



Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com



Original article

## Moderate exercise ameliorates osteoarthritis by reducing lipopolysaccharides from gut microbiota in mice

Kefeng Li <sup>a</sup>, Anli Liu <sup>b</sup>, Wenhao Zong <sup>a</sup>, Lulu Dai <sup>a</sup>, Yang Liu <sup>a</sup>, Renping Luo <sup>a</sup>, Shulin Ge <sup>c,\*</sup>, Guijun Dong <sup>a,\*</sup><sup>a</sup> College of Sports and Health, Shandong Sport University, Jinan 250102, China<sup>b</sup> Department of Acupuncture, Rizhao Hospital of Traditional Chinese Medicine, Rizhao 276800, China<sup>c</sup> School of Physical Education, Shandong Normal University, Jinan 250014, China

## ARTICLE INFO

## Article history:

Received 10 April 2020

Revised 13 August 2020

Accepted 16 August 2020

Available online 21 August 2020

## Keywords:

Berberine

Endotoxin

Unhealthy diets

Intestinal microorganisms

OA

## ABSTRACT

Lipopolysaccharides (LPSs) released by gut microbiota are correlated with the pathophysiology of osteoarthritis (OA). Exercise remodels the composition of gut microbiota. The present study investigated the hypothesis that wheel-running exercise prevents knee OA induced by high-fat diet (HFD) via reducing LPS from intestinal microorganisms. Male C57BL/6 J mice were treated with sedentary or wheel-running exercise, standard diet (13.5% kcal) or HFD (60% kcal), berberine or not according to their grouping. Knee OA severity, blood and synovial fluid LPS, cecal microbiota, and TLR4 and MMP-13 expression levels were determined. Our findings reveal that HFD treatment decreased gut microbial diversity. Increase in endotoxin-producing bacteria, decrease in gut barrier-protecting bacteria, high LPS levels in the blood and synovial fluid, high TLR4 and MMP-13 expression levels, and severe cartilage degeneration were observed. By contrast, voluntary wheel running caused high gut microbial diversity. The gut microbiota were reshaped, LPS levels in the blood and synovial fluid and TLR4 and MMP-13 expression levels were low, and cartilage degeneration was ameliorated. Berberine treatment reduced LPS levels in the samples, but decreased the diversity of intestinal flora with similar changes to that caused by HFD. In conclusion, unlike taking drugs, exercising can remodel gut microbial ecosystems, reduce the circulating levels of LPS, and thereby contribute to the relief of chronic inflammation and OA. Our findings showed that moderate exercise is a potential therapeutic approach for preventing and treating obesity-related OA.

© 2020 The Authors. Published by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

## 1. Introduction

Osteoarthritis (OA) is one common arthritic illness, but its etiology and pathogenesis are not fully understood. Current pharmacologic treatments may improve the pain but there is no effect on the progression of the illness. However, non-pharmacologic treatments in cartilage disease, such as physical activity, nutrients and vitamins, have been shown to affect the course of the disease (Ageberg and Roos, 2015; Messina et al., 2019). Obesity or overweight is considered to be a predisposing risk inductor for OA.

Nowadays, exercise is prescribed as an indispensable treatment. Apart from weight loss, exercise improves joint biomechanics and thus beneficial to OA treatment (Rios et al., 2018). However, the high frequency of OA occurrence in non-weight-bearing joints suggests that the investigation of the mechanism of exercise in OA treatment is incomplete (Leung et al., 2014).

In obesity, chronic inflammation, as a result of homeostasis imbalance between immune and metabolic responses, underlies many chronic metabolic diseases (Hotamisligil and Erbay, 2008). Emerging evidence suggests that enteric dysbacteriosis caused by high -fat diet (HFD) feeding is the potential drivers of metabolic inflammation, and perturbations in gut microbiota composition and intestinal barrier disruption can increase epithelial permeability and the translocation of lipopolysaccharides (LPSs) from the intestinal cavity to the circulatory system and activate innate immune responses (Hersoug et al., 2016). As a pathogen-related molecular pattern, the bacterial membrane component LPS can trigger many proinflammatory pathways, thereby initiating signaling cascades (Huang and Kraus, 2016). Toll-like receptors (TLRs) can recognize LPS, thereby activating the TLR pathways (Huang

\* Corresponding authors.

E-mail addresses: [1661650910@qq.com](mailto:1661650910@qq.com) (S. Ge), [dongguijun@sdpei.edu.cn](mailto:dongguijun@sdpei.edu.cn) (G. Dong).

Peer review under responsibility of King Saud University.

<https://doi.org/10.1016/j.sjbs.2020.08.027>

1319-562X/© 2020 The Authors. Published by Elsevier B.V. on behalf of King Saud University.

This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

and Kraus, 2016). In LPS/TLR4 pathways, the upregulated expression of TLR4 directly or indirectly activates matrix metalloproteinases (MMPs) and leads to cartilage degeneration (Matsumoto et al., 2008). Hence, high circulating LPS levels and imbalanced gut microbiota are presumed to be closely associated to the initiation of OA (Huang and Kraus, 2016; Robinson et al., 2016). Some animal experiments have supported this inference. In the rats fed with HFD, the serum LPS levels were higher than those fed with chow; moreover, the abundance in *Lactobacillus* spp. exhibited a significant negative relationship with the progression of OA, whereas the abundance in *Methanobrevibacter* spp. showed a strong positive relationship (Collins et al., 2015). Similarly, an HFD-induced increase in LPS levels, but different microbes, such as *Bacteroides/Prevotella*, *Bifidobacterium*, and *Roseburia*, were negatively related to OA (Rios et al., 2019).

Exercise improves human health and fitness, exemplified by reducing body fat and fasting blood insulin level. An experimental study proved that autonomous wheel movement relieves the development of OA (Griffin et al., 2012). The comprehensive treatment combined with exercise and dietary intake exerted better protective effect on knee health than that a single intervention (Rios et al., 2019). To date, OA is no longer considered as a simple cartilage degenerative disease, but as a global joint chaos with heterogeneity and multiple etiologies (Robinson et al., 2016). Similar to obesity, metabolic OA is a chronic, systemic, and low-grade inflammatory condition (Berenbaum, 2013; Robinson et al., 2016). Scientific evidence suggests that moderate-level running provides not only cyclical loading to the knee, which is important to maintaining cartilage homeostasis, but also improves metabolic state in individuals with chronic systemic inflammation (Ageberg and Roos, 2015; Uchiyama et al., 2019), wherein the improvement may be driven by reshaped gut microbiota. Exercise, unlike HFDs, can be a stronger modulator of gut intestinal homeostasis. *Akkermansia muciniphilla* abundance was found inversely correlated with obesity and associated metabolic disorders; interestingly, athletes with low body mass indices (BMIs) demonstrated higher *Akkermansia* levels than those with high BMIs (Clarke et al., 2014). *Lactobacillus* and *Bifidobacterium*, which have potential values in OA treatment (Schott et al., 2018), also can be positively regulated by exercise (Codella et al., 2018). Increasing evidence reveals that exercise can diversify intestinal microorganisms and reform the balance between the richness of beneficial and harmful microbes (Mailing et al., 2019; Matsumoto et al., 2008). Therefore, moderate running exercise, such as voluntary wheel running, seems to reduce systemic inflammation and metabolic dysregulation and thus contribute to OA prevention.

