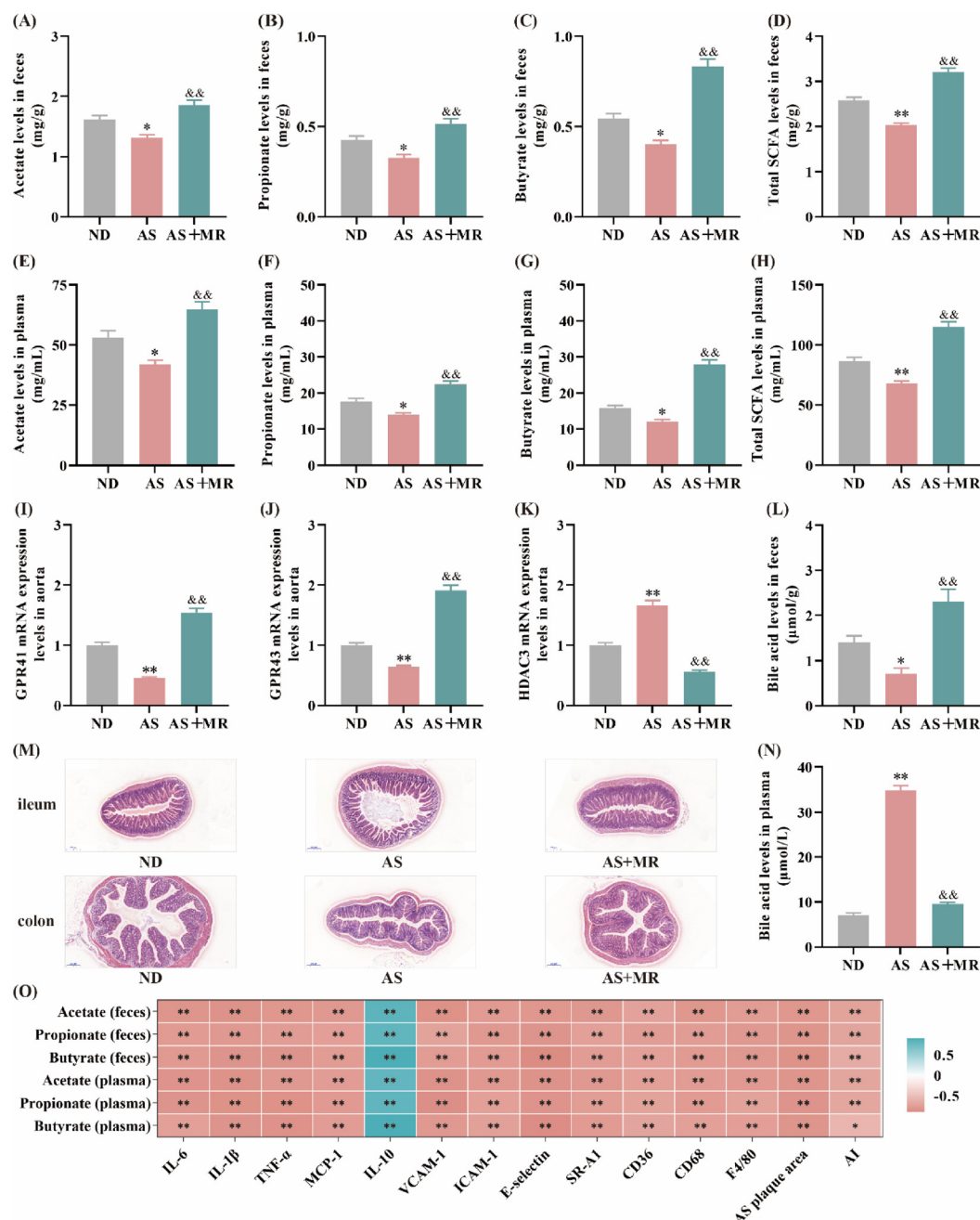


Fig. 10 The effects of MR on fecal acetate (A), propionate (B), butyrate (C), total SCFA (D), and bile acid (L) levels, and plasma acetate (E), propionate (F), butyrate (G), total SCFA (H), and bile acid (N) levels in AS mice. The effects of MR on mRNA expression levels of *GPR41* (I), *GPR43* (J), and *HDAC3* (K) in the aorta of AS mice. The effects of MR on the morphological structure of the ileum and colon tissues (M) in AS mice. Correlations (O) between fecal (acetate, propionate, butyrate)/plasma (acetate, propionate, butyrate) and *IL-6*, *IL-1β*, *TNF-α*, *MCP-1*, *IL-10*, *VCAM-1*, *ICAM-1*, *E-selectin*, *SR-A1*, *CD36*, *CD68*, *F4/80*, AS plaque area, and AI in AS mice. * $p < 0.05$, ** $p < 0.01$ (AS versus ND); ^ε $p < 0.05$, ^{εε} $p < 0.01$ (AS + MR versus AS).

deposition. In support of this, H&E staining of the aortic sinus exhibited that MR evidently reduced plaque lesions in the aortic sinus. This was further confirmed by ORO staining of the aortic sinus, which showed that MR markedly lowered the lesion area in the aortic sinus. Increasing lipid deposition is the initiating factor of AS and the foundation of its pathological changes.^{44,45} MR markedly reduced the body weight, body

weight change, fat mass, and percent fat mass, indicating that MR reduced lipid deposition in the body, which was in line with our previous findings that MR lowered lipid deposition in both adipose tissue and the liver of high-fat diet mice.²⁴ We also observed that MR markedly lowered the hepatic weight, hepatic TG, TC, and LDL-C levels, as well as the hepatic fatty infiltration area ratio, and increased hepatic HDL-C levels,

indicating that MR reduced hepatic lipid deposition. This gained further endorsement from the observations of the ORO and H&E stained micrographs of the liver, which accurately evaluated the hepatic lipid accumulation extent and demonstrated that MR improved the hepatic fatty infiltration area ratio. Notably, MR lowered intracellular lipid deposition, which exhibited as markedly reduced peritesticular fat and mesenteric fat weight, and adipocyte volume in the peritesticular fat and BAT tissue in AS mice. Increased lipid deposition in the body is often accompanied by hyperlipidemia, which leads to fat deposition in the inner walls of the aorta.^{46–48} In the current study, MR markedly lowered plasma TC, TG, and LDL-C levels and increased plasma HDL-C levels, indicating that MR reduced plasma lipid levels. The AI is an important predictor for assessing AS risk.⁴⁹ Our results showed that MR markedly reduced AI, which also supports the notion that MR improves AS. Interestingly, our findings showed that MR did not cause marked variations in the lean mass and crude protein levels in the forelimb, shoulder, hip, and hindlimb muscles, but it markedly increased the percent lean mass, indicating that MR did not result in a reduction in the lean mass and crude protein levels in muscle. Collectively, our findings indicated that MR reduced fat deposition in the body and alleviated AS.