Insights into the inflammatory pathophysiology underpinnings of OA suggest that alleviating inflammation may prevent the onset or minimize the progression of OA (Berenbaum, 2013; Griffin et al., 2012; Huang and Kraus, 2016). To clarify the mechanism of obesity-associated OA and find a potential therapeutic approach, we used male C57BL/6J mice to establish an OA model of obese mice with HFD feeding and investigated the difference in intestinal flora composition, LPS level in the blood and knee joint cavity, LPS/TLR4 pathway, and the degrading degree of OA after HFDs and/or voluntary wheel running. The data reveal that voluntary wheel running ameliorates OA by reducing LPS level in the blood and knee joint cavity. Whether the mechanism on how exercise reshapes intestinal flora community and alleviates inflammation is the same as that of intestinal -regulating drugs on intestinal flora must be investigated (Gao et al., 2017a). Therefore, berberine, which is an isoquinolinederivative alkaloid that has been traditionally used in the treatment of gastrointestinal infections, was selected as the control due to its antimicrobial properties. Berberine also regulates metabolic endotoxemia levels and demonstrates therapeutic potential for OA (Wong et al., 2019).

## 2. Materials and methods

### 2.1. Animals and treatment

The experimental protocol has been approved by Animal Care and Use Committee of Shandong Sport University. Dietary intake in this experiment included either standard diets (13.5% kcal) or HFDs (60% kcal; No. D12492, Beijing Keao Co-operative Feed Co., Ltd.). All the mice were allowed to eat freely, weighed once a week, kept one per cage throughout the experiment and maintained in the same environment. We used the standard = RAND () function in Microsoft Excel to realize the random grouping.

After 4 weeks of environmental acclimatization (1 rat per cage; fed with standard diet), 54 male C57BL/6J mice (body mass =  $20.1 \pm 2.0$  g) were randomly divided into either the standard diet group (control group, n = 18) or HFD group (fed with HFDs, n = 36) at 12 weeks of age. When the 8-week dietary intervention was ended, six mice in each group were randomly selected to take their knee joints for histological and histochemical analyses to verify whether knee OA has been successfully induced by the 8-week-old HFDs.

The remaining mice aged 20 weeks in the control group were randomly divided into RC\_Sed group (sedentary, n = 6) and RC\_Ex group (exercise, n = 6). In the HFD group, the obese mice with a body weight of 20% greater than that of the control group were divided into HF\_Sed group (sedentary, n = 6) and HF\_Ex group (exercise, n = 6). A free-wheel running device (with a diameter of 11.0 cm) was introduced to the exercise group but not to the sedentary group. The number of cycles was recorded through a magnetic inductor (Li et al., 2018). The wheel revolutions in the previous day were counted at 8:00 a.m. every day in the following 4-week experimental period. In the following description, exercise refers to voluntary wheel running, unless otherwise specified. Six obese mice were exposed to drug treatment (HF\_Bbr), with a dose of 150 mg/kg of berberine administered twice a week.

After the last exercise, the mice were subjected to 12 h fasting, but water was made available ad libitum. Blood samples were obtained by retro-orbital phlebotomy under ether anesthesia. The mice were then sacrificed through cervical dislocation. We obtained the following materials from the mice: 1) The left and right knee joints were obtained. 2) The knee -joint synovial fluid was collected from their knee joint cavities. We used a syringe to prick one side of the joint capsule along the frontal surface to form a "puncture hole." Endotoxin-free water (500  $\mu$ l) was slowly pushed into the joint cavity with the syringe on the opposite side of the "puncture hole" and then flowed from the "puncture hole" into an endotoxin-free tube. The fluid from both knee joints was pooled as one sample. 3) The cecum contents were collected in a sterile Eppendorf tube for the following intestinal flora composition analysis.

### 2.2. Bacterial community analysis

Microbial DNA was extracted from the cecum contents for bacterial analysis. Illumina MiSeq platform (Majorbio Co, Shanghai, China) was used to amplify and sequence the 16S rRNA gene of the bacterial microbes. The raw sequencing data were quality-filtered for further analysis on I-Sanger Platform. Operational taxonomic units were clustered with a similarity of 97%.

### 2.3. Histological and histochemical analyses

We used hematoxylin–eosin (HE) staining to assess the complete morphology and structural change of articular cartilage and toluidine blue staining to display proteoglycan content. The

muscles, ligaments, and patella around the joint were removed (by paying attention not to injure the cartilage surface). The whole left joint was fixed in 4% paraformaldehyde fixative solution. After half an hour of flushing with running tap water, the knee joint was cut along the median sagittal plane and decalcified in a solution of 10% EDTA (pH 7.4) for one month (Gao et al., 2017b). The decalcification solution was replaced every 3 days, the needle could easily penetrate the bone for complete decalcification, and the joint was paraffin-embedded. Then, these samples were sliced serially on the coronal plane with a thickness of 4  $\mu\text{m}$ . One section every 100  $\mu\text{m}$  was stained with HE and toluidine blue referring to the method of Schmitz et al. (2010) with minor modifications.

#### 2.4. Evaluation of OA

Sections stained with HE and Toluidine blue were evaluated for degenerative joint changes (at least 10 sections scored per knee) by three trained, blinded reviewers. Scores were recorded for four areas, lateral femur, lateral tibia, medial femur, and medial tibia according to a 14-point Mankin scale, which included structure, cellularity, staining intensity, and tidemark integrity (Mankin et al., 1971). Scores were averaged to determine Mankin scores for the entire joint with high values indicating severe cartilage degeneration.

#### 2.5. Quantification of cartilage thickness

Six sections of each joint (3 sections of the inner and outer joints) were collected. The thickness of cartilage layer (from the cartilage surface to tidemark) was measured at 11 points centered on the weight-bearing area of the tibial plateau. Five points were taken every 25  $\mu\text{m}$  left and right at  $\times 40$  magnification, and the average value was obtained.

#### 2.6. LPS determination in blood and knee joint synovial fluid

The serum samples were obtained by centrifugation of the blood samples at 3500 rpm for 20 min. To remove the cellular elements of synovial fluid, the sample was centrifuged at 13,500 rpm for 10 min and the supernatant was taken for further testing. LPS determination in blood and knee joint cavity fluid was conducted by the Limulus amoebocyte lysate chromogenic endpoint assay (HIT302; Hycult Biotech). The LPS concentration of samples was calculated by a logarithmic standard curve.

#### 2.7. Western blot analysis

We extracted total protein from articular cartilage homogenate with 1 ml of tissue lysate and protease inhibitor, and collected the supernatant after centrifugation at 12,000 rpm for 15 min at 4  $^{\circ}\text{C}$ , then determined the protein concentration via a BCA Protein Assay Kit (Beyotime Biotechnology Co.). An equal amount of protein was put into gels and resolved. Proteins were transferred to polyvinylidene difluoride membranes and exposed first to anti-MMP13 antibody ab39012, anti-TLR4 antibody ab13556, and anti-GAPDH antibody (ab8245) (Abcam, UK) and then to goat anti-rabbit IgG H&L (HRP) ab6721 (Abcam, MA). Bands were detected and analyzed using ChemiDoc<sup>TM</sup> XRS+ with Image Lab<sup>TM</sup> software (BIO-RAD, USA).

#### 2.8. Statistical analysis

The  $\alpha$ -diversity indices (Ace index and Simpson index) were calculated. Community bar plot analysis was conducted by calculating the average of the absolute abundance values within each group. SPSS version 20 (IBM; Chicago, IL) and GraphPad Prism V

6.02 (La Jolla, California, USA) were used for statistical analysis. LPS level in the blood is shown as the mean  $\pm$  SEM, and other data are shown as mean  $\pm$  SD. The two-way ANOVA with Tukey's multiple comparison post-test was used for the comparisons between multiple groups. For comparisons between two groups, Student's *t* test was used for parametric distribution data, and Mann-Whitney tests for non-parametric distribution data. Adjustment for multiple testing was estimated using false discovery rate functions. The differences were considered significant at  $p < 0.05$ .