MR ameliorated inflammatory responses and reduced cell adhesion molecules and foam cell formation. Inflammation serves as the underlying cause of physiological and pathological alterations during the onset and progression of AS.⁵⁰ Anti-inflammatory and pro-inflammatory cytokine levels reflect inflammatory activity in AS patients.^{51,52} MR markedly downregulated the pro-inflammatory cytokine *IL-6*, *IL-1 β* , *TNF- α* , and *MCP-1* mRNA expression levels in the aorta and upregulated the anti-inflammatory cytokine *IL-10* mRNA expression levels in the aorta, indicating that MR attenuated the inflammatory response in the aorta. This was further supported by the observation that MR markedly reduced pro-inflammatory cytokine *IL-6*, *IL-1 β* , and *TNF- α* levels in the plasma and increased anti-inflammatory cytokine *IL-10* levels in the plasma. The intricate and closely intertwined relationship between inflammation and oxidative stress is well established.⁵³ An important aspect of this relationship is that an improvement in inflammation frequently coincides with a reduction in oxidative stress, acting as a significant indicator of inflammation amelioration.²⁷ *Nrf2* serves as a pivotal transcription factor that regulates antioxidants, with *Nrf2* and its target genes *HO-1* and *NQO-1* playing crucial roles in antioxidant defense.^{54,55} *Keap1* is a negative regulatory factor of *Nrf2* and inhibits its activity by binding to it.⁵⁶ In the present study, MR markedly upregulated the mRNA expression levels of *Nrf2*, *HO-1*, and *NQO-1* and downregulated the mRNA expression levels of *Keap1* in the aorta, indicating that MR ameliorated oxidative stress, which also supports the conclusion of ameliorated aortic inflammation. In addition, MR markedly increased T-AOC, T-SOD, T-GSH, and GSH/GSSG levels in both plasma and hepatic tissues, increased hepatic GSH-Px levels, and lowered MDA levels in both plasma and hepatic tissues, which also support this notion. AS is a chronic

inflammatory condition, and extensive research has elucidated the molecular pathways by which inflammation leads to AS, primarily through the involvement of cell adhesion molecules and foam cell formation in the aorta.^{57–59} *E-selectin*, *VCAM-1*, and *ICAM-1* are primary cell adhesion molecules that have vital functions in the progression of AS.^{60,61} MR notably downregulated the mRNA expression levels of the cell adhesion molecules *VCAM-1*, *ICAM-1*, and *E-selectin* in the aorta, indicating that MR reduced cell adhesion molecules in the aorta. *SR-A1*, *CD36*, *CD68*, and *F4/80* are scavenger receptors, and the expression of these receptors is crucial for foam cell formation, which is intimately associated with AS development.^{62,63} MR markedly downregulated the mRNA expression levels of the foam cell formation *SR-A1*, *CD36*, *CD68*, and *F4/80* in the aorta, indicating that MR reduced foam cell formation in the aorta. Taken together, these data indicate that MR ameliorates AS by improving the inflammatory response and reducing cell adhesion molecules and foam cell formation in the aorta.

A novel and unexpected finding in this study was that MR altered the relative abundance of inflammation-, lipid metabolism-, and bile acid metabolism-related bacteria, which was associated with improved AS. First, MR markedly lowered the relative abundance of pro-inflammatory bacteria (*Faecalibaculum*, *Akkermansia*, *Bacteroides*, *Desulfovibrio*, *Maiella*, and *Bilophila*)^{64–69} and increased the relative abundance of anti-inflammatory bacteria (*Clostridioides*)⁷⁰ at the genus level. Notably, MR also markedly reduced the relative abundance of pro-inflammatory bacteria, including *Akkermansia muciniphila*, *Desulfovibrionaceae bacterium*, and *Akkermansia muciniphila* CAG:154, and increased the relative abundance of anti-inflammatory bacteria, including *Acetatifactor muris*, *Flavonifractor plautii*, *Parolsenella catena*, *Clostridioides difficile*, and *Dubosiella newyorkensis* at the species level, and these results support that MR ameliorated inflammatory responses. Second, MR markedly reduced the relative abundance of lipid metabolism-negatively related bacteria, including *Bacteroides* sp. CAG:927 and *Lachnospiraceae bacterium* 3-1, and increased the relative abundance of lipid metabolism-positively related bacteria, including *Firmicutes bacterium* M10-2, *Lachnospiraceae bacterium* 10-1, *Libanicoccus massiliensis*, *Firmicutes bacterium* CAG:129, *Oscillibacter* sp. PC13, *Pseudoflavonifractor* sp. An85, *Pseudoflavonifractor* sp. An44, *Pseudoflavonifractor* sp. An187, *Pseudoflavonifractor* sp. An176, and *Pseudoflavonifractor* sp. An184 at the species level. These results support that MR reduced lipid deposition. Finally, MR markedly reduced the relative abundance of bile acid metabolism-negatively related bacteria, including *Faecalibaculum rodentium* and *Bilophila wadsworthia*, and increased the relative abundance of bile acid metabolism-positively related bacteria, including [*Clostridium*] *innocuum* and *Coriobacteriaceae bacterium* at the species level. In addition, we also observed that MR promoted the excretion of intestinal bile acids, as evidenced by increased bile acid levels in feces and a reduction in plasma bile acid levels. Cholesterol is a precursor in the biosynthesis of bile acids, and earlier research has proven that increased excretion of bile acids can promote

cholesterol metabolism, thereby inhibiting the occurrence and progression of AS.^{71–73} MR can result in changes in bile acid metabolism through the gut microbiota and an increase in bile acid excretion, which may be an important reason why MR improves AS. Thus, the influence of MR on the aforementioned gut microbiota relative abundance is intimately associated with the improvement in inflammation, lipid metabolism, and cholesterol metabolism in AS mice.