### 3. Results

#### 3.1. The weight loss effect of exercise

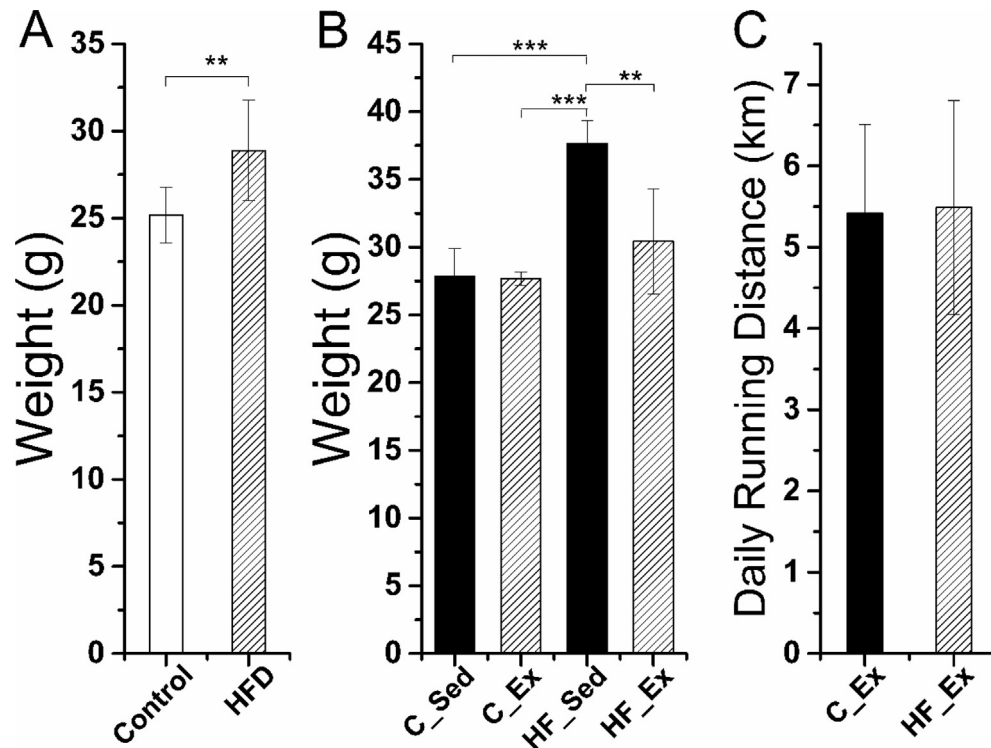
We fed the mice with HFDs from the age of 12 weeks to obtain obese mice. At 20 weeks old, the high-fat diet significantly increased the weight of the mice (Fig. 1A,  $p < 0.01$ ). With prolonged feeding time, the average body weight of HF\_Sed mice was 1.36 times that of RC\_Sed mice at 24 weeks of age with significant difference (Fig. 1B,  $p < 0.0001$ ). Thus, the obesity level of mice was increased. With exercise intervention, the weight of HF\_Ex mice decreased significantly (Fig. 1B,  $p < 0.01$ ), suggesting that 4-week free-wheel running played a role in weight loss. No significant difference were observed in the exercise distance between RC\_Ex and HF\_Ex groups (Fig. 1C), indicating that HFDs did not affect the activity of mice.

#### 3.2. Evaluation of knee OA

Severe articular cartilage fibrillation, loss of tidemark integrity, and surface irregularities were observed in the HF\_Sed group (Fig. 2E). The mice in the HF\_Ex group exhibited ameliorative cartilage degeneration compared with the sedentary mice. Compared with RC\_Sed, the Mankin scores of medial femur, medial tibia, and lateral tibia in HF\_Sed were significantly higher (Fig. 2A, B, and C). The score of lateral femur in HF\_Sed increased by an insignificant amount ( $p = 0.209$ ; Fig. 2D). On the contrary, the scores of medial tibia decreased significantly ( $p < 0.05$ , Fig. 2A), and those of medial femur, lateral tibia, and femur in HF\_Ex decreased insignificantly ( $p = 0.485, 0.270, 0.687$ ; Fig. 2B, C, and D) compared with those in the HF\_Sed group. Table 1 shows that HFDs could significantly reduce the cartilage thickness of medial joint and lateral joint (RC\_Sed vs. HF\_Sed), whereas exercise could increase the cartilage thickness of medial joint ( $p < 0.001$ ). These results indicated that HFDs lead to severe cartilage degeneration, whereas wheel running exercise could ameliorate cartilage degeneration.

#### 3.3. Microbial community diversity characterized by exercise, HFDs, and berberine

We examined whether the dietary and exercise interventions in mice could alter the gut microbial communities in the investigation of the latent mechanism of diet and exercise on OA. The results of bacterial community diversity (Fig. 3A; measured using the Ace index) revealed no difference in community richness between RC\_Sed and RC\_Ex groups. The diversity of HF\_Sed group was significantly lower than that of RC\_Sed group ( $p < 0.05$ ). However, the diversity of HF\_Ex group was significantly higher than that of RC\_Sed group and HF\_Sed group ( $p < 0.05, p < 0.01$ , respectively) groups. There was no statistically significant difference among groups tested with the Simpson diversity index (Fig. 3B). These results showed that HFDs can reduce the community richness of intestinal microflora in mice. Exercise had no effect on the microbiota richness in mice fed with normal diets but increased microbiota richness in the mice fed with HFDs.



**Fig. 1.** Effects of HFD and/or physical activity on the body weight of mice at 8 weeks (A), 12 weeks (B), and daily running distance (C). \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

We also investigated whether berberine has an effect on Intestinal microorganism. The biodiversity of HF\_Bbr group was significantly lower than that of HF\_Ex group ( $p < 0.01$ ). There was no change between HF\_Sed group and HF\_Bbr group. These results suggested that berberine treatments could not augment intestinal microbial diversity.

Distinct intestinal microbial populations were observed among the RC\_Sed, RC\_Ex, HF\_Sed, and HF\_Ex groups. Firmicutes, Bacteroidetes, and Proteobacteria are the three major phyla of gut microbiota. Obese mice in HF\_Sed demonstrated increased abundance in Firmicutes and Proteobacteria but decreased Bacteroidetes, and the Firmicutes/ Bacteroidetes ratio increased relative to that in RC\_Sed (Fig. 4A). Exercise intervention can reverse the pattern of increase or decrease caused by HFDs. Lower abundance in Firmicutes and Proteobacteria, higher abundance in Bacteroidetes and decreased Firmicutes/ Bacteroidetes ratio were observed in HF\_Ex compared with those in HF\_Sed. The performance of the phyla Firmicutes, Bacteroidetes, and Proteobacteria after berberine treatment (HF\_Bbr) was similar to that of the HF\_Sed group, wherein the distinct increases in phyla Firmicutes and Proteobacteria and distinct decrease in phylum Bacteroidetes were observed (Fig. 4A).

At the family level, the described reversal role of exercise was found in Bacteroidales\_S24-7, Lachnospiraceae, Desulfovibrionaceae, Ruminococcaceae, Lactobacillaceae, Prevotellaceae, Peptostreptococcaceae, Bifidobacteriaceae, and Staphylococcaceae (Fig. 4B). For instance, Bacteroidales\_S24-7, Prevotellaceae, and Bifidobacteriaceae appeared at the lower level in HF\_Sed compared with those in RC\_Sed and at the higher level in the HF\_Ex mice compared with that in the HF\_Sed ones. However, Desulfovibrionaceae and Peptostreptococcaceae indicated reverse patterns. Additionally, the performance of berberine treatment was the same as that of HFD and even led to the complete disappearance of certain bacteria, such as Bifidobacteriaceae and Prevotellaceae (Fig. 4B). This pattern indicated that exercise, unlike berberine, can remodel the composition of the intestinal microflora in HFD

animals. We further investigated which bacteria changed significantly due to exercise (Fig. 5). Compared with HF\_Sed, exercise led to a significant increase in some beneficial bacteria ( $p < 0.05$ ), such as two members of the Prevotellaceae family (*Prevotellaceae\_UCG-001* and another unidentified species) and an unidentified member of the family Bacteroidales\_S24-7.

### 3.4. LPS levels determined in the knee -joint synovial fluid and blood

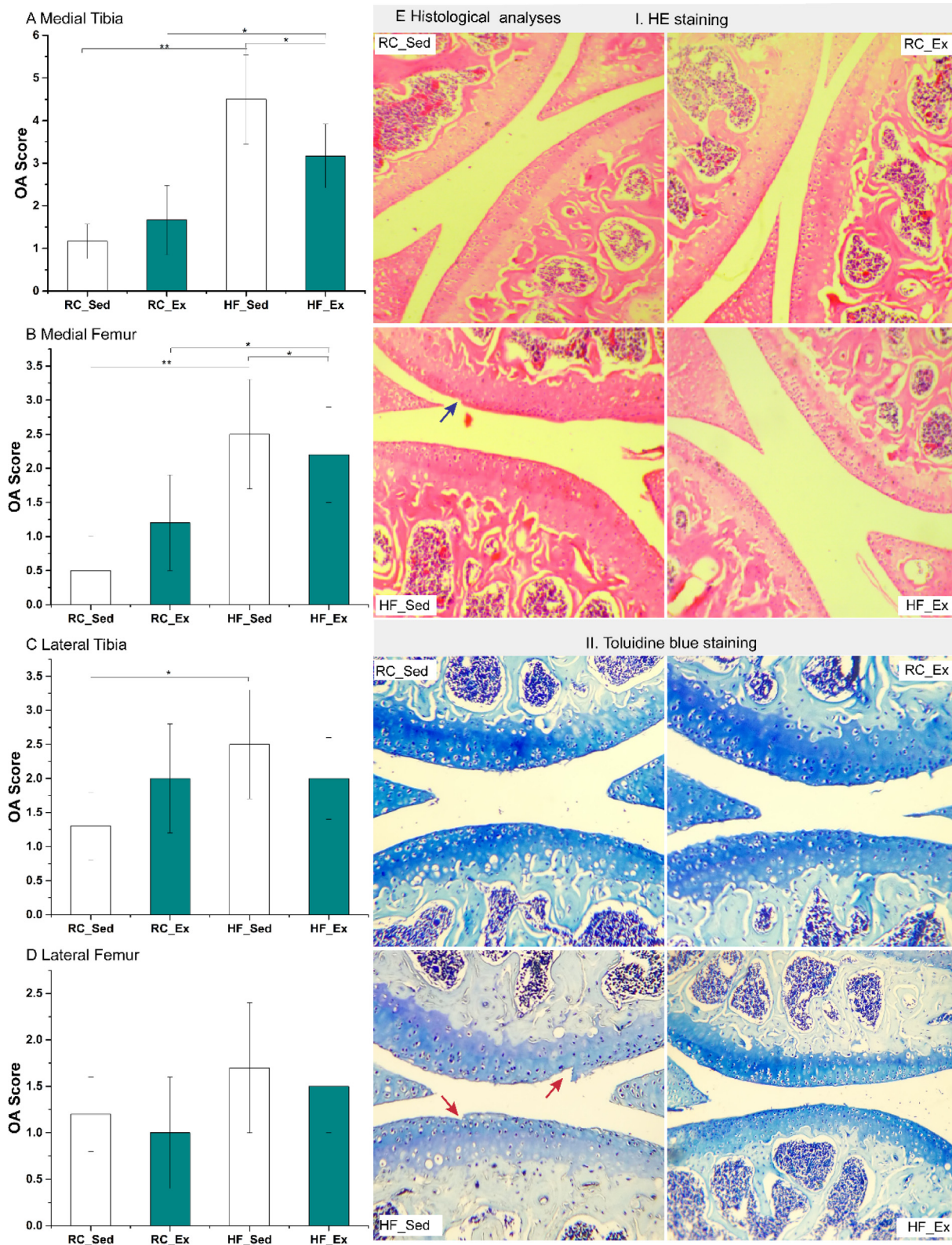
We measured the LPS levels in the samples to determine the effects of HFDs and exercise on endotoxin translocation or clearance. The results showed that the 12-week feeding of HFDs without exercise significantly increased the LPS levels in the blood and synovial fluid compared with that in RC\_Sed, RC\_Ex, and HF\_Ex (Fig. 6). The blood LPS level in HF\_Ex was significantly higher than that in RC\_Ex (Fig. 6A). These results suggested that HFDs contribute to the high LPS level in the blood.

The blood LPS level of RC\_Ex group was significantly lower than that of RC\_Sed group ( $p < 0.05$ , Fig. 6A), and that of HF\_Ex group was significantly lower than that of the HF\_Sed group ( $p < 0.01$ , Fig. 6A). The LPS level in the synovial fluid significantly decreased in the HF\_Ex group compared with that in the HF\_Sed group (Fig. 6B). No significant changes were found in the HF\_Ex and RC\_Ex mice. Our results indicated that exercise intervention could reduce the LPS concentrations in the blood and joint fluids.

Compared with HF\_Sed group, berberine treatment significantly decreased the LPS level in synovial fluid in the HF\_Bbr group ( $p < 0.001$ , Fig. 6B). Additionally, compared with HF\_Sed group, an insignificant decrease in blood LPS level was found in the HF\_Bbr group ( $p > 0.05$ , Fig. 6A).

### 3.5. TLR4 and MMP-13 expression profiles induced by HFD and exercise

The severity and progress of OA are closely related to the levels of TLR4 and MMP (such as MMP-13) in chondrocytes.