Interestingly, the aforementioned effects mediated by MR were related to an increase in the relative abundance of SCFA-producing bacteria. SCFA-producing bacteria regulate intestinal homeostasis and influence the abundance of bacteria linked to inflammation, lipid metabolism, and bile acid metabolism, ultimately reducing AS development.^{74–77} SCFA-producing bacteria, particularly *Bifidobacterium* and *Enterococcus*, are renowned for their multiple positive health impacts, encompassing anti-inflammatory effects and beneficial metabolic impacts.^{12,78} These beneficial microorganisms reduce the risk of AS by decreasing inflammatory responses and promoting lipid metabolism and bile acid metabolism.⁷⁹ *Oscillibacter*, *Roseburia*, *Pseudoflavonifractor*, *Bifidobacterium*, *Olsenella*, *Flavonifractor*, *Anaerotruncus*, *Faecalitalea*, *Collinsella*, *Intestinimonas*, *Libanicoccus*, *Enterococcus*, and *Bacillus* are positively associated with the production of SCFA.^{80–86} MR markedly increased the relative abundance of these bacteria at the genus level. Moreover, MR notably increased the relative abundance of SCFA-producing bacteria, comprising *Bifidobacterium pseudolongum*, *Roseburia* sp. 1XD42-69, *Roseburia* sp. 40_7, *Clostridiales bacterium*, *Butyricicoccus* sp. 1XD8-22, *Faecalitalea cylindroides*, *Lachnoclostridium* sp. An138, *Oscillibacter* sp. CAG:155, *Oscillibacter ruminantium*, *Intestinimonas massiliensis*, *Intestinimonas butyriciproducens*, *Anaerotruncus* sp. G3(2012), and *Olsenella umbonate* at the species level, indicating that MR increased the relative abundance of SCFA-producing bacteria. These bacteria, whose relative abundance increased, may regulate intestinal homeostasis, modulate the abundance of bacteria linked to inflammation, lipid metabolism, and bile acid metabolism, and improve the gut microbiota composition.

Another novel and unexpected finding was that MR increased intestinal SCFA production in AS mice, which is an important reason why MR alleviates AS. SCFA are generated by intestinal microorganisms during the fermentation process of undigested carbohydrates and amino acids in the gut.^{87,88} Intestinal microorganisms generate SCFA during energy metabolism, in which nucleic acid metabolism and membrane transport play crucial roles.^{89,90} In our study, the analysis of the KEGG pathways of the gut microbiota showed that MR markedly increased carbohydrate metabolism, energy metabolism, nucleotide metabolism, amino acid metabolism, and membrane transport at the species level, further demonstrating that MR promotes SCFA production in the gut. This was supported by our finding that MR markedly increased acetate, propionate, butyrate, and total SCFA levels in the feces. SCFA serve as the primary energy sources for intestinal epithelial cells and help maintain the structural integrity of tissues.⁹¹

MR improved the morphology of the ileum and colon, which is a key indicator of increased SCFA production. We also observed that MR markedly increased plasma acetate, propionate, butyrate, and total SCFA levels, further indicating that MR promoted SCFA production. In the internal circulation, SCFA have been reported to be able to reduce inflammation and promote lipid metabolism; these beneficial properties can aid in preventing and treating AS.⁹² Butyrate ameliorates AS by facilitating reverse cholesterol transport and modulating lipid metabolism.⁹³ Propionate improves cholesterol metabolism, thereby lowering plasma lipid levels and preventing the AS progression.⁹⁴ *GPR41* and *GPR43* are receptors for SCFA, and SCFA can decrease pro-inflammatory cytokine levels by stimulating *GPR41* and *GPR43*.^{95,96} SCFA have direct inhibitory effects on *HDAC3* activity, triggering histone acetylation and exerting an anti-inflammatory effect.^{97,98} In this study, MR markedly upregulated the mRNA expression levels of *GPR41* and *GPR43* and downregulated the mRNA expression levels of *HDAC3* in the aorta, indicating that MR promoted the utilization of SCFA and ameliorated aortic inflammation, thereby limiting the progression of AS. Furthermore, the correlation analysis indicated that the levels of SCFA in the feces and plasma were positively correlated with AS protective factors (*IL-10*) and negatively correlated with AS risk factors (*IL-6*, *IL-1 β* , *TNF- α* , *MCP-1*, *VCAM-1*, *ICAM-1*, *E-selectin*, *SR-A1*, *CD36*, *CD68*, *F4/80*, AS plaque area, and AI). Thus, these results indicated that MR alleviated AS, with an improvement in the gut microbiota composition and increased SCFA production, which played significant roles in this process.

5 Conclusions

In conclusion, our results demonstrated that MR reduced the body weight, fat deposition, plasma lipid levels, and aortic lipid deposition and lesion area. In addition, MR reduced aortic inflammatory responses, oxidative stress, cell adhesion molecules, and foam cell formation. Moreover, MR changed the relative abundance of inflammation-, lipid metabolism-, and bile acid metabolism-related bacteria. Furthermore, MR increased the relative abundance of SCFA-producing bacteria and SCFA levels in both feces and plasma, which are involved in the aforementioned beneficial effects. Consequently, MR ameliorates AS by remodeling the gut microbiota in ApoE^{−/−} mice. This research offers scientific and theoretical foundations for the possible utilization of MR as a dietary strategy for preventing or alleviating AS.

Ethical approval

All the procedures performed in studies involving animals were in accordance with the ethical standards laid down in the Guidelines for Laboratory Animal Care of Jiangsu Province (China) and in the 1964 Declaration of Helsinki and its later amendments.