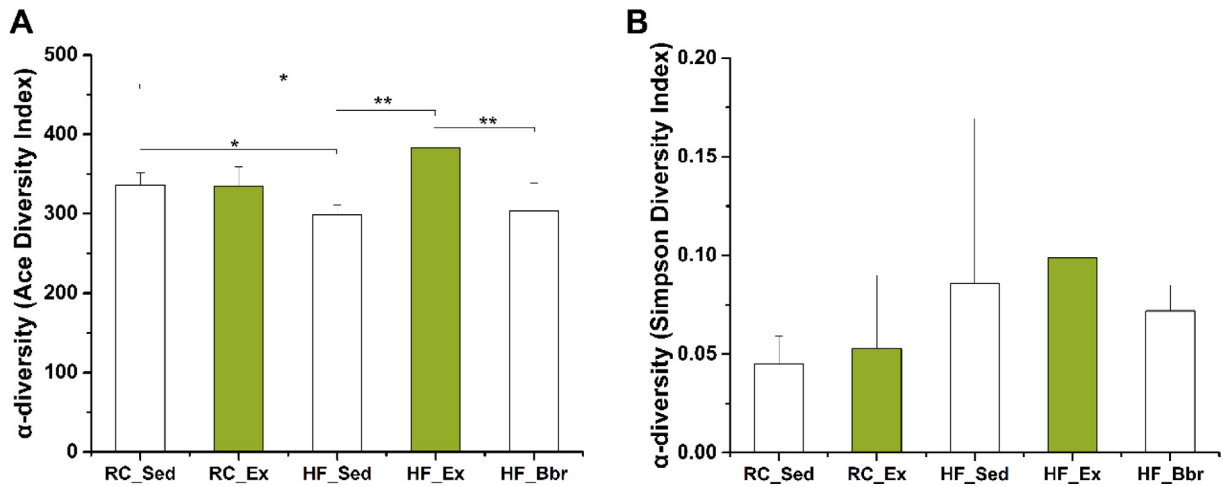
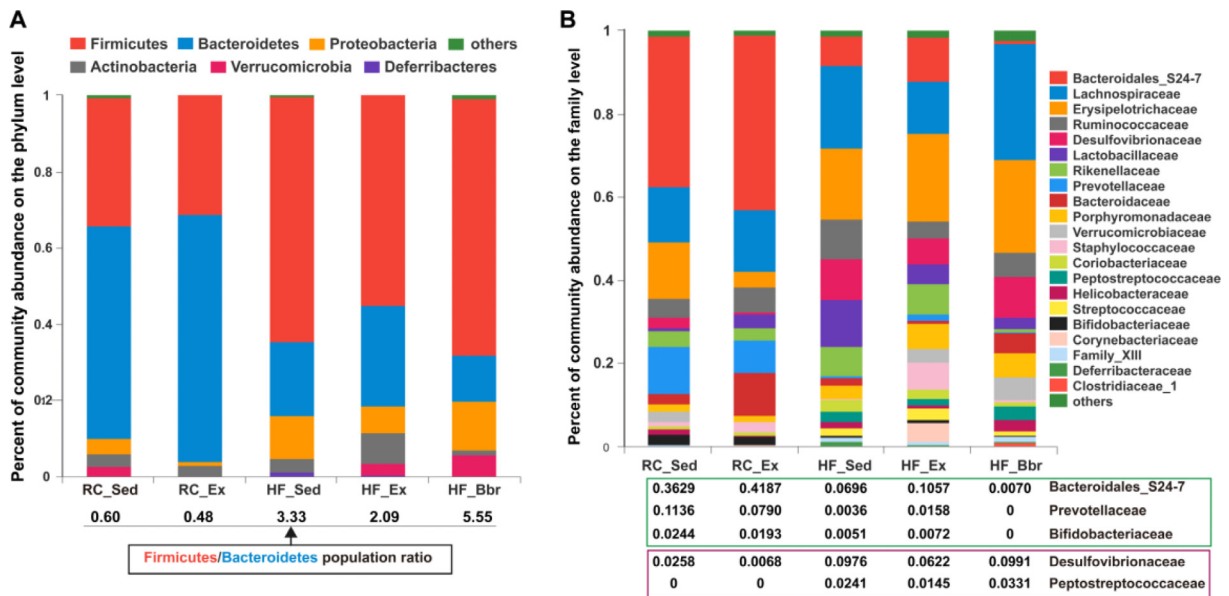
**Fig. 2.** Changes in articular cartilage with HFD and exercise interventions. Mankin scores of articular cartilage in medial tibia (A), medial femur (B), lateral tibia (C), and lateral femur (D); \* $p < 0.05$ , \*\* $p < 0.01$ . E. Histological changes in the effects of HFD and exercise for 12 weeks on articular cartilage at 400 $\times$  views; sheared articular surface (blue and red arrows) were observed in HF\_Sed group.

The association of HFDs and exercise with changes in the expression of TLR4 and MMP-13 in cartilage is depicted in Fig. 7. Compared with RC\_Sed group, the expression levels of TLR4 and MMP-13 were significantly upregulated in the HF\_Sed

group ( $p < 0.05$ ). The levels of HF\_Ex mice were significantly downregulated compared with those in HF\_Sed ( $p < 0.05$ ). No significant changes were observed between the RC\_Sed and HF\_Ex mice. These results indicated that HFDs led to severe

**Table 1**Comparison of cartilage thickness of medial and lateral joints ( $\mu\text{m}$ ; mean  $\pm$  SD).

	C_Sed	HF_Sed	C_Ex	HF_Ex
Medial joint	30.98 $\pm$ 4.67	26.35 $\pm$ 2.35 <sup>***</sup>	32.98 $\pm$ 4.67	30.00 $\pm$ 5.05 <sup>###</sup>
Lateral joint	33.54 $\pm$ 5.18	31.99 $\pm$ 3.18 <sup>*</sup>	33.54 $\pm$ 6.30	31.78 $\pm$ 5.66

Note: <sup>\*</sup>p < 0.05, <sup>\*\*\*</sup>p < 0.001, vs. C-Sed group; <sup>###</sup>p < 0.001, vs. HF-Sed group.**Fig. 3.** Community richness altered by exercise, HFDs, and berberine ( $\alpha$ -diversity).**Fig. 4.** The average relative abundance of each group is shown in the histogram. Firmicutes/Bacteroidetes (shown in A) and the five families (Bacteroidales\_S24-7, Prevotellaceae, Bifidobacteriaceae, Desulfovibrionaceae, and Peptostreptococcaceae; shown in B) for each group are calculated and averaged.

cartilage degeneration, whereas exercise might ameliorate this phenomenon.

#### 4. Discussion

HFDs are an unhealthy dietary pattern contributing to obesity. HFD can boost the proliferation of proinflammatory microbiota but inhibit the probiotics and prebiotics and increase the intestinal permeability and circulating levels of LPS. The absence of activity combined with obesity is a high risk factor of OA onset. According to our data, HFDs can significantly induce weight gain and OA

onset. It has been proved that the gut microbiota holds high -fat feeding, obesity, and OA together. Therefore, controlling obesity by modulating the intestinal microflora may be beneficial to prevent obesity-associated OA. Prior works have shown that voluntary exercise is more beneficial to the improvement of inflammation than compulsive exercise (Matsumoto et al., 2008). Hence, we used a free-wheel running protocol on the C57BL/6J mice to evaluate the role of exercise.

The intestinal microorganism of humans constitutes a superorganism. Thus, the balance between good and maleficent bacteria in the gut microbial community fluctuates due to the host diets. HFDs

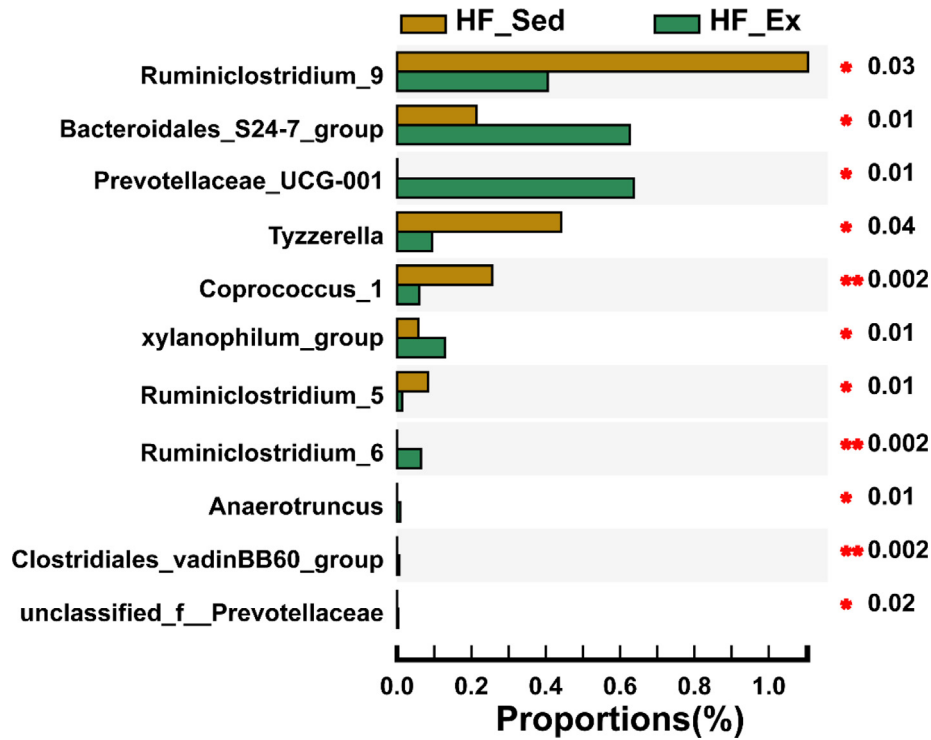


Fig. 5. Comparison of the relative abundance of the intestinal microorganism between HF\_Sed and HF\_Ex at the species level (top 11). \* $p < 0.05$ , \*\*  $p < 0.01$ .