Data availability

All relevant data are within the manuscript and its ESI.†

Conflicts of interest

There are no conflicts of interest to declare.

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References

- 1 L. Busetto, D. Dicker, G. Frühbeck, J. C. Halford, P. Sbraccia, V. Yumuk and G. H. Goossens, A new framework for the diagnosis, staging and management of obesity in adults, *Nat. Med.*, 2024, **30**, 2395–2399.
- 2 R. J. Loos and G. S. Yeo, The genetics of obesity: from discovery to biology, *Nat. Rev. Genet.*, 2022, **23**, 120–133.
- 3 J. Geng, Q. Ni, W. Sun, L. Li and X. Feng, The links between gut microbiota and obesity and obesity related diseases, *Biomed. Pharmacother.*, 2022, **147**, 112678.
- 4 P. B. Sandesara, S. S. Virani, S. Fazio and M. D. Shapiro, The forgotten lipids: triglycerides, remnant cholesterol, and atherosclerotic cardiovascular disease risk, *Endocr. Rev.*, 2019, **40**, 537–557.
- 5 L. Zeng, Y. Liu, X. Li, X. Gong, M. Tian, P. Yang, Q. Cai, G. Wu and C. Zeng, Comprehensive scRNA-seq Model Reveals Artery Endothelial Cell Heterogeneity and Metabolic Preference in Human Vascular Disease, *Interdiscip. Sci.: Comput. Life Sci.*, 2024, **16**, 104–122.
- 6 M. Batty, M. R. Bennett and E. Yu, The role of oxidative stress in atherosclerosis, *Cells*, 2022, **11**, 3843.
- 7 Y.-H. Chan and D. P. Ramji, in *Atherosclerosis: Methods and Protocols*, Springer, 2022, vol. 2419, pp. 3–19.
- 8 X. Zhu, L. Zhao, Y. Wang, X. Hu, Y. Zhu and X. Yang, Dietary titanium dioxide particles (E171) promote diet-induced atherosclerosis through reprogramming gut microbiota-mediated choline metabolism in APOE^{-/-} mice, *J. Hazard. Mater.*, 2022, **436**, 129179.
- 9 Z. He, W. Hao, E. Kwek, L. Lei, J. Liu, H. Zhu, K. Y. Ma, Y. Zhao, H. M. Ho and W.-S. He, Fish oil is more potent than flaxseed oil in modulating gut microbiota and reducing trimethylamine-N-oxide-exacerbated atherogenesis, *J. Agric. Food Chem.*, 2019, **67**, 13635–13647.
- 10 H. Cao, Y. Zhu, G. Hu, Q. Zhang and L. Zheng, Gut microbiome and metabolites, the future direction of diagnosis and treatment of atherosclerosis?, *Pharmacol. Res.*, 2023, **187**, 106586.
- 11 M. L. Battson, D. M. Lee, T. L. Weir and C. L. Gentile, The gut microbiota as a novel regulator of cardiovascular function and disease, *J. Nutr. Biochem.*, 2018, **56**, 1–15.
- 12 Y. Zhu, C. Yin and Y. Wang, Probiotic *Enterococcus Faecium* Attenuated Atherosclerosis by Improving SCFAs Associated with Gut Microbiota in ApoE^{-/-} Mice, *Bioengineering*, 2024, **11**, 1033.
- 13 M. Dekker Nitert, Knights in shining armor: short chain fatty acid producers to prevent atherosclerotic plaques?, *Circ. Res.*, 2019, **124**, 12–14.
- 14 W. W. Tang, T. Kitai and S. L. Hazen, Gut microbiota in cardiovascular health and disease, *Circ. Res.*, 2017, **120**, 1183–1196.
- 15 J. Li, S. Yang, D. Liu, Q. Yan, H. Guo and Z. Jiang, Neoagarotetraose Alleviates Atherosclerosis via Modulating Cholesterol and Bile Acid Metabolism in ApoE^{-/-} Mice, *Nutrients*, 2024, **16**, 1502.
- 16 J. Fu, Y. Liang, Y. Shi, D. Yu, Y. Wang, P. Chen, S. Liu and F. Lu, HuangQi ChiFeng decoction maintains gut microbiota and bile acid homeostasis through FXR signaling to improve atherosclerosis, *Heliyon*, 2023, **9**, e21935.
- 17 H. Fang, K. P. Stone, D. Wanders, L. A. Forney and T. W. Gettys, The origins, evolution, and future of dietary methionine restriction, *Annu. Rev. Nutr.*, 2022, **42**, 201–226.
- 18 A. Nagarajan, A. T. Lasher, C. D. Morrow and L. Y. Sun, Long term methionine restriction: Influence on gut microbiome and metabolic characteristics, *Aging Cell*, 2024, **23**, e14051.
- 19 Y. Yang, M. Lu, Y. Xu, J. Qian, G. Le and Y. Xie, Dietary methionine via dose-dependent inhibition of short-chain fatty acid production capacity contributed to a potential risk of cognitive dysfunction in mice, *J. Agric. Food Chem.*, 2022, **70**, 15225–15243.
- 20 C. L. Green, D. W. Lamming and L. Fontana, Molecular mechanisms of dietary restriction promoting health and longevity, *Nat. Rev. Mol. Cell Biol.*, 2022, **23**, 56–73.
- 21 L. Han, G. Wu, C. Feng, Y. Yang, B. Li, Y. Ge, Y. Jiang, Y. Shi and G. Le, Dietary methionine restriction improves the impairment of cardiac function in middle-aged obese mice, *Food Funct.*, 2020, **11**, 1764–1778.
- 22 S. Maddineni, S. Nichenametla, R. Sinha, R. P. Wilson and J. P. Richie Jr, Methionine restriction affects oxidative stress and glutathione-related redox pathways in the rat, *Exp. Biol. Med.*, 2013, **238**, 392–399.
- 23 Y. Yang, Y. Wang, J. Sun, J. Zhang, H. Guo, Y. Shi, X. Cheng, X. Tang and G. Le, Dietary methionine restriction reduces hepatic steatosis and oxidative stress in high-fat-fed mice by promoting H₂S production, *Food Funct.*, 2019, **10**, 61–77.
- 24 Y. Yang, M. Lu, J. Qian, Y. Xu, B. Li, G. Le and Y. Xie, Dietary methionine restriction promotes fat browning and attenuates hepatic lipid accumulation in high-choline-fed

- mice associated with the improvement of thyroid function, *J. Agric. Food Chem.*, 2023, **71**, 1447–1463.
- 25 A. Longchamp, T. Mirabella, A. Arduini, M. R. MacArthur, A. Das, J. H. Trevino-Villarreal, C. Hine, I. Ben-Sahra, N. H. Knudsen and L. E. Brace, Amino acid restriction triggers angiogenesis via GCN2/ATF4 regulation of VEGF and H₂S production, *Cell*, 2018, **173**, 117–129.
 - 26 M. Lu, Y. Yang, Y. Xu, X. Wang, B. Li, G. Le and Y. Xie, Dietary methionine restriction alleviates choline-induced tri-methylamine-N-oxide (TMAO) elevation by manipulating gut microbiota in mice, *Nutrients*, 2023, **15**, 206.
 - 27 Y. Yang, Y. Zhang, Y. Xu, T. Luo, Y. Ge, Y. Jiang, Y. Shi, J. Sun and G. Le, Dietary methionine restriction improves the gut microbiota and reduces intestinal permeability and inflammation in high-fat-fed mice, *Food Funct.*, 2019, **10**, 5952–5968.
 - 28 S. G. Andrews, A. M. Koehle, D. Paudel, T. Neuberger, A. C. Ross, V. Singh, T. Bottiglieri and R. Castro, Diet-Induced Severe Hyperhomocysteinemia Promotes Atherosclerosis Progression and Dysregulates the Plasma Metabolome in Apolipoprotein-E-Deficient Mice, *Nutrients*, 2024, **16**, 330.
 - 29 M. Niimi, Y. Chen, H. Zhao, X. Tang, F. Matsuhisa, H. Zhou, H. Yan, L. Chen, S. Kitajima and A. Sato, Enhanced atherosclerosis in apolipoprotein E knockout rabbits: role of apoB48-rich remnant lipoproteins, *Front. Cardiovasc. Med.*, 2024, **11**, 1424064.
 - 30 J. S. Saulnier-Blache, R. Wilson, K. Klavins, D. Graham, I. Alesutan, G. Kastenmüller, R. Wang-Sattler, J. Adamski, M. Roden and W. Rathmann, Ldlr^{-/-} and ApoE^{-/-} mice better mimic the human metabolite signature of increased carotid intima media thickness compared to other animal models of cardiovascular disease, *Atherosclerosis*, 2018, **276**, 140–147.
 - 31 Y. Qi, Z. Zhang, S. Liu, Z. Aluo, L. Zhang, L. Yu, Y. Li, Z. Song and L. Zhou, Zinc supplementation alleviates lipid and glucose metabolic disorders induced by a high-fat diet, *J. Agric. Food Chem.*, 2020, **68**, 5189–5200.
 - 32 M. Gacias, S. Gaspari, P.-M. G. Santos, S. Tamburini, M. Andrade, F. Zhang, N. Shen, V. Tolstikov, M. A. Kiebish and J. L. Dupree, Microbiota-driven transcriptional changes in prefrontal cortex override genetic differences in social behavior, *eLife*, 2016, **5**, e13442.
 - 33 Y. Li, Z. Yu, Y. Liu, T. Wang, Y. Liu, Z. Bai, Y. Ren, H. Ma, T. Bao and H. Lu, Dietary α -Linolenic Acid-Rich Flaxseed Oil Ameliorates High-Fat Diet-Induced Atherosclerosis via Gut Microbiota-Inflammation-Artery Axis in ApoE^{-/-} Mice, *Front. Cardiovasc. Med.*, 2022, **9**, 830781.
 - 34 M. U. Ijaz, M. I. Ahmad, M. Hussain, I. A. Khan, D. Zhao and C. Li, Meat protein in high-fat diet induces adipogenesis and dyslipidemia by altering gut microbiota and endocannabinoid dysregulation in the adipose tissue of mice, *J. Agric. Food Chem.*, 2020, **68**, 3933–3946.
 - 35 S. D. Parlee, S. I. Lentz, H. Mori and O. A. MacDougald, in *Methods of Adipose Tissue Biology*, Pt A, ed. O. A. MacDougald, 2014, vol. 537, pp. 93–122.