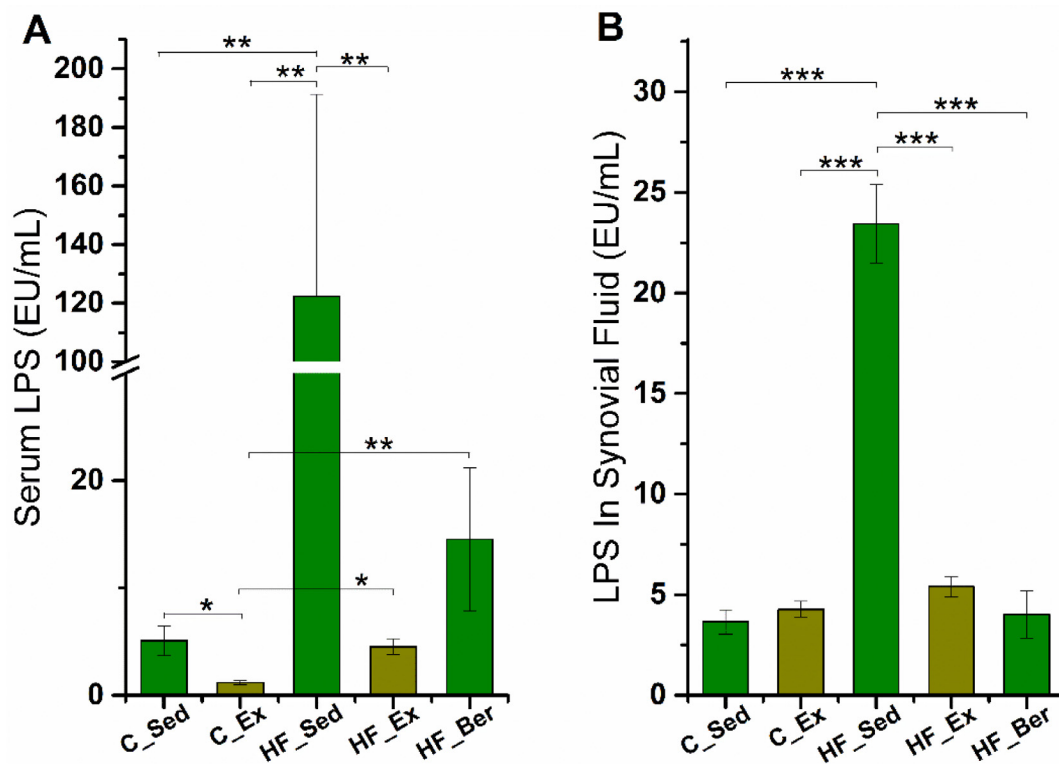


Fig. 6. Changes in the LPS levels in the (A) blood and (B) synovial fluid \* $p < 0.05$ ,  $n = 6$ .

can alter the microbial community structure and reduce the microbial diversity, whereas exercise can diversify the gut microbiota (Codella et al., 2018). It has been hypothesized that Firmicutes are more effective as an energy source than Bacteroidetes, thus

promoting more energy harvest from colonic fermentation and subsequently gaining weight (Koliada et al., 2017). Fluctuations in phyla Bacteroidetes and Firmicutes are often used to analyze the changes in intestinal flora. Although with inconsistencies, the

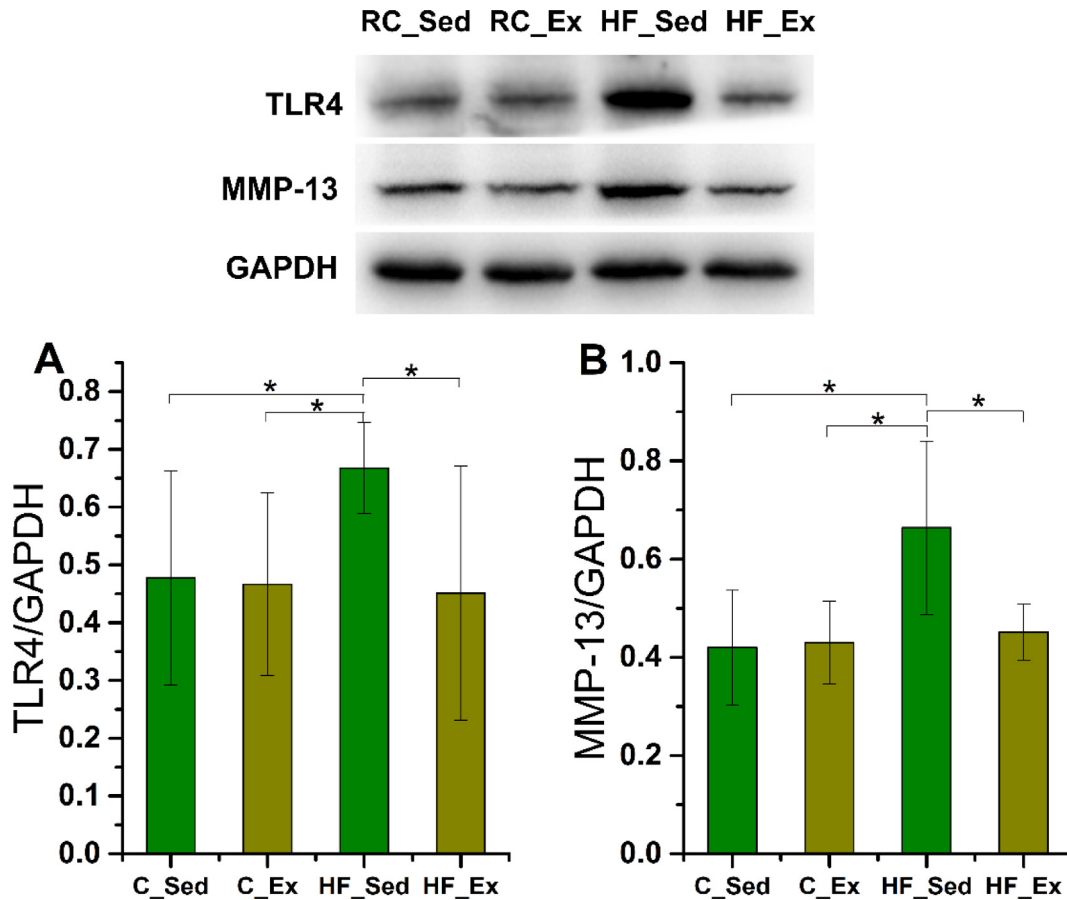


Fig. 7. Changes in the protein expression levels of (A) TLR4 and (B) MMP-13 induced by HFD and exercise. Anti-GAPDH antibodies acted as the loading controls.