 - 36 E. Murphy, P. Cotter, S. Healy, T. M. Marques, O. O'sullivan, F. Fouhy, S. Clarke, P. O'toole, E. M. Quigley and C. Stanton, Composition and energy harvesting capacity of the gut microbiota: relationship to diet, obesity and time in mouse models, *Gut*, 2010, **59**, 1635–1642.
 - 37 B. Zhao, J. Wu, J. Li, Y. Bai, Y. Luo, B. Ji, B. Xia, Z. Liu, X. Tan and J. Lv, Lycopene alleviates DSS-induced colitis and behavioral disorders via mediating microbes-gut-brain axis balance, *J. Agric. Food Chem.*, 2020, **68**, 3963–3975.
 - 38 K. Peng, J. Deng, N. Zou, X. Sun, W. Huang, R. Li and X. Yang, Emergence of the fourth mobile sulfonamide resistance gene sul4 in clinical Salmonella enterica, *Front. Microbiol.*, 2023, **14**, 1242369.
 - 39 H. Gao, C. He, R. Hua, C. Liang, B. Wang, Y. Du, S. Xin, Y. Guo, L. Gao and L. Zhang, Underlying beneficial effects of Rhubarb on constipation-induced inflammation, disorder of gut microbiome and metabolism, *Front. Pharmacol.*, 2022, **13**, 1048134.
 - 40 J. I. van der Vaart, R. van Eenige, P. C. Rensen and S. Kooijman, Atherosclerosis: an overview of mouse models and a detailed methodology to quantify lesions in the aortic root, *Vasc. Biol.*, 2024, **6**, e230017.
 - 41 Q. Zhou, Y. Wang, Y. Cheng, J. Zhou, W. Liu, X. Ma, S. Tang, S. Tang and C. Tang, Ilexgenin A inhibits lipid accumulation in macrophages and reduces the progression of atherosclerosis through PTPN2/ERK1/2/ABCA1 signalling pathway, *Biochim. Biophys. Acta, Mol. Cell Biol. Lipids*, 2024, **1869**, 159533.
 - 42 W. Lei, S. Qian, X. Zhu and J. Hu, Haemodynamic effects on the development and stability of atherosclerotic plaques in arterial blood vessel, *Interdiscip. Sci.: Comput. Life Sci.*, 2023, **15**, 616–632.
 - 43 M. Zhai, S. Gong, P. Luan, Y. Shi, W. Kou, Y. Zeng, J. Shi, G. Yu, J. Hou and Q. Yu, Extracellular traps from activated vascular smooth muscle cells drive the progression of atherosclerosis, *Nat. Commun.*, 2022, **13**, 7500.
 - 44 J. Xie, L. Peng, C. Yang, N. Chen, X. Feng, T. Wu, T. Xu and Y. Chen, QiShenYiQi pill inhibits atherosclerosis by promoting reverse cholesterol transport PPAR γ -LXR α / β -ABCA1 pathway, *J. Ethnopharmacol.*, 2023, **315**, 116684.
 - 45 Y. Xie, H. Chen, P. Qu, X. Qiao, L. Guo and L. Liu, Novel insight on the role of Macrophages in atherosclerosis: Focus on polarization, apoptosis and efferocytosis, *Int. Immunopharmacol.*, 2022, **113**, 109260.
 - 46 A. Tomaszewska, W. Gonciarz, T. Rechcinski, M. Chmiela, A. K. Kurdowska and A. Krupa, Helicobacter pylori components increase the severity of metabolic syndrome and its hepatic manifestations induced by a high fat diet, *Sci. Rep.*, 2024, **14**, 5764.
 - 47 B. Yao, J. Ma, Q. Ran, H. Chen and X. He, Mechanism of Valeriana officinalis L. extract improving atherosclerosis by regulating PGC-1 α /Sirt3/Epac1 pathway, *Front. Pharmacol.*, 2024, **15**, 1483518.
 - 48 S. Zhao, J. Zhong, C. Sun and J. Zhang, Effects of aerobic exercise on TC, HDL-C, LDL-C and TG in patients with

- hyperlipidemia: A protocol of systematic review and meta-analysis, *Medicine*, 2021, **100**, e25103.
- 49 X.-Y. Yang, D.-Y. Zhong, G.-L. Wang, R.-G. Zhang and Y.-L. Zhang, Effect of walnut meal peptides on hyperlipidemia and hepatic lipid metabolism in rats fed a high-fat diet, *Nutrients*, 2021, **13**, 1410.
 - 50 P. Libby, P. M. Ridker, G. K. Hansson and L. T. N. o. Atherothrombosis, Inflammation in atherosclerosis: from pathophysiology to practice, *J. Am. Coll. Cardiol.*, 2009, **54**, 2129–2138.
 - 51 A. Edsfieldt, H. Grufman, G. Ascitto, M. Nitulescu, A. Persson, M. Nilsson, J. Nilsson and I. Gonçalves, Circulating cytokines reflect the expression of pro-inflammatory cytokines in atherosclerotic plaques, *Atherosclerosis*, 2015, **241**, 443–449.
 - 52 P. Tsioufis, P. Theofilis, K. Tsioufis and D. Tousoulis, The impact of cytokines in coronary atherosclerotic plaque: current therapeutic approaches, *Int. J. Mol. Sci.*, 2022, **23**, 15937.
 - 53 I. Bondia-Pons, L. Ryan and J. A. Martinez, Oxidative stress and inflammation interactions in human obesity, *J. Physiol. Biochem.*, 2012, **68**, 701–711.
 - 54 A. Loboda, M. Damulewicz, E. Pyza, A. Jozkowicz and J. Dulak, Role of Nrf2/HO-1 system in development, oxidative stress response and diseases: an evolutionarily conserved mechanism, *Cell. Mol. Life Sci.*, 2016, **73**, 3221–3247.
 - 55 S. Saha, B. Buttari, E. Panieri, E. Profumo and L. Saso, An overview of Nrf2 signaling pathway and its role in inflammation, *Molecules*, 2020, **25**, 5474.
 - 56 Y.-S. Keum and B. Y. Choi, Molecular and chemical regulation of the Keap1-Nrf2 signaling pathway, *Molecules*, 2014, **19**, 10074–10089.
 - 57 Y. Zhu, X. Xian, Z. Wang, Y. Bi, Q. Chen, X. Han, D. Tang and R. Chen, Research progress on the relationship between atherosclerosis and inflammation, *Biomolecules*, 2018, **8**, 80.
 - 58 J. Moriya, Critical roles of inflammation in atherosclerosis, *J. Cardiol.*, 2019, **73**, 22–27.
 - 59 E. Punch, J. Klein, P. Diaba-Nuhoho, H. Morawietz and M. Garelnabi, Effects of PCSK9 targeting: alleviating oxidation, inflammation, and atherosclerosis, *J. Am. Heart Assoc.*, 2022, **11**, e023328.
 - 60 V. Singh, R. Kaur, P. Kumari, C. Pasricha and R. Singh, ICAM-1 and VCAM-1: Gatekeepers in various inflammatory and cardiovascular disorders, *Clin. Chim. Acta*, 2023, **548**, 117487.
 - 61 S. Marinović Kulišić, M. Takahashi, M. Himelreich Perić, V. Mužić Radović and R. Jurakić Tončić, Immunohistochemical analysis of adhesion molecules e-selectin, intercellular adhesion molecule-1, and vascular cell adhesion molecule-1 in inflammatory lesions of atopic dermatitis, *Life*, 2023, **13**, 933.
 - 62 X. Jian, Y. Liu, Z. Zhao, L. Zhao, D. Wang and Q. Liu, The role of traditional Chinese medicine in the treatment of atherosclerosis through the regulation of macrophage activity, *Biomed. Pharmacother.*, 2019, **118**, 109375.
 - 63 N. Schwarz, S. Fernando, Y. C. Chen, T. Salagaras, S. R. Rao, S. Liyanage, A. E. Williamson, D. Toledo-Flores, C. Dimasi and T. J. Sargeant, Colchicine exerts anti-atherosclerotic and-plaque-stabilizing effects targeting foam cell formation, *FASEB J.*, 2023, **37**, e22846.
 - 64 J. Yu, H. Sun, Y. Yang and Y. Yan, Sesamolin alleviates non-alcoholic fatty liver disease through modulating gut microbiota and metabolites in high-fat and high-fructose diet-fed mice, *Int. J. Mol. Sci.*, 2022, **23**, 13853.
 - 65 K. Zhang, X. Qin, J. Qiu, T. Sun, K. Qu, A. U. Din, W. Yan, T. Li, Y. Chen and W. Gu, Desulfovibrio desulfuricans aggravates atherosclerosis by enhancing intestinal permeability and endothelial TLR4/NF-κB pathway in ApoE^{−/−} mice, *Genes Dis.*, 2023, **10**, 239–253.
 - 66 P. Chen, M. Ma, X. Chen, Z. Xu, J. Guo, X. Hu, L. Lv, J. Guo and G. Liu, Foodborne Carbon Dots-Induced Gut Microbiota Dysbiosis Lead to Reproductive Toxicity in Male Mice, *Fertil. Steril.*, 2024, **1**, 131.
 - 67 Y. Zhang, C. Wang, H. Lang, H. Yu, M. Zhou, X. Rao, Q. Zhang, L. Yi, J. Zhu and M. Mi, The Contrasting Effects of Two Distinct Exercise Training Modalities on Exhaustive Exercise-Induced Muscle Damage in Mice May Be Associated with Alterations in the Gut Microbiota, *Int. J. Mol. Sci.*, 2024, **25**, 7837.
 - 68 M. Schneeberger, A. Everard, A. G. Gómez-Valadés, S. Matamoros, S. Ramírez, N. M. Delzenne, R. Gomis, M. Claret and P. D. Cani, Akkermansia muciniphila inversely correlates with the onset of inflammation, altered adipose tissue metabolism and metabolic disorders during obesity in mice, *Sci. Rep.*, 2015, **5**, 16643.
 - 69 G. Shi, Y. Lin, Y. Wu, J. Zhou, L. Cao, J. Chen, Y. Li, N. Tan and S. Zhong, Bacteroides fragilis supplementation deteriorated metabolic dysfunction, inflammation, and aorta atherosclerosis by inducing gut microbiota dysbiosis in animal model, *Nutrients*, 2022, **14**, 2199.
 - 70 J. R. Fletcher, C. M. Pike, R. J. Parsons, A. J. Rivera, M. H. Foley, M. R. McLaren, S. A. Montgomery and C. M. Theriot, Clostridioides difficile exploits toxin-mediated inflammation to alter the host nutritional landscape and exclude competitors from the gut microbiota, *Nat. Commun.*, 2021, **12**, 462.
 - 71 R. Z. Vlahcevic, D. M. Heuman and P. B. Hylemon, Regulation of bile acid synthesis, *Hepatology*, 1991, **13**, 590–600.
 - 72 Y. Zhang, X. Jiang, W. Wang, L. Lei, R. Sheng, S. Li, J. Luo, H. Liu, J. Zhang and X. Han, ASGR1 Deficiency Inhibits Atherosclerosis in Western Diet-Fed ApoE^{−/−} Mice by Regulating Lipoprotein Metabolism and Promoting Cholesterol Efflux, *Arterioscler., Thromb., Vasc. Biol.*, 2024, **44**, 2428–2449.
 - 73 T.-t. Wang, C.-y. Yang, L. Peng, L. Li, N.-t. Chen, X. Feng, J. Xie, T.-c. Wu, T. Xu and Y.-z. Chen, QiShenYiQi pill inhibits atherosclerosis by promoting TTC39B-LXR mediated reverse cholesterol transport in liver, *Phytomedicine*, 2024, **123**, 155192.
 - 74 C. Martin-Gallausiaux, L. Marinelli, H. M. Blottière, P. Larraufie and N. Lapaque, SCFA: mechanisms and func-