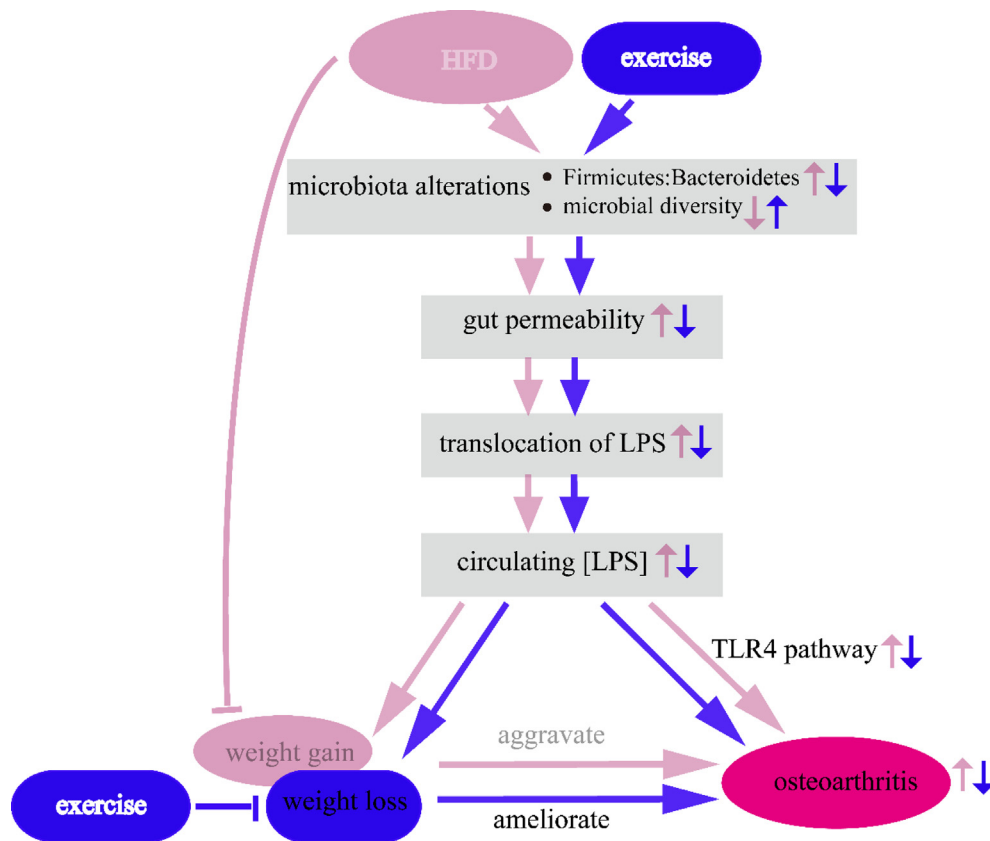
majority of results showed that HFDs and obesity increase the Firmicutes/Bacteroidetes ratio whereas exercise decreases this value (Codella et al., 2018). This viewpoint was validated in our study. We observed a significantly low diversity in the mice fed with HFDs and no movements. Exercise had no effect on the microbiota richness in the mice fed with normal diets but could increase the richness in the mice fed with HFDs. The changes in microbial ecology induced by HFDs and exercise were shown by the comparison of dominant phyla with HFDs or exercise intervention. The results revealed that Firmicutes, Bacteroidetes, and Proteobacteria are susceptible to the treatment with exercise and HFDs. HFDs increased Firmicutes and Proteobacteria and reduced Bacteroidetes. Conversely, exercise increased Bacteroidetes and reduced Firmicutes and Proteobacteria.

At the family level, we also observed the remodeling of the flora driven by exercise. The family Bacteroidales\_S24-7, Prevotellaceae, and Bifidobacteriaceae decreased but Desulfovibrionaceae and Peptostreptococcaceae increased in the HFD-fed mice. However, exercise tended to reverse these changes induced by HFD. The families Desulfovibrionaceae and Peptostreptococcaceae have been identified as endotoxin-producing bacteria (Schott et al., 2018; Zhang et al., 2009), and Bifidobacteriaceae and Prevotellaceae can strengthen the intestinal barrier function, reduce the endotoxin level, and ameliorate metabolic inflammation (Cani et al., 2007). Notably, an uncultured family S24-7 was dominant in the mouse gut microbiota (Ormerod et al., 2016), and the emerging data suggest that these microbes are associated with positive health effects. HFD reduced the abundance of S24-7, whereas exercise substantially rescued its loss in obesity. Additionally, few Bacteroidetes

and substantial Firmicutes are associated with the increased gut permeability and LPS translocation (Cani et al., 2007; Hersoug et al., 2016). Therefore, the above results suggested that exercise might facilitate the enhancement of intestinal barrier but inhibit LPS translocation.

LPS serves as a key mediator of metabolic perturbations in obesity and OA (Cani et al., 2007; Huang and Kraus, 2016). High endotoxin levels have been observed in a sedentary lifestyle; conversely, low endotoxin levels are induced by physical activity (Lira et al., 2010). This phenomenon suggests that physical activity may be beneficial to eliminating LPS in vivo. However, different patterns and intensities of exercise may have various effects on the intestinal flora; for example, voluntary wheel running decreases the Firmicutes/Bacteroidetes ratio, whereas forced treadmill running increases this value (Codella et al., 2018; Mailing et al., 2019). Exercise intensity has similar effects on the intestinal permeability and LPS levels. The intestinal permeability (Pals et al., 1997) and LPS level (Antunes et al., 2019) increase after heavy exercise. Therefore, a compulsory high-intensity exercise seems to be a proinflammatory factor in the body. By contrast, moderate running exercise, such as voluntary wheel running, may contribute to the relief of inflammation, maintenance of metabolic homeostasis (Griffin et al., 2012), and protection against OA. Our results in the LPS measurement concur with this hypothesis, that is, exercise intervention can reduce the high LPS concentrations induced by HFDs in the blood and joint fluids. The increased protein expression levels of TLR4 and MMP-13 induced by HFDs was also decreased by exercise. The histologic assessment showed that exercise could reduce the high





**Fig. 8.** Exercise imparts protective effects on cartilage via its effects on the modification of gut microbiota and reduction of circulating LPS in addition to losing weight.

rate of OA induced by HFDs, increase cartilage thickness, and ameliorate cartilage degeneration.

We further tested the role of berberine on the gut microbiota and LPS clearance in this experiment to determine whether or not exercise had the same effect. We found berberine could reduce the level of LPS in the serum and synovial fluid, which is similar to the effect of exercise. However, the mechanism of berberine on LPS may be different from that of exercise. Berberine reduces the diversity of intestinal flora along with substantial Firmicutes and few Bacteroidetes. These results are similar to those of HFDs but different from those of exercise. The clearance mechanism of LPS through exercise is different from that via drugs. The results of blood LPS determination indicated that berberine showed a less scavenging effect on LPS compared with exercise. Based on the above results and discussions, exercise is highly conducive to the reconstruction of healthy intestinal flora and the elimination of LPS in vivo.

The appropriate modality and intensity of exercise have positive and multifaceted effects on OA. In addition to losing weight (benefits of biomechanics), exercise can alleviate OA by reducing the LPS production (improving intestinal flora) and transport (strengthening intestinal barrier) and improving the LPS clearance in the circulatory system (Fig. 8). However, many problems need to be discussed further. For example, the microbiota is altered as a result of physical activity, but the positive modulation of such activity remains unclear. A one-unit decrease in pH (from 6.5 to 5.5) has an important selective effect on the microbiota and is conducive to the reproduction of probiotics (Duncan et al., 2009). Thus, could exercise directly or indirectly alter the intestinal pH or could the biomechanic force from exercise increase the gut motility and then accelerate the mixing of intestinal contents? Whether exercise can directly or indirectly reduce the circulating LPS (activate

LPS clearance or metabolic mechanism) when the microbiota is unregulated also remains unclear.

## 5. Conclusions

LPS is recently considered as a trigger for the pathology of OA. The LPS level of HFD-fed mice increased but decreased after exercise intervention. These changes were highly related to the alteration of the intestinal microorganism. The HFD treatment resulted in decreased gut microbial diversity, increases in endotoxin-producing bacteria, and decrease in gut barrier-protecting bacteria. Voluntary wheel running caused high gut microbial diversity and reshaped gut microbiota. Microbiota alterations caused by exercise were completely different from those induced by intestinal -regulating drugs. The increased protein expression levels of TLR4 and MMP-13 induced by HFDs decreased because of exercise. The histologic assessment showed that exercise can reduce OA rate induced by HFDs, increase cartilage thickness, and ameliorate cartilage degeneration. This study revealed that, apart from losing weight, exercise has protective effects on the cartilage by influencing the modification of the gut microbiota and reducing circulating LPS level. We proposed that voluntary wheel running should be an intervention strategy for the prevention and treatment of OA. Moreover, microbiome monitoring can be translated into diagnostic and clinical practice.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

This work was supported by the Natural Science Foundation of Shandong Province, China (Grant No. ZR2017LC012) and A Project of Shandong Province Higher Educational Science and Technology Program (Grant No. J16LE14).