- tional importance in the gut, *Proc. Nutr. Soc.*, 2021, **80**, 37–49.
- 75 X. Zhou, S. Pak, D. Li, L. Dong, F. Chen, X. Hu and L. Ma, Bamboo shoots modulate gut microbiota, eliminate obesity in high-fat-diet-fed mice and improve lipid metabolism, *Foods*, 2023, **12**, 1380.
 - 76 M. Hemmati, S. Kashanipoor, P. Mazaheri, F. Alibabaei, A. Babaeizad, S. Asli, S. Mohammadi, A. H. Gorgin, K. Ghods and B. Yousefi, Importance of gut microbiota metabolites in the development of cardiovascular diseases (CVD), *Life Sci.*, 2023, **329**, 121947.
 - 77 X. Liang, X. Zheng, P. Wang, H. Zhang, Y. Ma, H. Liang and Z. Zhang, *Bifidobacterium animalis* subsp. *lactis* F1–7 Alleviates Lipid Accumulation in Atherosclerotic Mice via Modulating Bile Acid Metabolites to Downregulate Intestinal FXR, *J. Agric. Food Chem.*, 2024, **72**, 2585–2597.
 - 78 B. J. Verhaar, A. Prodan, M. Nieuwdorp and M. Muller, Gut microbiota in hypertension and atherosclerosis: a review, *Nutrients*, 2020, **12**, 2982.
 - 79 M. Abdi, H. E. G. Ghaleh and R. Ranjbar, *Lactobacilli* and *Bifidobacterium* as anti-atherosclerotic agents, *Iran. J. Basic Med. Sci.*, 2022, **25**, 934.
 - 80 F. Yang, C. Liu, X. Lu, S. Liu, Y. Zhang, Y. Yu and Y. Long, *Cistanche deserticola* improves postmenopausal osteoporosis through gut microbiota and short-chain fatty acids, *J. Funct. Foods*, 2024, **112**, 105925.
 - 81 Y. Sun, Z. Li, M. Yan, H. Zhao, Z. He and M. Zhu, Responses of Intestinal Antioxidant Capacity, Morphology, Barrier Function, Immunity, and Microbial Diversity to Chlorogenic Acid in Late-Peak Laying Hens, *Animals*, 2024, **14**, 2957.
 - 82 J. Paek, L. Bai, Y. Shin, H. Kim, S. H. Kim, J. H. Shin, J.-K. Kook and Y.-H. Chang, Description of *Olsenella absiana* sp. nov., an anaerobic bacterium isolated from pig faeces, *Int. J. Syst. Evol. Microbiol.*, 2024, **74**, 006579.
 - 83 Q. Zhang, S. Zhang, S. Wu, M. H. Madsen and S. Shi, Supplementing the early diet of broilers with soy protein concentrate can improve intestinal development and enhance short-chain fatty acid-producing microbes and short-chain fatty acids, especially butyric acid, *J. Anim. Sci. Biotechnol.*, 2022, **13**, 97.
 - 84 M. Zhao, Y. Zhang, Y. Li, K. Liu, K. Bao and G. Li, Impact of *Pediococcus acidilactici* GLP06 supplementation on gut microbes and metabolites in adult beagles: a comparative analysis, *Front. Microbiol.*, 2024, **15**, 1369402.
 - 85 I. Calvete-Torre, C. Sabater, M. Villamiel, A. Margolles, P. Méndez-Albiñana, L. Ruiz and O. Hernandez-Hernandez, Exploring the modulatory effect of trehalose-derived galactooligosaccharides on key gut microbiota groups, *Int. J. Biol. Macromol.*, 2024, **273**, 133053.
 - 86 X. Pi, W. Teng, D. Fei, G. Zhao and W. Liu, Effects of live combined *Bacillus subtilis* and *Enterococcus faecium* on gut microbiota composition in C57BL/6 mice and in humans, *Front. Cell. Infect. Microbiol.*, 2022, **12**, 821662.
 - 87 D. Yao, M. Wu, Y. Dong, L. Ma, X. Wang, L. Xu, Q. Yu and X. Zheng, In vitro fermentation of fructooligosaccharide and galactooligosaccharide and their effects on gut microbiota and SCFAs in infants, *J. Funct. Foods*, 2022, **99**, 105329.
 - 88 J. Song, Q. Li, N. Everaert, R. Liu, M. Zheng, G. Zhao and J. Wen, Dietary inulin supplementation modulates short-chain fatty acid levels and cecum microbiota composition and function in chickens infected with *Salmonella*, *Front. Microbiol.*, 2020, **11**, 584380.
 - 89 J. G. LeBlanc, F. Chain, R. Martín, L. G. Bermúdez-Humarán, S. Courau and P. Langella, Beneficial effects on host energy metabolism of short-chain fatty acids and vitamins produced by commensal and probiotic bacteria, *Microb. Cell Fact.*, 2017, **16**, 1–10.
 - 90 Y. Liu, X. Jin, Y. Ma, Z. Jian, Z. Wei, L. Xiang, Q. Sun, S. Qi, K. Wang and H. Li, Short-chain fatty acids reduced renal calcium oxalate stones by regulating the expression of intestinal oxalate transporter SLC26A6, *Msystems*, 2021, **6**, e01045–e01021.
 - 91 D. Parada Venegas, M. K. De la Fuente, G. Landskron, M. J. González, R. Quera, G. Dijkstra, H. J. Harmsen, K. N. Faber and M. A. Hermoso, Short chain fatty acids (SCFAs)-mediated gut epithelial and immune regulation and its relevance for inflammatory bowel diseases, *Front. Immunol.*, 2019, **10**, 277.
 - 92 Y. Feng and D. Xu, Short-chain fatty acids are potential goalkeepers of atherosclerosis, *Front. Pharmacol.*, 2023, **14**, 1271001.
 - 93 Y. Du, X. Li, C. Su, M. Xi, X. Zhang, Z. Jiang, L. Wang and B. Hong, Butyrate protects against high-fat diet-induced atherosclerosis via up-regulating ABCA1 expression in apolipoprotein E-deficiency mice, *Br. J. Pharmacol.*, 2020, **177**, 1754–1772.
 - 94 A. Haghikia, F. Zimmermann, P. Schumann, A. Jasina, J. Roessler, D. Schmidt, P. Heinze, J. Kaisler, V. Nageswaran and A. Aigner, Propionate attenuates atherosclerosis by immune-dependent regulation of intestinal cholesterol metabolism, *Eur. Heart J.*, 2022, **43**, 518–533.
 - 95 J. Moleón, C. González-Correa, I. Robles-Vera, S. Miñano, N. de la Visitación, A. M. Barranco, N. Martín-Morales, F. O'Valle, L. Mayo-Martínez and A. García, Targeting the gut microbiota with dietary fibers: A novel approach to prevent the development cardiovascular complications linked to systemic lupus erythematosus in a preclinical study, *Gut Microbes*, 2023, **15**, 2247053.
 - 96 H. Duan, L. Wang, M. Huangfu and H. Li, The impact of microbiota-derived short-chain fatty acids on macrophage activities in disease: Mechanisms and therapeutic potentials, *Biomed. Pharmacother.*, 2023, **165**, 115276.
 - 97 H. Ma, L. Yang, Y. Liu, R. Yan, R. Wang, P. Zhang, Z. Bai, Y. Liu, Y. Ren and Y. Li, Butyrate suppresses atherosclerotic inflammation by regulating macrophages and polarization via GPR43/HDAC-miRNAs axis in ApoE^{−/−} mice, *PLoS One*, 2023, **18**, e0282685.
 - 98 R. Fellows, J. Denizot, C. Stellato, A. Cuomo, P. Jain, E. Stoyanova, S. Balázs, Z. Hajnád, A. Liebert and J. Kazakevych, Microbiota derived short chain fatty acids promote histone crotonylation in the colon through histone deacetylases, *Nat. Commun.*, 2018, **9**, 105.