## References

- Ageberg, E., Roos, E.M., 2015. Neuromuscular exercise as treatment of degenerative knee disease. *Exerc. Sport Sci. Rev.* 43, 14–22.
- Antunes, B.M., Campos, E.Z., Dos Santos, R.V.T., Rosa-Neto, J.C., Franchini, E., Bishop, N.C., et al., 2019. Anti-inflammatory response to acute exercise is related with intensity and physical fitness. *J. Cell. Biochem.* 120, 5333–5342.
- Berenbaum, F., 2013. Osteoarthritis as an inflammatory disease (osteoarthritis is not osteoarthrosis!). *Osteoarthritis Cartilage* 21, 16–21.
- Cani, P.D., Amar, J., Iglesias, M.A., Poggi, M., Knauf, C., Bastelica, D., et al., 2007. Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes* 56, 1761–1772.
- Clarke, S.F., Murphy, E.F., O'Sullivan, O., Lucey, A.J., Humphreys, M., Hogan, A., et al., 2014. Exercise and associated dietary extremes impact on gut microbial diversity. *Gut* 63, 1913–1920.
- Codella, R., Luzzi, L., Terruzzi, I., 2018. Exercise has the guts: How physical activity may positively modulate gut microbiota in chronic and immune-based diseases. *Dig. Liver Dis.* 50, 331–341.
- Collins, K.H., Paul, H.A., Reimer, R.A., Seerattan, R.A., Hart, D.A., Herzog, W., 2015. Relationship between inflammation, the gut microbiota, and metabolic osteoarthritis development: Studies in a rat model. *Osteoarthritis Cartilage* 23, 1989–1998.
- Duncan, S.H., Louis, P., Thomson, J.M., Flint, H.J., 2009. The role of pH in determining the species composition of the human colonic microbiota. *Environ. Microbiol.* 11, 2112–2122.
- Gao, W., Baig, A.Q., Ali, H., Sajjad, W., Farahani, M.R., 2017a. Margin based ontology sparse vector learning algorithm and applied in biology science. *Saudi J. Biol. Sci.* 24 (1), 132–138.
- Gao, W., Wang, Y., Basavanagoud, B., Jamil, M.K., 2017b. Characteristics studies of molecular structures in drugs. *Saudi. Pharm. J.* 25 (4), 580–586.
- Griffin, T.M., Huebner, J.L., Kraus, V.B., Yan, Z., Guilak, F., 2012. Induction of osteoarthritis and metabolic inflammation by a very high-fat diet in mice: Effects of short-term exercise. *Arthritis Rheum.* 64, 443–453.
- Hersoug, L.G., Moller, P., Loft, S., 2016. Gut microbiota-derived lipopolysaccharide uptake and trafficking to adipose tissue: implications for inflammation and obesity. *Obes. Rev.* 17, 297–312.
- Hotamisligil, G.S., Erbay, E., 2008. Nutrient sensing and inflammation in metabolic diseases. *Nat. Rev. Immunol.* 8, 923–934.
- Huang, Z., Kraus, V.B., 2016. Does lipopolysaccharide-mediated inflammation have a role in OA? *Nat. Rev. Rheumatol.* 12, 123–129.
- Koliada, A., Syzenko, G., Moseiko, V., Budovska, L., Puchkov, K., Perederiy, V., et al., 2017. Association between body mass index and Firmicutes/Bacteroidetes ratio in an adult Ukrainian population. *BMC Microbiol.* 17, 120.
- Leung, G.J., Rainsford, K.D., Kean, W.F., 2014. Osteoarthritis of the hand I: aetiology and pathogenesis, risk factors, investigation and diagnosis. *J. Pharm. Pharmacol.* 66, 339–346.
- Li, W., Jia, M., Deng, J., Wang, J., Lin, Q., Liu, C., et al., 2018. Isolation, genetic identification and degradation characteristics of COD-degrading bacterial strain in slaughter wastewater. *Saudi J. Biol. Sci.* 12 (25), 1800–1805.
- Lira, F.S., Rosa, J.C., Pimentel, G.D., Souza, H.A., Caperuto, E.C., Carnevali, L.C., et al., 2010. Endotoxin levels correlate positively with a sedentary lifestyle and negatively with highly trained subjects. *Lipids Health Dis.* 9, 82.
- Mailing, L.J., Allen, J.M., Buford, T.W., Fields, C.J., Woods, J.A., 2019. Exercise and the gut microbiome: A review of the evidence, potential mechanisms, and implications for human health. *Exerc. Sport Sci. Rev.* 47, 75–85.
- Mankin, H.J., Dorfman, H., Lippiello, L., Zarins, A., 1971. Biochemical and metabolic abnormalities in articular cartilage from osteo-arthritic human hips. II. Correlation of morphology with biochemical and metabolic data. *J. Bone Joint Surg. Am.* 53, 523–537.
- Matsumoto, M., Inoue, R., Tsukahara, T., Ushida, K., Chiji, H., Matsubara, N., et al., 2008. Voluntary running exercise alters microbiota composition and increases n-butyrate concentration in the rat cecum. *Biosci. Biotechnol. Biochem.* 72, 572–576.
- Messina, O.D., Vidal Wilman, M., Vidal Neira, L.F., 2019. Nutrition, osteoarthritis and cartilage metabolism. *Aging Clin. Exp. Res.* 31 (6), 807–813.
- Ormerod, K.L., Wood, D.L., Lachner, N., Gellatly, S.L., Daly, J.N., Parsons, J.D., et al., 2016. Genomic characterization of the uncultured Bacteroidales family S24-7 inhabiting the guts of homeothermic animals. *Microbiome* 4, 36.
- Pals, K.L., Chang, R.T., Ryan, A.J., Gisolfi, C.V., 1997. Effect of running intensity on intestinal permeability. *J. Appl. Physiol.* 1985 (82), 571–576.
- Rios, J.L., Boldt, K.R., Mather, J.W., Seerattan, R.A., Hart, D.A., Herzog, W., 2018. Quantifying the effects of different treadmill training speeds and durations on the health of rat knee joints. *Sports Med. Open.* 4 (1), 15.
- Rios, J.L., Bomhof, M.R., Reimer, R.A., Hart, D.A., Collins, K.H., Herzog, W., 2019. Protective effect of prebiotic and exercise intervention on knee health in a rat model of diet-induced obesity. *Sci. Rep.* 9, 3893.
- Robinson, W.H., Lepus, C.M., Wang, Q., Raghu, H., Mao, R., Lindstrom, T.M., et al., 2016. Low-grade inflammation as a key mediator of the pathogenesis of osteoarthritis. *Nat. Rev. Rheumatol.* 12, 580–592.
- Schmitz, N., Laverty, S., Kraus, V.B., Aigner, T., 2010. Basic methods in histopathology of joint tissues. *Osteoarthritis Cartilage* 18 (Suppl 3), S113–116.
- Schott, E.M., Farnsworth, C.W., Grier, A., Lillis, J.A., Soniwal, S., Dadourian, G.H., et al., 2018. Targeting the gut microbiome to treat the osteoarthritis of obesity. *JCI Insight* 3, (8) e95997.
- Uchiyama, K., Naito, Y., Takagi, T., 2019. Intestinal microbiome as a novel therapeutic target for local and systemic inflammation. *Pharmacol. Ther.* 199, 164–172.
- Wong, S.K., Chin, K.Y., Ima-Nirwana, S., 2019. Berberine and musculoskeletal disorders: The therapeutic potential and underlying molecular mechanisms. *Phytomedicine* 73, 152892.
- Zhang, C., Zhang, M., Wang, S., Han, R., Cao, Y., Hua, W., et al., 2009. Interactions between gut microbiota, host genetics and diet relevant to development of metabolic syndromes in mice. *ISME J.* 4, 232–241.